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MISCELLANEOUS PUBLICATION 20

IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY

1. BACTERIAL STUDIES

Warren R. Sanborn

MARCH 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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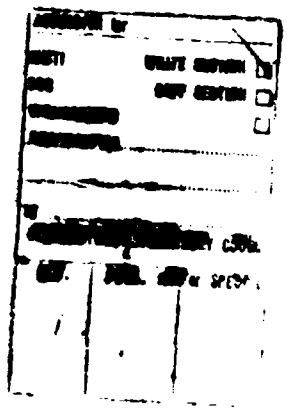
I. BACTERIAL STUDIES



Warren R. Sanborn

March 1968

Technical Information Division
AEROBIOLOGY AND EVALUATION LABORATORY

FOREWORD

The use of immunofluorescence, or fluorescent antibody, was initiated by Dr. Albert H. Coons and his co-workers in 1942. Dr. Coons has modestly stated that making antibodies fluorescent was "simply another variation of their use as reagents for the identification of specific antigen. . . ." However, this "variation" has proved to be one of immense significance to modern immunology. Its importance lies in the wedding of the two broad areas of investigation, morphology and immunology, thus allowing the detection of immunologic reactions at the cellular level.

The volume of literature related to immunofluorescence or fluorescent antibody and covering use of this technique has expanded explosively over the relatively few years since its inception. This expanding literature volume bears witness to the basic value of the technique. Through 1954, only about 40 articles had been published. In the next two years, 58 were added. During 1957 and 1958 there were 83 and 96, respectively. By 1961 the annual figure had reached more than 260 articles. For this supplementary second edition, the figures for 1963, 1964, and 1965 are 551, 764, and 678, respectively. These totals are testimony to Dr. Coons' genius.

Although it would be virtually impossible to cite every article that refers to the use of immunofluorescence, an attempt has been made to approach that limit. To that end, more than 445 journals were searched. In addition, six abstracting journals and the computer system of the National Library of Medicine, MEDLARS, were employed. Fifteen languages are represented. Translations were provided by colleagues of the compiler, government translating services, abstractors, and the compiler. The earliest entry in the original edition was 1905. In the present edition, entries covering the years 1963, 1964, and 1965 are the primary ones included, but there are also a few earlier entries not listed in the first edition. Further entries for 1966 and 1967 are now being compiled; these will be incorporated into further revisions of this bibliography.

The bibliography is intended to aid investigators in following the expanding mass of literature on the technique and to improve their skill in its use. This entire second edition, Miscellaneous Publication 20, has the same overall title, "Immunofluorescence, an Annotated Bibliography," as the first edition (Miscellaneous Publication 3). The present edition also has the same six-volume structure: Volume I, "Bacterial Studies"; Volume II, "Viral Studies"; Volume III, "Studies of Fungi, Metazoa, Protozoa, and Rickettsiae"; Volume IV, "Studies of Animal Physiology"; Volume V, "Diagnostic Applications and Review Articles"; and Volume VI, "Technical Procedures." Each of the volumes is subdivided into subject categories that should, hopefully, aid the reader in finding pertinent information in his field of interest without his spending undue time in scanning superfluous citations. Articles within subject categories are arranged alphabetically by senior author. A seventh volume, "Author and Subject Indexes," has been added to further aid the investigator in his search for articles relevant to his interest area.

Abstracts for citations in this edition have been prepared or modified in keeping with the central theme, the application of immunofluorescence to various problems. If the primary emphasis in the original article was immunofluorescence and the author's summary reflected this, the summary was generally left unchanged, except for minor changes and abbreviations simply to save space. In other instances, it was necessary to write a new abstract in order to indicate the proper place of immunofluorescent technique in the study. At the same time, the main point of such articles was maintained in abbreviated form in the abstract. Hopefully, this approach will be successful in bringing the application of immunofluorescence to the attention of the reader, while preserving each author's ideas at the same time. It is further hoped that this bibliography will aid investigators in avoiding duplication of effort and thus contribute to even greater and more imaginative applications of immunofluorescence.

Accession numbers have been assigned consecutively to citations throughout all six volumes of this edition. The plan for further future volumes allows this simple system. Entries applicable to more than one subject category appear more than once, and these will have an accession number for each placement in each volume.

A complete author index is included in each volume; the author's name is listed with the accession numbers of the entries with which he is associated. The asterisk designates those for which he is senior author.

To avoid excess duplication and unwieldy size, the second parts of Volumes V and VI contain only basic citations for articles printed in the other four volumes. However, titles of articles are included to assist the reader in selection of those citations of possible interest. As in the other volumes, the references are placed in subject categories and are arranged alphabetically by senior author within categories. The authors, the year of publication, and the volume and accession number are shown to indicate where the complete entry can be found.

For brevity, certain abbreviations in common usage in this field have been used rather than the more ponderous forms. For unmistakable identification, they are listed below.

BSA	bovine serum albumin
DANS	a. 1-dimethylaminonaphthalene-5-sulfonic acid b. 5-dimethylamino-1-naphthalene sulfonic acid or chloride form.
FA	fluorescent antibody
FIC	fluorescein isocyanate
FITC	fluorescein isothiocyanate
FTA	fluorescent treponemal antibody
FTA abs	fluorescent treponemal antibody absorbed
FTA-200	a modification of the above based on serum dilution
PAP	primary atypical pneumonia

PAS	para-aminosalicylic acid
PBS	phosphate-buffered saline
RB 200	a. lissamine rhodamine RB 200 b. lissamine rhodamine B 200 c. lissamine rhodamine B d. sulphorhodamine B e. acid rhodamine B
TPFA	Treponema pallidum fluorescent antibody
TPI	Treponema pallidum immobilization

Generally, the citations follow the format prescribed by the second edition of Style Manual for Biological Journals, American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D.C., 20036. Abbreviations follow "American Standard for Periodical Title Abbreviations," Z39.5-1963, American Standards Association Incorporated, New York.

The compiler began to collect this immunofluorescence literature in 1957 while he was stationed at U.S. Navy Preventive Medicine Unit No. 2, Norfolk, Virginia. The literature collection became more intense and organized after 1959 when he was transferred to Fort Detrick, Frederick, Maryland. Following his further transfer to the Microbiology Department of the Naval Medical Research Institute, Bethesda, Maryland, in 1963, he continued this work with the encouragement and support of both of these latter installations. Work on the second edition began in 1964, and it has continued through support from both the U.S. Army and the Bureau of Medicine and Surgery of the U.S. Navy. This volume was completed while the compiler was assigned to U.S. Navy Medical Research Unit No. 3, FPO, New York, 09527, where he is currently serving as head of the Bacteriology Department.

The information in these volumes was originally recorded on coded marginal punch cards. With the compilation of this publication, the citations and annotations have been transcribed on punched tape for conversion to automatic data processing and for use in updating later editions. Each entry is coded for recall by authors, date, title, and source publication to allow compilation of more selective listings.

Readers are invited to report errors or suggest added entries to the compiler or to Editorial Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701, for improvement of the subsequent editions. Reader assistance in this area will be deeply appreciated.

ACKNOWLEDGMENTS

The essential team effort required for development of this immunofluorescence bibliography cannot be overstressed. As with many projects of this nature, the talents, advice, guidance, and assistance of many people led to the completion of this second edition. The compiler is deeply grateful to the many people who have contributed.

Financial support for this project at first was absorbed by the Pathology Division and the Walter Reed Army Medical Unit, Fort Detrick. However, completion of the first edition (through 1962) was made possible by special financial assistance from Physical Defense Division, Fort Detrick, under Dr. Charles R. Phillips. I am extremely grateful to him for his aid. Expenses for this second edition were primarily met through a generous grant from U.S. Navy Bureau of Medicine and Surgery, Preventive Medicine Division, under CAPT J. Millar, MC, USN. Many administration expenses also were borne by the Naval Medical Research Institute and by Fort Detrick.

A number of libraries kindly donated their services. In spite of the unusual requests required by this project, these libraries were very helpful and willingly assisted, often providing valuable suggestions. Libraries primarily involved were the Technical Library, Fort Detrick, under Mr. Charles N. Bebee and later Miss Joyce A. Wolfe, and the Technical Reference Library, Naval Medical Research Institute, Mrs. T.P. Robinson, librarian. Much valuable assistance also was rendered by the National Institutes of Health Library, Miss R. Connelly, reference librarian, the National Library of Medicine, and the library of the Walter Reed Army Medical Unit, Fort Detrick. The staff members of these libraries were both helpful and patient. Without such fine assistance, the work could not have been completed.

It is a pleasure to acknowledge the highly competent secretarial help. Secretaries providing their capable and untiring talents were: Miss Sandra Rosenblatt, Miss Linda L. Zimmerman, Mrs. Marguerite M. Matovich, Mrs. Gene Heaven, Mrs. Linda Franklin, Mrs. Alberta Brown, Mrs. Margaret Raheb, and a number of others. Valuable assistance in double-checking problem references was provided by Mrs. Catherine F. Eaves and Mrs. Mary J. Gretzinger. Dr. George H. Nelson was a willing consultant for classification problems. Dr. Harold W. Batchelor provided an essential key to the development of this work by introducing the compiler to marginal punch card systems and guiding him in their application.

The Technical Information Division, under Mr. Gerald W. Beveridge, continually provided all types of assistance in addition to a home base from which to work. My gratitude for this cannot be fully expressed.

Last, but by no means least, the essential editorial work receives my highest praise. The tireless efforts, patience, and driving force supplied by these people were the prime factors in bringing this edition to completion. Mrs. Madeline Warnock Harp, in charge, Mrs. Mary D. Nelson, and Mrs. Ruth P. Zmudzinski all spent many hard weeks of work on this project. I shall always be indebted to them.

ABSTRACT

This volume is one of a series of six in the second edition of an annotated bibliography on various aspects of immunofluorescence and its use. The first six-volume edition was published in 1965 and included citations for the period 1905 through 1962. The present edition covers the period 1963 through 1965; Volume I contains 490 annotated literature citations, arranged according to major subject areas, and a complete author index.

CONTENTS

I. ACTINOMYCETALES

5001

Al-Doory, Y.; Csizmas, L. 1964. Fluorescent antibody studies with Nocardia asteroides. Bacteriol. Proc. M165:75.

Fluorescent antibody techniques were employed in differentiating N. asteroides from other branching gram-positive filaments with the aim of reducing diagnostic uncertainty arising from morphological similarities. Rabbit antiserum for N. asteriodes was produced by subcutaneous inoculation of an emulsified suspension composed of incomplete Freund adjuvant and heat-killed, acetone-dried fungal powder. Using the homologous antigen, whole serum was titrated for precipitins and tested by the indirect staining method. The globulin fraction of one sample was obtained by precipitation with half-saturated ammonium sulfate. To reduce nonspecific reactions, another sample of the same serum was fractionated by continuous-flow paper electrophoresis, from which alpha, beta, and gamma globulin fractions were collected. Using both direct and indirect staining methods, a comparison was made of the three fractions and their conjugates. This was done by testing their precipitin titer and their reactions against two strains of N. asteroides and a strain each of ten different species of closely related organisms. The two fractions of the second sample gave the most specific staining. Two methods of antibody absorption, one of which, the column method, represents a new approach, were applied to eliminate cross-reactions.

5002

Azoury, F.J.; Jones, H.E.; Gum, O.B. 1965. Comparative study of antinuclear factors and intradermal tests in systemic lupus erythematosus and antinuclear factors in nephrotic syndrome and leprosy. Arth. Rheum. 8:428-429.

A comparative study of the significance of different antinuclear factors and different intradermal tests in the diagnosis of systemic lupus erythematosus (SLE) was carried out. Of 42 patients with SLE, 31 had positive LE tests and 40 had positive antinuclear factors. Of these 40, 23 had only homogeneous pattern, 10 had shaggy and homogeneous pattern, and seven had speckled pattern. The seven patients with a speckled pattern had nephrotic syndrome. Twelve patients with nephrotic syndrome secondary to other diseases had no antinuclear factors. The presence of the shaggy pattern correlated well with acute lupus or lupus nephritis. Of ten patients with leprosy, two had antinuclear factors and arthritic manifestations. Concurrently, intradermal testing was carried out on 22 patients with SLE, using homologous leukocytes, nucleoprotein, histone, and DNA. The ten patients positive to intradermal DNA had either acute lupus or lupus nephritis confirmed by renal biopsy; the shaggy pattern was present in all positives for antinuclear factors.

5003

Barker, M.; Humphries, J.C.; Scherago, M. 1963. Uptake of fluorescein-labeled PPD by leukocytes from normal and tuberculous guinea pigs. Bacteriol. Proc. M19:59.

This study was made to determine if the leukocytes of normal and tuberculous animals can be differentiated by fluorescein-labeled purified protein derivative (PPD). PPD was labeled with fluorescein by the Celite method. Excess dye was removed with Dowex 2-X4 resin. Tuberculous, PPD-positive and nontuberculous, PPD-negative guinea pigs were used as leukocyte sources. Migration inhibition demonstrated the leukocytes of the tuberculous animals to be PPD-sensitive and those of the uninfected animals PPD-insensitive. The labeled PPD was allowed to react with lymphocytes and polymorphonuclear leukocytes from uninfected and infected guinea pigs for various times, then wet mounts were examined with a fluorescence microscope. Leukocytes from both normal and infected guinea pigs took up the labeled PPD in like degree over the range 1.0 to 0.125 mg per ml. Uptake time was less than 1 hour but more than 30 minutes. Both cell types took up labeled PPD equally well and fluorescence was evenly distributed throughout both. By light microscopy the labeled-PPD leukocytes from tuberculous animals appeared more granular than those from normal animals. Repeated exposure of labeled-PPD leukocytes to Hanks', Ringer's, and saline solutions for time periods up to 24 hours failed to elute the PPD.

5004

Bonomo, L.; Tursi, A.; Trimigliozzi, G.; Dammacco, F. 1965. LE cells and antinuclear factors in leprosy. Brit. Med. J. 2:689-690.

Serum antinuclear factors as detected by FA were found in 16 of 55 cases of leprosy. LE cells or 'rosettes' were present in the blood specimens of 4 of 10 leprosy patients with serum antinuclear factors.

5005

Clark, H.F.; Shepard, C.C. 1963. A dialysis technique for preparing fluorescent antibody. Virology 20:642-644.

A dialysis method is described for conjugating serum proteins with FITC. Antisera against rabies, polio, rickettsiae, and mycobacteria were used. Human, monkey, guinea pig, sheep, and rabbit globulins were conjugated by this method. Good specific FA staining with minimal nonspecific staining was obtained.

5006

Cottenot, F. 1964. Quantitative evaluation of murine leprosy bacillus for detection of serum antibody in human leprosy. Compt. Rend. Soc. Biol. 158:1004-1005. In French.

Indirect immunofluorescence of Stephansky's bacillus was always positive with sera from leprosy patients and was never positive with normal serum. Serum antibodies are therefore present in large numbers, especially in sera from patients with lepromatous forms or borderline cases recently detected and with positive bacteriological findings on direct examination. With effective treatment, a distinct decrease sets in. The titer is on the whole lower in tuberculoid leprosy, and particularly in indeterminate leprosy treated at an early stage.

5007

Cummins, C.S. 1965. Chemical and antigenic studies on cell walls of mycobacteria, corynebacteria, and nocardias. Amer. Rev. Resp. Dis. 92(Suppl.): 63-72.

As a portion of this study, indirect FA was used to detect the uptake of anti-smegma serum by various bacteria strains. In general FA results and agglutination results agreed. Negative FA results were seen against M. avium, M. fortuitum, M. smegmatis (fluorescent dots at surface), and Nocardia brasiliensis. It was felt that the negative FA results where positives were expected resulted from a lack of accessibility of the antigen to the serum. Purified cell walls of M. fortuitum and M. smegmatis gave strong FA reactions.

5008

Gillissen, G. 1963. Preparation of mycobacteria by a fluorescence serological method. Zentralbl. Bakteriol. Parasitenk. Infektionskrankh. Hyg. 188:81-91.

Mycobacteria were prepared using the fluorescence serological sandwich method after elimination of the nonspecific dyeing effects. The complement binding with labeled anti-complements was demonstrated.

5009

Gillissen, G. 1964. The fluorescent serological demonstration of a complement agglutination through cell-associated antibodies in tuberculosis. Z. Hyg. Infektionskrankh. 150:194-198. In German.

Washed peritoneal cells of tuberculin-sensitized rabbits agglutinate following contact with tuberculin-specific guinea pig complement. This was shown with fixed cells and an anticomplement serum conjugated with FITC.

5010

Jones, W.D., Jr.; Saito, H.; Kubica, G.P. 1965. Fluorescent antibody techniques with mycobacteria. Amer. Rev. Resp. Dis. 92:255-260.

The direct and indirect fluorescent antibody procedures have been successfully applied both to smears of intact cells prepared from pure cultures of mycobacteria and to mycobacteria present in the sputum. Cross-reactions among antisera of M. tuberculosis, M. kansasii, and M. phlei, when present, could be eliminated by the use of proper dilutions of antisera. Cross-reactions between the antisera of M. avium and Group III nonphotochromogens did present a problem, and further investigation of these two groups is needed.

5011

Kuroda, S. 1963. Serological study of antagonistic Streptomyces by fluorescent antibody technique. Chiba Igakukai Zasshi 38:456-466. In Japanese.

Some basic studies with the indirect technique using several known Streptomyces species which produce anti-tumor antibiotics are reported. Identification of 12 strains of antigen-producing Streptomyces species was made, and results were compared with agglutination titers. There was little difference in antigen from aerial vs. culture mycelia used in the tests. One case of autofluorescence in S. aburaviensis was noted. Serological and biological characteristics of Streptomyces species producing actinomycin, puromycin, and quinoxalines are reported and evaluated. The incidence of Streptomyces strains producing anti-tumor antigens was small compared to the incidence with antagonistic properties against bacteria. Fluorescent antibody is a valuable tool for identification of fungal species and products when used in conjunction with products of known strains at first-stage screening and with the available spectrum of known antagonistic streptomycetes.

5012

Martins, A.B.; Moore, W.D.; Dickinson, J.B.; Raffel, S. 1964. Cellular activities in hypersensitive reactions: III. Specifically reactive cells in delayed hypersensitivity: Tuberculin hypersensitivity. J. Immunol. 93:953-959.

Small lymphocytes from lymph nodes of guinea pigs with tuberculin hypersensitivity react specifically with tuberculoproteins, as revealed by fluoresceinated guinea pig antituberculoprotein serum. Fluorescence occurs as a rimming or halo effect in 5 to 20 per cent of such cells. Occasional glowing cells are seen in normal cell preparations at a lower level of intensity of fluorescence. Although the methods employed suggest that antibody globulin adsorbed to cells, or produced by them, is not implicated in the reactive property, this possibility cannot be excluded.

5013

Merklen, F.-P.; Cottenot, F.; Galistin, P. 1963. Antibodies demonstrated by immunofluorescence in the sera of human leprosy patients. Compt. Rend. 257:2212-2213. In French.

With immunofluorescent methods, both direct and indirect, the authors were able to demonstrate an antibacillary antibody in the sera of human leprosy patients, using Hansen's bacilli from smears of nasal mucus of lepromatous form. Stefansky's bacillus, also called the bacillus of rat leprosy, proved to be equally able to fix the antibacillary antibody of the sera of human leprosy. In sera from subjects with evolutive tuberculosis, an antibacillary antibody was found whose fixation to the bacillus of Hansen or the bacillus of Stefansky could be demonstrated by immunofluorescence; this antibody disappears as the result of previous absorption of the tuberculosis serum by a BCG culture. These investigations have not only led to the demonstration of an antibacillary antibody in the sera of human leprosy by immunofluorescence, but they have also revealed the antigenic relationship among the bacilli of Hansen, Stefansky, and Koch.

5014

Ritz, H.L. 1963. Localization of Nocardia in dental plaque by immunofluorescence. Proc. Soc. Exp. Biol. Med. 113:925-929.

Seventy-five organisms were isolated from dental plaque samples from 30 subjects and identified on the basis of their colonial morphology, micromorphology, and biochemical reactions. When stained by the indirect technique with an antinocardial serum, all 29 oral Nocardia strains isolated in this laboratory and four obtained from other laboratories stained brilliantly, although heterologous organisms showed much lower intensities of staining. The staining characteristics of paraffin sections of plaque by this technique were suggestive of the possible influence of the aerobic Nocardia on the rate of plaque formation.

5015

Saito, H.; Jones, W.D., Jr.; Kubica, G.P. 1964. Fluorescent antibody techniques with mycobacteria. Amer. Rev. Resp. Dis. 90:305-306.

Studies on mycobacterial agglutination have led to an FA technique for staining intact organisms. Actively growing cultures in Tween-albumin broth were centrifuged and resuspended in saline to an optical density of 0.1 (525 mu). At weekly intervals this viable suspension was injected into rabbits intravenously in graded dosage. If agglutinin titers were 1:1280 or greater 5 days after the fourth injection, serum was collected. The gamma globulin was fractionated from the serum and labeled with FITC. The fixation method that has proved most effective for all species of mycobacteria studied is gentle heating (65 C for

2 hours). Direct and indirect FA were tried. Duplicate microscopy preparations of the organisms being studied are prepared on one slide and fixed at 65 C for 2 hours. One smear is flooded with labeled homologous gamma globulin, the other with labeled normal gamma globulin (control). Smears are incubated in a moist chamber at 37 C for 20 minutes, washed in BS, rinsed in distilled water, gently blotted, cover slip mounted in pH 9 buffered glycerin, and examined. For examination of fluorescence, the OG-1 eyepiece and the BG-12 barrier filters have been most effective. Although cross-staining has been observed with heterologous sera, this nonspecific reaction often may be eliminated by dilution of sera. This technique has been successfully tried on pure cultures grown in Tween-albumin broth or on Loewenstein-Jensen egg slants. Organisms in a sputum preparation also have been stained. Complete article.

5016

White, R.G. 1963. The applications of fluorescent antibody techniques in bacteriology and virology; the fluorescent antibody technique in the detection of localization of bacterial antigens: Application to mycobacteria, Nocardia, and corynebacteria. Proc. Roy. Soc. Med. 56:474-478.

The use of a double-layer fluorescent antibody technique with rabbit antiserum to Mycobacterium smegmatis results in a pattern of staining of corynebacteria, nocardias, and some mycobacteria species that conforms with previously reported results of cell-wall analyses for sugars, amino sugars, and amino acids and of cell wall agglutination tests. It presumably depends upon the existence of a common antigenic determinant identical with that present in the glycopeptide moiety of wax D of human strains of M. tuberculosis. The failure of the fluorescent antibody method to stain M. smegmatis, M. avium, M. lepraemurium, and a strain of M. phlei is attributed to the inaccessibility of this antigen to antibody at the surface of these organisms. Antigen is accessible in cell-wall preparations. The widespread occurrence of this antigen throughout mycobacteria, corynebacteria, and nocardia species is of importance in the specific diagnosis of antigens or bacteria in these genera by the fluorescent antibody method.

II. BACILLACEAE

5017

Aleksevich, Va.I.; Kishko, Va.G. 1964. Application of the fluorescent antibody method for detecting tetanus bacilli. Voenno-Med. Zh. 10:47-50. In Russian.

Three series of tests for the detection of Clostridium tetani were carried out. In the first series an examination was made of laboratory strains of C. tetani in pure culture and in mixtures with C. perfringens, C. sporogenes, and B. subtilis. A clear and specific fluorescence was obtained with C. tetani cultures. Controls of C. perfringens, processed in the same manner, produced only slight fluorescence. The second stage was a study of the possible detection of C. tetani in soil. In the third series of tests a separate suspension of C. tetani as well as C. tetani in a mixture with C. perfringens and C. sporogenes were introduced into artificial wounds on the extremities of white mice and rats. An FA examination of the wound surface preparations inoculated with a mixture of tetanus microbes and C. perfringens and C. sporogenes revealed a clear glow of only the C. tetani. Examination by phase contrast revealed a large number of bacilliform microorganisms. The antitetanus agglutinating serum, obtained by immunizing rabbits under the newly developed scheme, combined with labeled antirabbit globulin conjugate, made it possible to detect the tetanus agent in pure and mixed laboratory cultures, various soil samples, and artificially inoculated wounds of test animals. The examination of wounds required 1 hour, that of the soil, 1 day.

5018

Avakyan, A.A.; Katz, L.N.; Levina, K.N.; Pavlova, I.B. 1965. Structure and composition of the Bacillus anthracis capsule. J. Bacteriol. 90:1082-1095.

Observations by phase contrast, dark-field, and fluorescence revealed the complex structure of the Bacillus anthracis capsule, which changes regularly during the growth cycle of the culture. Special cytological methods of staining the capsule made it possible to study its fine structure. The capsule shows a membrane-like surface, fine transverse lines, and interruptions and transverse septa traversing the entire capsule. The capsule has a stratified structure. The various layers differ in the value of the isoelectric point, metrachromatic ability, sensitivity to various enzymes, and, consequently, chemical composition. The membrane-like surface of the capsule consists of peptides and neutral mucopolysaccharides. The middle part of the capsule is a complex of substances, both polysaccharide and protein, and the inner part consists of acid mucopolysaccharides. Observation of the capsular forms of B. anthracis with an electron microscope revealed differences in

the osmiophilia and submicroscopic structure of the membrane-like surface and the middle and inner parts of the capsule. Immunochemical studies conducted by the fluorescent antibody method revealed localization of antigens in different parts of the capsule and made it possible to differentiate the capsular antigens according to their serum-staining ability and their relationships to enzymes.

5019

Batty, I.; Walker, P.D. 1963. Differentiation of Clostridium septicum and Clostridium chauvoei by the use of fluorescent labelled antibodies. J. Pathol. Bacteriol. 85:517-521.

Fluorescent labeled antibodies may be used as specific stains to differentiate between Clostridium chauvoei and C. septicum in smears and in tissue sections. Strains that take up homologous fluorescent antibody are also agglutinated by it. The technique provides a rapid method for the detection of these organisms in pathological material.

5020

Batty, I.; Walker, P.D. 1964. The identification of Clostridium novyi (Clostridium oedematiens) and Clostridium tetani by the use of fluorescent labeled antibodies. J. Pathol. Bacteriol. 88:327-328.

Fluorescein-labeled antibodies have been used as specific stains to identify Clostridium novyi and C. tetani.

5021

Batty, I.; Walker, P.D. 1965. Colonial morphology and fluorescent-labeled antibody staining in the identification of species of the genus Clostridium. J. Appl. Bacteriol. 28:112-118.

The morphological and colonial appearances of a number of species of Clostridium are described and illustrated. The advantages of surface culture on appropriately supplemented media on stiff agar plates for the isolation and purification of anaerobic organisms are listed. The use of fluorescein-labeled antibody in the diagnosis of anaerobic disease is discussed.

5022

Biegeleisen, J.Z., Jr. 1964. Immunofluorescent staining of Bacillus anthracis in dried beef. J. Bacteriol. 88:260-261.

FA proved to be a simple screening procedure for Bacillus anthracis in tissue. Material involved in an anthrax outbreak was successfully tested.

5023

Boikov, Ye.I. 1964. A discussion of the use of the method of fluorescing antibodies for identifying Bacillus anthracis in the soil and dust. Tr. Vses. Nauch. Issled Inst. Vet. Sanit. 24:125-132. In Russian.

The globulin fraction of anthrax-precipitating serum bound with fluorochrome was used. There was luminescence not only of the anthrax bacilli but of the anthracoids when these sera were used. Adsorbed sera were more specific. However, they also caused luminescence in heterogenic cultures. BA-47-58334.

5024

Boothroyd, M.; Georgala, D.L. 1964. Immunofluorescent identification of Clostridium botulinum. Nature 202:515-516.

Specific staining obtained with absorbed antisera suggests that the immunofluorescence technique would be useful for the rapid identification of C. botulinum in culture media or in foodstuffs. Vegetative cells of C. botulinum A were successfully detected in toxic meat, previously inoculated with spores of this organism. This examination was made on smears taken directly from the meat, and provided the identification within one hour of sampling, as opposed to the 12 to 24 hours that is required for the demonstration of C. botulinum toxin by mouse inoculation test. This technique might also be valuable for the very rapid detection and typing of C. botulinum in foods examined during suspected incidents of botulism.

5025

Chung, K.L.; Hawirko, R.Z.; Isaac, P.K. 1964. Cell wall replication: I. Cell wall growth of Bacillus cereus and Bacillus megaterium. Can. J. Microbiol. 10:43-48.

Cell wall replication of Bacillus cereus and Bacillus megaterium was studied by differential labeling with fluorescent and nonfluorescent antibody. Growth of new cell wall in B. cereus was initiated near the poles. In the old wall, additional new wall segments gradually developed to form an alternating pattern of new and old wall segments. Further growth elongated the new wall and pushed the old segments apart. Separation of daughter cells appeared to involve splitting of the transverse septa laid down at or near the old wall segments. Growth of new cell wall of B. megaterium was initiated either at one of the poles or at the central area of the cell. Multiple segments of new and old wall appeared along the cell length. Further elongation was followed by formation of transverse septa and separation of daughter cells incorporating either old or new wall segments. Our evidence clearly shows that growth and elongation of the two bacilli do not occur by diffuse intercalation of new cell wall into the old.

5026

Cole, R.M. 1965. Symposium on the fine structure and replication of bacteria and their parts: III. Bacterial cell wall replication followed by immunofluorescence. Bacteriol. Rev. 29:326-344.

This is an interpretive and critical report. The author urges further application of FA to the study of surface-antigen replication in walled microorganisms. Confirmation or denial of controversial points in this study area will follow only from such further study. FA has clear advantages over any other method for cell wall study. The chief advantage is the ability to apply a specific label to the wall of a living cell.

5027

D'Antona, D. 1964. Anaerobic and aerobic tetanus bacillus studied with the immunofluorescence reaction: Contribution to the study of the genesis of tetanus toxin. Nuovi Ann. Ig. Microbiol. 15:254-262. In Italian.

The results demonstrate the usefulness and reliability of the immunofluorescent test, using tetanus antitoxin conjugated with fluorescein isothiocyanate, in recognizing and differentiating anaerobic and aerobic strains of the tetanus bacillus. The presence and localization of tetanus toxin in the bacterial cells in the anaerobic (pathogenic) strains, and its absence in the aerobic (nonpathogenic) strains, are rapidly shown by FA. The loss of every pathogenic and toxic power during the passage of Nicolaier bacillus from anaerobic or aerobic life is confirmed. Existence of a somatic agglutinogen of tetanus organisms in both the anaerobic and aerobic phase is demonstrated by the immunofluorescent test applied through cross tests with anti-anaerobic and aerobic agglutinating sera.

5028

Pey, H. 1965. Fluorescence microscopy identification of unusual colonies of clostridia. Pathol. Microbiol. 28:225-228. In German.

Since using trypticase soy agar as a base for blood agar, we have observed that, although C. feseri strains produce normal colonies with strong hemolysis, their gram-stained rods show yeast-like ballooning and thickening. Using fluorescent microscopy with anti-C. feseri serum marked with RB 200, rapid identification of these abnormal forms as C. feseri organisms was possible.

5029

Franek, J. 1964. Application of fluorescent antibodies for demonstrating B. anthracis in the organs of infected animals. J. Hyg. Epidemiol. Microbiol. Immunol. 8:111-119.

The possibilities of using anticapsular serum for the detection of B. anthracis in the organs of experimental animals were tested. Although the serum alone does not give a strictly specific reaction with bacterial suspensions, it proved absolutely satisfactory for the identification of anthrax bacilli in organ imprints. The development of anthrax in experimental animals was studied by the indirect fluorescent antibody method. When applied to spleen imprints, fluorescent antibody gives two basic, characteristic pictures, intensively fluorescent granules, localized mainly within the cells, during the first hours of the infectious process, and rods with progressively developing capsules during the later phases. RB200-BSA was successful as a counterstain.

5030

Franek, J. 1965. Use of fluorescent antibodies for the rapid diagnosis of infections caused by B. anthracis and P. tularensis. J. Hyg. Epidemiol. Microbiol. Immunol. 9:160-168.

In experiments with virulent strains of B. anthracis and P. tularensis the possibility of speeding up biological tests by FA was studied. It was possible to detect 150 anthrax bacilli and 500 pasteurellae in the smears from suspensions of organs. These results were obtained with concentrations of microorganisms reaching approximately 10^4 to 5×10^5 of the organ suspensions under investigation. Thus it was possible to finish the biological test for B. anthracis within 18 to 24 hours and that for P. tularensis within 72 hours. In order to speed up the biological tests still more and to increase the reliability of detection of even small quantities of microorganisms, the use of cortisone in mice brought good results.

5031

Hendrix, C.E.; Tew, R.W. 1965. A photographic method for determination of fluorescence intensity. Bacteriol. Proc. M129:61.

This describes a photographic method for quantitative measurement of the brightness of preparations stained with fluorescent antibody. The theory of the procedure is based on determinations of the exposure time (minimal exposure time) necessary to reach a reference point on the Hurter-Driffield curve of the photographic emulsion. The point chosen is at the toe of the curve, which for practical purposes represents the exposure necessary to give a barely discernible image. To insure comparability of results, illumination should be measured

prior to each determination of fluorescence brightness. We employed the method in a study of staining time and titer. Polaroid 3000 speed film was used for photography with UV dark-field illumination and 1,200X magnification. Results of staining Bacillus globigii spores with two dilutions of a fluoreacent conjugate are given in terms of staining times in minutes, visual estimates of brightness, and minimal exposure times in seconds. The data show that visual observations correlated rather well with photographic measurements.

5032

Hill, E.O.; Lewis, S. 1964. L forms of bacteria isolated from surgical infections. Bacteriol. Proc. M26:48.

Transitional and stable L forms have been isolated from a variety of specimens obtained from patients: blood; spinal, pleural, and synovial fluids; abscesses; lymph nodes; and acne pustules. So called transitional L forms of Clostridium perfringens, C. sporogenes, Peptostreptococcus sp., and Sphaerophorus sp. have been recovered on primary culture media without use of inducing or selective agents. Aerobic and anaerobic cultures were planted on brain heart infusion agar, PPLO agar, Brewer-modified thioglycolate medium, and Castaneda blood culture medium, with and without supplements of yeast extract, human blood, ascitic fluid, or PPLO serum fraction. Use of 2,3,5-triphenyltetrazolium chloride at 0.0025 per cent in the agar media aided in the detection of L colonies. Evidence is presented for the in vivo occurrence of L forms in a patient with thrombophlebitis. L forms were observed in direct mounts of spinal fluid. Organisms of similar morphology were recovered from cultures of the spinal fluid, thrombus, and blood. Immunofluorescence indicated that the L forms recovered from the thrombus were serologically related to S. necrophorus, and transitional L forms of Sphaerophorus sp. were isolated from blood cultures. L forms have persisted in the blood stream of this patient for 5 months postoperatively.

5033

Hovnanian, H.P.; Brennan, T.A.; Botan, E.A. 1964. Quantitative rapid immunofluorescence microscopy. J. Bacteriol. 87:473-476.

An elaborate electronic-optical instrument for detection and measurement of FA stain reactions is described. It consists of a UV source, UV monochromator, UV filter system, bright-field fluorescence microscope, secondary filter system, a UV television camera, a microspot scanner, a quantitative light-reading device, television monitor, and an oscilloscope. Satisfactory tests were made with FA systems for E. coli, S. lutea, and B. globigii.

5034

Klotz, A.W. 1965. Application of FA techniques to detection of Clostridium perfringens. Public Health Rep. 80:305-311.

One hundred and fifty strains of Clostridium perfringens (toxigenic Types A through F) were obtained from a variety of sources. Employing formalin-treated antigens, sera were prepared in rabbits for 56 of these strains. The sera and conjugates derived from them were arranged into five pools for screening cultures by slide agglutination and fluorescent antibody tests. These reagents were specific for the capsular antigen by capsular swelling and FA tests. C. perfringens was grown in a variety of foods and the encapsulated organisms were stained by the appropriate FA reagent in smears made directly from the food. Mixed strains of C. perfringens were commonly found in human feces, but the organisms apparently were not well encapsulated. Their presence was easily demonstrated by a combination of enrichment and FA techniques without the necessity of obtaining pure cultures. FA may prove to be a valuable tool for the rapid identification and enumeration of C. perfringens in food and feces during investigations of food poisoning outbreaks.

5035

Klotz, A.W. 1965. Application of fluorescent antibody techniques to the study of Clostridium perfringens. Diss. Abstr. 26:1290.

A variety of sources supplied 150 strains of C. perfringens (toxigenic Types A-F). The identity of these cultures was confirmed by standard bacteriological techniques. Formalinized antigens were prepared from all strains and rabbit antisera of satisfactory quality were produced. FA conjugates were prepared and divided into five pools for screening purposes. C. perfringens strains fluoresced brilliantly when stained with homologous conjugates. FA techniques and Quellung tests showed that the antibodies produced were directed primarily against the capsules. Capsular antigens were very stable, withstanding 5.0 per cent formalinization, treatment with dilute alkalies, and autoclaving in saline or water for an hour at 245 F. Antigen suspensions stored at 5 C in 0.4 per cent formalinized buffered (pH 7.4) saline were stable for 6 months or more. FA slide agglutination tests confirmed the findings of previous researchers that C. perfringens strains are antigenically diverse. Antibodies produced were not in all cases specific for the individual toxigenic types (A-F). Type B organisms appeared to be more closely related antigenically than the other types. By the use of 56 antisera and conjugates, only 34 of a total of 79 cultures were serotypeable. Members of the Hobbs strains accounted for 21 of the 34 typeable strains. Tests were conducted to check the specificity of C. perfringens antisera and conjugates against other closely associated bacteria. These were negative, with the exception of a possible antigenic relationship between C. perfringens and certain strains of C. multifermentans

and C. septicum. Mixed strains of C. perfringens were found commonly in human feces but apparently were not well encapsulated. Their presence was easily demonstrated by a combination of cultural and FA techniques. C. perfringens produced capsules when it grew in foods. Food samples could be preserved with 1.0 to 5.0 per cent formalin for at least 20 days at 37 C without impairing the FA staining characteristics.

5036

Nikitin, V.M. 1964. The use of immunofluorescent paper disks for the rapid detection of pathogenic microbes. VoennoMed. Zh. 11:55-58. In Russian.

Instead of the liquid form of labeled gamma globulins, strips or disks of various kinds of paper saturated with fluorescent conjugates were prepared. Best results were obtained with filter paper disks. One drop of fluorescent immune serum of certain specificity was applied to each side of the disks measuring 1 cm in diameter. The disks were dried and stored at 4 C. Trials on cultures of Flexner's bacillus, Escherichia coli, tularemia vaccine, and anthrax vaccine were carried out both by the direct and the indirect methods. The disks kept their immunological capacity for specific staining up to 15 months, although exposure time had to be increased as storage time was increased. The use of these disks simplifies storage, application, and transportation of the fluorescent antibodies and facilitates their standardization. The recommendation of their use in field conditions is justified.

5037

Petty, C.S. 1965. Botulism: The disease and the toxin. Amer. J. Med. Sci. 249:345-359.

As a portion of this extensive review of botulism, FA is mentioned as a method for identifying the organism.

5038

Ponomareva, T.N. 1963. On bacteriological diagnosis of anthrax. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:5:107-112. In Russian.

The paper reports on the difficulties encountered during laboratory diagnosis of anthrax. Anthrax bacilli should be identified by a complex of signs: morphology of colonies and bacilli, spore formation and capsule formation in the animal organism, the absence of motility, the character of growth on gelatin, the absence of hemolysis, pathogenicity for guinea pigs, and positive precipitation reaction according to Ascoli. The bead test, fluorescence microscopy, and the test with a specific phage facilitate diagnosis. All the anthrax strains isolated from human beings possessed a high virulence for albino mice and guinea pigs and were typical in their principal properties, exclu-

ding one strain isolated from a patient after a 4-day penicillin treatment. From the anthrax patients treated with antibiotics, it was possible to isolate organisms not later than 4 days from the beginning of treatment. An anthrax culture is described that was isolated from the soil sample of a former cattle burial place. This culture had altered properties: the absence of capacity to form a capsule in the animal, a reduced virulence for guinea pigs and albino mice, and a capacity to provoke hemolysis and form smooth colonies.

5039

Szanto, R.; Geck, P. 1965. Immunofluorescent identification of Clostridium perfringens in various secretions. Orv. Hetilap 106:313-314. In Hungarian.

Clostridium perfringens was demonstrated in various human secretions by the immunofluorescent method, using no preliminary preparation. The usual bacteriological culture was performed by the anaerobic method for comparison. Of the 100 samples, 4 per cent positive indications were obtained with the anaerobic culture method compared with 19 per cent positive results with the immunofluorescent method. The advantages of this method lie not only in the greater sensitivity but also in the reduction of time required to obtain results.

5040

Walker, P.D.; Batty, I. 1963. Facets of Clostridium sporogenes and Cl. botulinum as shown by fluorescent labeled antibodies. J. Appl. Bacteriol. 26:v.

Some of the antigenic changes occurring on the surface of Clostridium sporogenes during sporulation and germination were followed by means of spore antisera coupled with FITC and vegetative cell antisera coupled with RB 200. The first apparent change during germination was a loss in some areas of the spore antigens, probably synchronous with the splitting of the spore coat. Following this, vegetative antigens emerged, and the antigen of the spore gradually disappeared until only the vegetative cell antigens remained. During sporulation, spore antigens appeared on the surface of the vegetative cell only after the organism had become fully permeable to spore strains. Fluorescent labeled antisera to heat killed organisms were used to discriminate between the various types of C. botulinum. An antiserum prepared against a Type A organism stained all strains of Types A, B, and F, but none of Types C, D, and E. Antisera prepared against Type C and D gave complete cross-reaction with both these types and with no other type. An antiserum prepared against Type E stained organisms of Type E only. The antisera stained flagella as well as the cell wall. Considerable overlap of flagella staining was noted in the various types. Complete article.

5041

Walker, P.D.; Batty, I. 1964. Fluorescent studies in the genus Clostridium: I. The location of antigens on the surface of Clostridium sporogenes during sporulation and germination. J. Appl. Bacteriol. 27:137-139.

Fluorescent labeled specific antisera against spores and vegetative cells have been used as stains to follow the antigenic changes that occur on the surface of Clostridium sporogenes during sporulation and germination.

5042

Walker, P.D.; Batty, I. 1964. Fluorescent studies in the genus Colostridium: II. A rapid method for differentiating Clostridium botulinum types A, B and F, types C and D, and Type E. J. Appl. Bacteriol. 27:140-142.

Fluorescent labeled specific antisera have been used to distinguish among Types A, B, and F, Types C and D, and Type E of Clostridium botulinum.

5043

Walker, P.D.; Batty, I. 1965. Surface antigenic changes in Bacillus cereus during germination and sporulation as shown by fluorescent staining. J. Appl. Bacteriol. 28:194-196.

Antigenic changes occurring on the surface of Bacillus cereus during sporulation and germination have been followed using FA.

5044

Zacks, S.I.; Sheff, M.F. 1965. Studies on tetanus toxin: III. Intercellular localization of fluorescent-labeled tetanus toxin and antitoxin in mice. Acta Neuropathol. 4:267-277.

In a series of in vivo and in vitro experiments employing crude and purified tetanus toxin labeled with fluorescent dyes, the following observations were made. Tetanus toxin is bound to muscle and brain, but not to liver, cardiac muscle, spleen, kidney, or lung. Since these tissues will not bind serum albumin or tetanus antitoxin or rhodamine to the same degree, even when these are present at 20 times the concentration of the toxin, this binding indicates tissue selectivity towards tetanus toxin. Mitochondria isolated from brain were stained by RB 200-labeled tetanus toxin, but liver mitochondria were not stained. Treatment of the test animals with either antitoxin or toxin before sacrifice reduced the apparent amount of toxin bound to muscle in vitro but had little effect on the ability of brain to bind the toxin.

After toxin was bound to both muscle and brain in vitro, valences were still available for the binding of antitoxin. Since the amount of antitoxin bound to muscle or brain was greatly increased by prior incubation of the sections with toxin it follows that this is an example of specific immunological binding.

III. BEDSONIA (MIYAGAWANELLA)

5045

Bates, H.A.; Pomeroy, B.S.; Seal, U.S.; Jay, A.R. 1965. Ornithosis: Experimental immunofluorescent studies. Avian Dis. 9:24-30.

Ion exchange chromatography and gel filtration yielded a turkey serum fraction that had apparently lost little of its DCF antibody activity and, after conjugation to fluorescein isothiocyanate, stained with specificity tissues obtained from infected mice and turkeys. This positive fluorescent staining could be correlated with gross lesions and isolation of an ornithosis agent from these tissues.

5046

Bernkopf, H.; Treu, G.; Maythar, B. 1964. Human infection experiments with three cell-cultured trachoma agents. Arch. Ophthalmol. 71:693-700.

Three trachoma strains adapted to continuous growth in FL cell cultures were examined for their pathogenicity for the human eye. Trachoma developed after infection with each of the three strains studied. Considerable individual differences in the clinical picture were observed. A specific antibody response was elicited in each case, as demonstrated by the indirect fluorescent antibody technique as well as by complement fixation and neutralization tests.

5047

Dunlop, E.M.C.; Al-Hussaini, M.; Garland, J.; Treharne, J.; Harper, I.; Jones, B. 1965. Infection of urethra by TRIC agent in men presenting because of nonspecific urethritis. Lancet 1:1125-1128.

Nine men with nonspecific urethritis were studied by cultural isolation microscopic examination for inclusion bodies, and microscopic evidence of cellular change. Three isolations were made. Four specimens contained inclusion bodies; two of these were also positive for isolation of TRIC agent. Four female sexual partners were examined and one inclusion was found. Among other test procedures, FA was used to demonstrate that the isolates were members of the Bedsonia group.

5048

Goldin, R.B.; Krasnik, F.I. 1963. Specific staining of ornithosis virus by fluorescein-labeled incomplete antibodies. Acta Virol. 7:561.

Sera from pigeons recovered from ornithosis served as source of antibodies. The presence in these sera of complete and incomplete antibodies was estimated by the direct and indirect modifications of the

complement fixation reaction. The conjugates were all equally sufficiently specific, clearly stained homologous viral bodies, and induced no fluorescence of antigenically distinct microorganisms. Conjugates prepared from incomplete antibodies stained homologous antigens more rapidly, in higher dilution, and stained more particles than conjugates prepared from complete antibodies.

5049

Goldin, R.B.; Krasnik, F.I. 1963. Use of complete and incomplete fluorescent antibodies for detection of virus of ornithosis (experimental material). Tr. Inst. Epidemiol. Mikrobiol. Gig. Leningrad 25:251-259. In Russian.

Specific fluorescent sera were prepared for the immunofluorescent staining of members of the ornithosis group. For direct staining we used ornithosis convalescent sera from doves. For indirect FA antisera obtained by immunizing rabbits with human globulin, dove sera and duck sera were used. The gamma globulin of dove serum containing incomplete specific antibodies against the ornithosis virus was tagged, and the immunochemical and dyeing properties of the conjugates were studied. Incomplete fluorescent antibodies stained homologous particles, with higher dilutions of conjugates, two to three times faster than the corresponding fluorescent sera containing complete antibodies. This shows the greater avidity of conjugates with incomplete antibodies. Conjugates from complete and incomplete antibodies are sufficiently specific to stain ornithosis agent, and do not produce fluorescence with viruses, rickettsiae, or bacteria. Use of this method allows direct exposure of virus particles in smear imprints from infected animals and chick embryos. The simplicity, specificity, and rapidity of the FA method recommend it for diagnosis and study of ornithosis infection.

5050

Hahon, N.; Nakamura, R.M. 1964. Quantitative assay of psittacosis virus by the fluorescent cell-counting technique. Virology 23:203-208.

An assay technique for psittacosis virus is described that is based on the enumeration of fluorescent cells within 24 hours after infection of McCoy cell monolayers. The distribution of infected cells is random; the relationship of cell counts to virus concentration is linear. The quantity of psittacosis virus neutralized by dilutions of antiserum is shown by this assay technique to be a linear function.

5051

Mahon, N.; Cooke, K.O. 1965. Fluorescent cell-counting neutralization test for psittacosis. J. Bacteriol. 89:1465-1471.

A sensitive, precise, and specific serological procedure, the fluorescent cell-counting neutralization test, was developed to detect and to measure quantitatively psittacosis serum-neutralizing antibodies within 24 hours. The test is based on the reduction of fluorescent cells in McCoy cell monolayers resulting from the neutralization of infective agent particles by specific antiserum. Small but significant rises in neutralizing titers were measured in serum specimens from monkeys previously exposed to the psittacosis agent and from humans with diagnoses of subclinical or established psittacosis infections.

5052

Hanna, L.; Bernkopf, H. 1964. Trachoma viruses isolated in the United States: VIII. Separation of TRIC viruses from related agents by immunofluorescence. Proc. Soc. Exp. Biol. Med. 116:827-831.

Yolk sacs infected with trachoma, inclusion conjunctivitis, lymphogranuloma venereum, psittacosis 6BC, or meningopneumonitis were extracted repeatedly with fluorocarbon. The resulting suspension of elementary bodies contained little egg material. The particles were distinct, and reacted with strain-specific rabbit antisera as measured by indirect fluorescence. The homologous antiserum usually yielded the highest titer; heterologous antisera showed equal, intermediate, or no reaction; antisera to normal yolk sac were negative. Psittacosis 6BC and meningopneumonitis could be separated easily from TRIC agents. LGV, an IC isolate, and BOUR, a trachoma isolate, showed much cross-reaction, but BOUR could be differentiated regularly from another trachoma isolate, ASGH.

5053

Hanna, L.; Bernkopf, H. 1964. Immunofluorescence for the separation of TRIC viruses from related agents. Bacteriol. Proc. V16:117.

There is no acceptable serological test to differentiate TRIC from other members of the group, although species-specific antigens have been demonstrated in cell walls of some pneumonitis viruses. Pools of infected yolk sacs were extracted repeatedly with a Freon-113 and heptane mixture (specific gravity 1.3) and concentrated by centrifugation. The resulting concentrated antigen suspension contained very little egg material; individual particles were sharply defined, and reacted readily with strain-specific high-titered rabbit antisera by the indirect fluorescent antibody technique. The homologous antiserum usually yielded the highest titer, 1:64 to 1:1,000; heterologous antisera gave equal, intermediate, or no reactions. Normal yolk sac antisera showed no reactivity. The results to date permit

an unequivocal separation of meningopneumonitis and psittacosis 6BC from TRIC agents. One trachoma isolate, BOUR, is separable from another, ASGH. One strain of lymphogranuloma venereum and an inclusion conjunctivitis isolate, CAL 3, cannot be separated, and overlap much more with others.

5054

Hanna, L.; Okumoto, M.; Thygeson, P.; Rose, L.; Dawson, C.R. 1965. TRIC agents isolated in the United States: X. Immunofluorescence in the diagnosis of TRIC agent infection in man. Proc. Soc. Exp. Biol. Med. 119:722-728.

Conjunctival scrapings from volunteers experimentally infected with TRIC agents and from patients with spontaneous eye disease were examined by immunofluorescence. Brightly fluorescing inclusions were demonstrated with hyperimmune TRIC antisera. The findings were compared with those obtained by Giemsa stain, iodine stain, or isolation of the infectious agent in embryonated eggs. Immunofluorescence appears to offer an improved method for laboratory diagnosis of TRIC infections.

5055

Jawetz, E.; Rose, L.; Hanna, L.; Thygeson, P. 1965. Experimental inclusion conjunctivitis in man: Measurements of infectivity and resistance. J. Amer. Med. Ass. 194:620-632.

Two isolates from inclusion conjunctivitis of the newborn were inoculated into one eye of volunteers one or more times. In 33 volunteers, 41 infections were produced and followed for up to ten months. The infective dose for the adult eye consisted of 500 to 1500 particles which constituted 4 to 24 egg infective doses. After prolonged infection with one isolate, volunteers were fully resistant to reinoculation with the same, but not a different, isolate. This apparent strain-specific immunity may have important implications for the development of immunizing procedures against TRIC agents. Signs, symptoms, and dose effects are described. FA tests were not able to distinguish the trachoma and inclusion conjunctivitis agents.

5056

Katzenelson, E.; Bernkopf, H. 1965. Serologic differentiation of trachoma strains and other agents of the psittacosis-lymphogranuloma venerum-trachoma group with the aid of the direct fluorescent antibody method. J. Immunol. 94:467-474.

The serologic relationship of various strains of the psittacosis-lymphogranuloma venereum-trachoma group of agents was examined with the aid of rabbit-immune sera and antigens partially purified with fluorocarbon or diethylaminoethyl cellulose. The direct fluorescent

antibody technique was employed. Major sharing of antigens between trachoma and inclusion conjunctivitis (TRIC) agents and two strains of lymphogranuloma venereum was demonstrated No cross-reactions were observed between these strains and a strain of psittacosis. By the use of cross-absorbed immune sera, a subdivision of TRIC agents was obtained according to which strains Dari and Camal, isolated in Israel, and strains Bour and Tang fell in Group I and strains Asgh, SA-1, and G-17 in Group II. The strain of inclusion conjunctivitis IC Cal-3 showed cross-reactions with both groups of trachoma and, in addition, some specificity of its own. With the aid of cross-absorbed rabbit-immune sera it was possible to group trachoma agents directly from infected yolk sac smears.

5057

Mordhorst, C.H. 1965. Trachoma agents isolated in the United States: IX. Complement-fixing and immunofluorescent antibodies in parenterally injected monkeys. Amer. J. Ophthalmol. 59:769-773.

All injected animals developed similar levels of antibodies measured by the indirect immunofluorescence test. Antibody titer was unrelated to the observed partial resistance to infection. Recent studies in this laboratory have suggested far-reaching overlap of antigenic responses to the inclusion conjunctivitis isolate IC-Cal 3 and the trachoma isolate ASGH, so that, in fact, these two agents cannot be distinguished by immunofluorescence examination of fluorocarbon-treated antigens. An important and significant finding in the present experiments was the unequivocal ability of the immunofluorescence test to distinguish between antiyolk sac and anti-TRIC antibodies, both of which were stimulated by immunization with infected yolk sac material The complement fixation test with group antigen was incapable of such a distinction. The results suggest that eye infection was a less potent stimulus to TRIC antibodies than repeated intramuscular administration of antigens. Elevated antibody titers persisted for only a very few weeks.

5058

Murray, E.S. 1964. Guinea pig inclusion conjunctivitis virus: I. Isolation and identification as a member of the psittacosis-lymphogranuloma-trachoma group. J. Infect. Dis. 114.1-12

A conjunctivitis caused by a psittacosis-lymphogranuloma-trachoma (PLT) group virus has been shown to occur as an enzootic, epizootic disease in certain herds of guinea pigs. It occurs commonly between the 4th and 8th weeks of life. The association of the guinea pig inclusion conjunctivitis (gp-ic) viruses with the PLT group was demonstrated by two serologic tests: inclusion bodies in infected guinea pig conjunctival cells as well as elementary bodies in infected yolk sacs of embryonated eggs fluoresce when stained with conjugated lymphogranuloma venereum sera; and, upon boiling, the gp-ic viruses yield the common

PLT group antigen that fixes complement with convalescent sera from patients with lymphogranuloma venereum, trachoma, and psittacosis. Furthermore, boiled group antigens processed from trachoma or psittacosis viruses fix complement with sera from many, but not all, guinea pigs convalescent from a conjunctivitis caused by reinfections with a gp-ic virus.

5059

Nichols, R.L.; McComb, D.E. 1964. Serologic strain differentiation in trachoma. J. Exp. Med. 120:639-654.

An in vitro test for the differentiation of trachoma strain isolates is described. The method was based on absorption of trachoma antiserum followed by immunofluorescent staining of individual trachoma strains. Nineteen trachoma strains were studied; two main types were identified. Evidence for quantitative and qualitative antigenic variation between the two strains is presented. The limitations in practice and in theory of the test are emphasized.

5060

Parikh, G.; Shechmeister, I.L. 1964. Interaction of meningopneumonitis virus with white blood cells: II. Antigenic subunits of meningopneumonitis virus. Virology 22:177-185.

Fluorocarbon extract of concentrated meningopneumonitis virus contained multifunctional subunits that exhibited precipitating, leucoagglutinating, and complement-fixing activities. Further concentration of these subunits was achieved by ether extraction, particularly in the case of the complement-fixing antigen. FA was applied to quantitative determination of virus antigens on paper. FA was more sensitive than the Ouchterlony test, and detected minute amounts of antigen.

5061

Ross, M.R.; Borman, E.K. 1963. Direct and indirect fluorescent antibody techniques for the psittacosis-lymphogranuloma venereum-trachoma group of agents. J. Bacteriol. 85:851-858.

Direct and indirect FA techniques were developed for the detection of group antigen in infected tissue cultures and the titration of group antibody in human antiserum. The growth of the agent of meningopneumonitis, MP, in mouse embryo lung cell monolayers was followed by infectivity and CF antigen titrations and by cytological examination of FA-stained cultures. Although infectivity and CF antigen reached a peak at 2 days and remained constant for an additional 3 days, only cells tested 2 to 3 days after infection were suitable for FA staining with labeled anti-MP serum because of excessive artifacts in the older cultures. FITC-labeled rooster and guinea pig anti-MP

sera and human anti-psittacosis sera were titrated in direct FA and hemagglutination inhibition, HI, tests. The rooster conjugate showed brighter staining and higher antibody titers than the guinea pig or human conjugates. FA staining reactions with labeled rooster serum were inhibited by unlabeled rooster serum, but clearcut inhibition with human anti-psittacosis serum could not be demonstrated. A comparison of the indirect FA, HI, and CF tests showed the indirect FA technique to be intermediate in sensitivity between the HI and CF tests. None of the three tests showed significant cross-reactions with human sera reactive against other agents.

5063

Serbezov, V.; Ognanov, D.; Matova, E.; Alexandrov, E.; Makaveyeva, E.; Nedeltseva, N. 1965. Detection of ornithosis virus by the fluorescent antibody method, using convalescent antivirus abortion sheep sera. J. Hyg. Epidemiol. Microbiol. Immunol. 9:253-255.

The findings indicate that conjugates prepared with anti-VAS sheep sera can be used for studying viruses of the psittacosis-ornithosis group by the fluorescent antibody method.

5063

Terskikh, I.I.; Zairov, G.K. 1964. A comparative study of trachoma and ornithosis viruses. Vop. Virusol. 9:674-677. In Russian.

The development of some strains of ornithosis and trachoma viruses, chlamydozoa group, was studied by fluorescent microscopy in primary and continuously cultivated tissue cultures. According to histochemical and morphological findings, cytoplasmic inclusions of ornithosis and trachoma have three stages depending on the amount of RNA and DNA therein. Clear differences are observed between trachoma and ornithosis viruses in tissue culture, which can be used for comparative studies of newly isolated agents of this group and as diagnostic and laboratory tests to determine a stage of virus development in the cell.

5064

Tokarevich, K.N.; Krasnik, F.I.; Goldin, R.B. 1963. The use of fluorescent antibody technique in serological diagnosis of ornithosis. Acta Virol. 7:478.

The FA technique makes it possible to demonstrate both complete and incomplete antibodies, unifying therefore the diagnostic possibilities offered by both modifications of the complement fixation test. The FA technique can thus be recommended as a rapid and universal method in serological diagnosis of ornithosis.

5065

Tokarevich, K.N.; Krasnik, F.I.; Goldin, R.B. 1963. Serodiagnosis of ornithosis infection by the immunofluorescent method. Tr. Inst. Epidemiol. Mikrobiol. Gig. Leningrad 25:245-250. In Russian.

As a result of the parallel investigation of hundreds of sera from humans and birds by the immunofluorescent method and by the complement fixation test in two modifications, we demonstrated a high degree of correlation in the results of these immunological methods. It was also established that the immunofluorescent method makes it possible to detect not only CF antibodies but also inhibitor-specific immune bodies. This method therefore combines the diagnostic capabilities of both modifications of the complement fixation reaction.

5066

Zelenkova, L.; Strauss, J. 1963. Fluorescent antibody tests in the diagnosis of ornithosis. Cesk. Epidemiol. 12:140-144. In Czech.

The test of fluorescent ornithosis antibodies was first tried experimentally on impression smears from mouse brains and pigeon spleen and liver infected with ornithosis virus. Later, the method was tried in practice in ducks considered to have latent ornithosis. The impression smears were treated by direct and indirect FA using labeled human convalescent or rabbit antihuman gamma globulin. Labeled antibodies were the most reliable index of both acute and latent infection. Simultaneous examination of 44 ducks' spleen and liver by FA and of their sera by the inhibition complement fixation reaction showed FA to be a more sensitive technique by 30 per cent. It is suggested that FA should be introduced on a large scale into veterinary practice for purposes of constant effectiveness of antibiotic treatment and prevention.

IV. BRUCELLACEAE

5067

Ananova, Ye.V.; Yemelyanova, O.S. 1964. Application of the fluorescence serological method for detection of the tularemia microbe. Lab. Delo 10:35-39. In Russian.

The fluorescence serological method is specific for tularemia. Its sensitivity threshold in regular detection of the tularemia microbe in pure suspensions and in mixtures with other bacteria is 1 million microbial cells in 1 ml of physiological solution or distilled water. The method is more sensitive than ordinary microscopic examination of smear imprints from the organs of animals stained with the Romanovsky-Giemsa stain. It can be compared with the method of detecting the tularemia microbe by seeding on nutrient media. This method has an advantage over other methods of detecting tularemia in the rapidity of the response obtained, and can be recommended for rapid detection of this causative agent.

5068

Anonymous. 1965. Echo of historic epidemics: Six plague cases reported in nation. J. Amer. Med. Ass. 194:21-23.

Plague was contracted by five children in New Mexico and one boy in California. Wild rodents were the source of infection. FA demonstrated Pasteurella pestis in tissue.

5069

Axt, J.; Jentzsch, K.D. 1962. Study of brucellae with fluorescent antibodies: 1. Conjugation of immune globulin with DANS. Arch. Exper. Veterinaermed. 16:1309-1316. In German.

This is a description of Brucella antiserum with DANS. This process decreased the agglutinin titer considerably. Preservation of antisera with 0.5 per cent phenol did not affect the conjugation.

5070

Balandin, G.A.; Sazykin, S.P. 1964. On postvaccinal allergy in brucellosis: II. Communication. Zh. Mikrobiol. Epidemiol. i Immunobiol. 41:2:80-84. In Russian.

Live Brucella abortus vaccine strains 19 and 19-BA, evidently not pathogenic, not only determine the development of specific antibrucellosis immunity, in subjects vaccinated with them, but at the same time tend to sensitize them specifically. The extent and depth of this

sensitization is in direct relationship to the degree of antigenic stimulation of those vaccinated. This should be taken into consideration in revaccination against this infection.

5071

Berenznitskaya, E.R. 1964. A method for selective extinction of the parafluorescence of cocci in preparations stained with pertussis and parapertussis fluorescent sera. Zh. Mikrobiol. Epidemiol. i Immunobiol. 41:71-74.

A method has been developed for selective extinction of the parafluorescence of cocci in preparations first stained with fluorescent pertussis and parapertussis sera, and then Gram-stained. Treatment of the preparations with gentian violet and iodine, serving as fluorescence extinction agents, has made it possible to eliminate the fluorescence of the Gram-positive parafluorescent cocci, and to retain at the same time the fluorescence of the Gram-negative pertussis and parapertussis bacteria.

5072

Biegeleisen, J.Z., Jr.; Mitchell, M.S.; Marcus, B.B.; Rhoden, D.L.; Blumberg, R.W. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: I. Clinical evaluation of conjugates on smears prepared directly from cerebrospinal fluid sediments. J. Lab. Clin. Med. 65:976-989.

Techniques were described for the cultivation and immunofluorescent identification of Hemophilus influenzae, Diplococcus pneumoniae, Neisseria meningitidis, and eight less common pathogens in specimens of cerebrospinal fluid from 100 patients with bacterial meningitis. A comparison of the results obtained by conventional methods and by immunofluorescent staining indicated that the latter method was fully as sensitive as the former and was more accurate in treated cases. Some of the dangers involved in the use of the Gram stain of the sediment as a tool for presumptive diagnosis were discussed, as were shortcomings of fluorescent antibody staining, particularly in infections caused by uncommon gram-negative organisms. The immunofluorescent staining technique was recommended for the rapid screening of spinal fluid specimens, as well as of cultural isolates.

5073

Franek, J. 1965. Use of fluorescent antibodies for the rapid diagnosis of infections caused by B. anthracis and P. tularensis. J. Hyg. Epidemiol. Microbiol. Immunol. 9:160-168.

In experiments with virulent strains of B. anthracis and P. tularensis the possibility of speeding up biological tests by FA was studied. It was possible to detect 150 anthrax bacilli and 500 pasteurellae in the smears from suspensions of organs. These results were obtained with

concentrations of microorganisms reaching approximately 10^4 to 5×10^5 of the organ suspensions under investigation. Thus it was possible to finish the biological test for B. anthracis within 18 to 24 hours and that for P. tularensis within 72 hours. In order to speed up the biological tests still more and to increase the reliability of detection of even small quantities of microorganisms, the use of cortisone in mice brought good results.

5074

Franek, J.; Prochazka, O. 1965. Fluorescent antibody demonstration of Pasteurella tularensis. Folia Microbiol. 10:77-84.

The authors studied certain factors determining the possibility of the demonstration of Pasteurella tularensis by the immunofluorescence technique and the sensitivity, specificity, and reliability of the reaction under experimental conditions. On the basis of the results they regard this method as suitable for rapid microbiological diagnosis, both for demonstrating Pasteurella tularensis in material from patients and infected animals and for detecting antibodies in the serum of convalescents.

5075

Franek, J.; Wolfova, J. 1965. Use of the immunofluorescence method in an epidemic focus of tularemia. Folia Microbiol. 10:85-92.

Four hares found dead and 32 animals caught in a focus of tularemia were examined by the immunofluorescence method. In full agreement with the biological test, the use of fluorescent antibodies showed the presence of Pasteurella tularensis in the hares, even when the organs were massively contaminated with other bacteria, making cultivation tests quite impossible. In the other animals, all the test methods had negative results. Tests of sera from 149 convalescent patients and 59 control subjects showed complete agreement between the indirect immunofluorescence method and the agglutination reaction. The question of the titers found in the indirect immunofluorescence reaction are discussed. The first results with the use of fluorescent antibodies in practice thus confirm that this is a suitable method for examining animals in epizootic foci and for detecting specific antibodies in human serum.

5076

Grossman, M.; Sussman, S.; Gottfried, D.; Quock, C.; Ticknor, W. 1964. Immunofluorescent techniques in bacterial meningitis: Identification of Neisseria meningitidis and Hemophilus influenzae. Amer. J. Dis. Children 107:356-362.

This study was designed to investigate the application of the fluorescent antibody technique to the rapid diagnosis of meningococcal and H. influenzae meningitis by staining cerebrospinal fluid smears. Conjugates of polyvalent meningococcal and H. influenzae antisera were

of excellent potency and specificity. The application of these conjugates to the diagnosis of meningitis from initial cerebrospinal fluid smears was disappointing. In the case of meningococci, the fluorescent antibody technique was comparable to Gram stain and in the case of H. influenzae it was not quite as good as Gram stain. The application of the fluorescent antibody technique to cerebrospinal fluid smears shares the limitation of other direct fluorescent antibody examination of clinical material in bacterial disease. Further trial of this method seems warranted, perhaps with the modification of incubating the cerebrospinal fluid for a few hours before examination.

5077

Hatten, B.A.; Sulkin, S.E. 1965. Intracellular induction and survival of Brucella abortus L forms. Bacteriol. Proc. M111:58.

Persistence of Brucella abortus infections even in the presence of antibodies or therapeutic agents suggests that altered forms may be involved. Hamster kidney cells infected with B. abortus and maintained under various conditions were examined periodically for the presence of bacteria and L forms by May-Grunwald-Giemsa and fluorescent antibody staining and by subculture. B. abortus were present in cells for only a limited time when combinations of penicillin and streptomycin in concentrations of 2.5 to 20 ug per ml were added. L forms persisted in cells exposed as long as 2 weeks to the same antibiotic concentrations. Induction of L forms was stimulated by either antibiotic alone during the first few days after infection. L-phase growth usually occurred as two distinct types. One appeared 4 to 5 days after infection as small, granular, intracellular organisms producing typical L forms or transitional forms upon cultivation in artificial media and frequently gave rise to typical bacterial colonies. The second type appeared later as large bodies, grew poorly on artificial media, and seldom reverted to the bacterial phase.

5078

Holwerda, J.; Eldering, G. 1963. Culture and fluorescent antibody methods in diagnosis of whooping cough. J. Bacteriol. 86:449-451.

Nasopharyngeal swabs from 517 suspected whooping-cough patients were examined by culture and by FA staining procedures applied to direct slide preparations. A total of 138 were positive by both methods, 25 by culture only, and 25 by FA only. The FA technique was also used in the identification of young cultures. It was shown that a positive culture report could be speeded up by about 1 day by this method. Without FA, one-half the positive culture reports were made in 3 days; with FA, three-fourths were reported in a similar period.

5079

Jentzsch, K.D. 1963. Study of brucellae with fluorescent antibody: 3. Indirect staining of Brucella antibody in bovine serum with fluorescent conjugates. Arch. Exp. Veterinaermed. 17:922-935. In German.

Antibodies were studied in 500 sera from cattle by indirect FA method. Results were compared with those of agglutination and complement fixation. There was 87.8 per cent agreement. In five cases the FA method failed. Failures by agglutination were 2.4 per cent and complement fixation 0.6 per cent. It was easy to obtain a diagnosis by FA with sera showing a prozone phenomenon. The FA technique is simple, rapid, and specific.

5080

Jentzsch, K. D. 1964. Demonstration of Brucella with fluorescent antibodies: 4. 'Complement staining' for the detection of complement-fixing Brucella antibodies in bovine sera. Arch. Exp. Veterinaermed. 18:967-979. In German.

For comparison, 200 samples of bovine serum were examined for Brucella antibodies by means of slow serum agglutination, complement-fixation reaction (CFR), and complement staining with special emphasis on the last. Its results agreed up to 94 per cent with those of the fixation test. In eight sera the test failed because they had been too much diluted. In another four a hemolytically mute CFR was highly probable as the complement staining had proved positive at negative CFR; the indirect identification and the agglutination tests confirmed that these sera came from infected cattle. The results of the fluorescence tests agreed with each other to a much higher degree (96 per cent) than those of the slow agglutination and CFS (85.5 per cent). BA-46-26150.

5081

Jentzsch, K.D.; Axt, J. 1963. Study of brucellae with fluorescent antibodies: 2. Proof of the specificity of labeled antisera. Arch. Exper. Veterinaermed. 17:173-180. In German.

Conjugation of Brucella antiserum with DANS and examinations of its specificity were made. There were stained 39 distinct strains of the genus Brucella studied, including all three types (abortus, suis, melitensis). There was no staining of 145 heterologous strains of bacteria including Salmonella, Pasteurella, Listeria, Streptococcus, Bacillus and Mycobacterium. The absorption test confirmed the specificity of the conjugated Brucella antiserum. This serum was suitable for work in routine diagnosis.

5082

Karakawa, W.W.; Sedgwick, A.K.; Borman, E.K. 1964. Typing of Haemophilus influenzae with fluorescent antibody reagent. Health Lab. Sci. 1:114-118.

Immunization of rabbits over a 4-week period with vaccine harvested from 6-hour cultures on Levinthal agar of Haemophilus influenzae Type B yields antiserum of high type specificity. Antibodies to somatic antigens common to all types are present in minimal concentration as compared with homologous-type, anticapsular antibodies. This antiserum yields fluorescein-labeled conjugates that stain smooth Type B strains in high titer and are rendered completely type-specific by minimal adsorption with heterologous-type cells from smooth cultures or by use of a blocking anti-rough serum in a two-step inhibition technique. Rapid identification of H. influenzae in clinical material, particularly the demonstration of Type B in spinal fluid, is readily and surely achieved by use of carefully prepared and standardized FA reagents. Other available serological techniques are more susceptible to uncontrollable errors arising from less than optimal antigen-antibody ratios, a factor not operative in the direct FA method.

5083

Kartman, L. 1960. The role of rabbits in sylvatic plague epidemiology, with special attention to human cases in New Mexico and use of fluorescent antibody technique for detection of Pasteurella pestis in field specimens. Zoonoses Res. 1:1-27.

The epidemiological role of rabbits in sylvatic plague was evaluated to define their position in the natural plague cycle and in public health. A review of the literature indicated that little is known, but that numerous observations, especially in South America, suggest that rabbits become infected during epizootics and may convey the infection to man. The fluorescent antibody staining technique was used to identify plague organisms in animals found dead in the field. Bone marrow provided the most useful tissue for application of this method to material from cadavers of varying degrees of decomposition.

5084

Klugerman, M.R. 1965. Chemical and physical variables affecting the properties of fluorescein isothiocyanate and its protein conjugates. J. Immunol. 95:1165-1173.

The effect of pH, time, temperature, and buffer systems on solutions of fluorescein isothiocyanate and its protein conjugates was examined fluorometrically. The fluorescence of the isothiocyanate and of the protein conjugate was maximal at about pH 8.7 and pH 10.7, respectively. The stability of their fluorescence above pH 7 was affected adversely by increases in pH or temperature. The protein conjugate, however, showed maximum stability at pH 10.5 and above. The type of buffer - carbonate, phosphate, borate, Tris or barbiturate - did not affect the fluorescence of

the free dye significantly. On the other hand, increasing the molarity of the buffer caused a decrease in stability of fluorescence of the free dye but did not seriously affect the fluorescence of the conjugate. The absorbance of fluorescein isothiocyanate and its conjugates increased with increasing pH. With increased pH or temperature of the reaction mixture during conjugation, fluorescein isothiocyanate reacted more readily with the protein. Conditions may be selected to obtain the desired degree of label with short conjugation periods. Conjugation of a bovine anti-Brucella abortus globulin sample for 30 minutes at pH 9.45 and room temperature was as effective as conjugation at pH 8.75 for 18 hours at 5 C. No apparent loss of biologic activity was observed as the result of conjugation.

5085

Lambert, H.J. 1965. Epidemiology of a small pertussis outbreak in Kent County, Michigan. Public Health Rep. 80:365-369.

The 89 families in which at least one member harbored Bordetella pertussis formed the group studied in the 1962 pertussis outbreak in Kent County, Michigan. Approximately one-half of the 474 family members had been previously vaccinated with three or more injections of pertussis vaccine. All susceptible unvaccinated persons acquired pertussis during the study, as did 46 per cent of vaccinated family members. B. pertussis was isolated from two healthy as well as 69 ill vaccinated persons. The increased incidence of pertussis in vaccinated persons was directly related to the interval since the last injection of pertussis vaccine.

5086

Marie, J.; Herzog, F.; Badillet, M.; Gaiffe, M. 1964. Diagnosis of whooping cough by the immunofluorescence technique. Pediatrie 19:53-59. In French.

A direct fluorescent antibody test on B. pertussis in nasopharyngeal mucus was compared with the bacteriological diagnosis in 1,000 suspected cases. The fluorescence technique gave a greater proportion of positive results in early cases and took 1 hour instead of 2 to 3 days.

5087

Marshall, J.D., Jr. 1964. The use of immunofluorescence for the identification of members of the genus Pasteurella in chemically fixed tissues. Diss. Abstr. 24:4921.

FA, cultural, and histologic methods were used to determine the effectiveness of FA for diagnosis of pasteurellosis. Specimens were obtained from animals infected with Pasteurella gallinarum, P. haemolytica, P. multocida, P. novicida, P. pestis, P. pseudotuberculosis, and P. tularensis. FA provided rapid, reliable identification of all species in pure or mixed cultures or from untreated clinical material. P. pestis and P. pseudotuberculosis

cross-reacted serologically. Fraction I antiserum from P. pestis was specific. Ethanol (95 or 70 per cent), methanol, chloroform, or 10 per cent formalin were satisfactory fixatives for all species except P. pestis and P. pseudotuberculosis. Methanol was the only satisfactory fixative for these. Histopathologic studies using FA are described.

5088

Mayorova, G.F.; Korn, M.Ya. 1963. A study of the antipertussis fluorescent serum specificity. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:9:42-48. In Russian.

In studies on the specificity of fluorescent serological examination of H. pertussis with the aid of antipertussis fluorescent serum, it was impossible to detect H. pertussis with the aid of heterologous sera. Antipertussis fluorescent serum stained some species of microorganisms nonspecifically: P. pestis, E. coli, Bacillus brucellosis, P. tularensis, and others. Staphylococci and streptococci were stained specifically. The method of fixation also influenced the results of the investigation. Rough fixation by flame provoked microbial staining of H. pertussis with heterologous fluorescent sera. Identification of H. pertussis in practical conditions by FA at present is fraught with some difficulties, since the Staphylococcus and Streptococcus may be stained simultaneously. It was impossible to distinguish H. parapertussis from H. pertussis by the indirect method.

5089

Mitchell, M.S.; Biegeleisen, J.Z., Jr. 1965. The effect of penicillin on immunofluorescent staining of Diplococcus pneumoniae, Neisseria meningitidis, and Hemophilus influenzae in cerebrospinal fluid in vitro. J. Lab. Clin. Med. 66:53-63.

Several strains of Diplococcus pneumoniae, Neisseria meningitidis, and Hemophilus influenzae were exposed to a range of concentrations of penicillin G in pooled human cerebrospinal fluid at 37 C in vitro. Pneumococci were killed and lysed at bactericidal concentrations of penicillin, but a large percentage of these cells seemed to be unchanged in appearance from the untreated state on immunofluorescent staining. At subinhibitory concentrations, organisms of this species may have been slightly enlarged, but capsular staining was again totally unaffected. Neisseria meningitidis, Groups A and C, became enlarged and bloated, and lost most of their capsular staining at bactericidal concentrations of penicillin, but the only difference observed at subinhibitory concentrations was the enlargement of some cells. Neisseria meningitidis, Group B, seemed unchanged in size and staining characteristics at all concentrations of the antibiotic. Hemophilus influenzae, Type b, organisms lost all capsular staining, but were not disrupted at bactericidal concentrations of penicillin. At subinhibitory concentrations, generally little or no change in morphology and integrity of capsular antigen was observed, although 1 to 2 per cent of the cells were long forms with somewhat shaggy capsules.

5090

Mitchell, M.S.; Marcus, B.B.; Biegelaisen, J.Z., Jr. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: II. Growth, viability, and immunofluorescent staining of Hemophilus influenzae, Neisseria meningitidis, and Diplococcus pneumoniae in cerebrospinal fluid. J. Lab. Clin. Med. 65:990-1003.

H. influenzae, N. meningitidis, and D. pneumoniae were studied. H. influenzae, Type b, could be isolated in 7 to 9 days at 4 or 25 C, and intense FA staining of capsules was observed for as long as 2 weeks. At 37 C the organism could not be cultured beyond 2 to 3 days. The capsules appeared ragged, as judged by the FA staining reaction, and by 18 to 24 hours, fluorescence was considerably diminished. Ability to be stained was lost in most cases after 48 hours of incubation at 37 C. N. meningitidis, Group B, could be cultured from specimens of CSF for 7 to 8 days when stored at 4 or 25 C and an average of 15 days at 37 C. FA staining seemed equally bright indefinitely at all storage temperatures, although after 5 to 7 days the staining may well have been somatic rather than capsular. Pneumococci were cultured from CSF specimens for an average of 17 days at 4 or 37 C and an average of 21 days at 25 C. FA staining remained. In normal cerebrospinal fluid seeded with cultures of the above three species of organisms in vitro, immunofluorescent staining characteristics were very similar to those in clinical specimens, but viability was, in almost all instances, considerably shorter. All cultures tested multiplied consistently with the exception of meningococci of Groups A and C, which only rarely grew in CSF.

5091

Nelson, J.D.; Hempstead, B.; Tanaka, R.; Pauls, F.P. 1964. Fluorescent antibody diagnosis of infections. J. Amer. Med. Ass. 188:1121-1124.

In areas without bacteriology laboratory facilities, certain infections can be diagnosed by the fluorescent antibody technique from specimens mailed to a central laboratory. An outbreak of respiratory illness occurred in a remote area of Alaska. Nasopharyngeal swab specimens applied to microscope slides were sent to laboratories in Anchorage, Alaska, and Dallas, Texas. Positive fluorescent antibody identification of Bordetella pertussis was made in 14 specimens by the Anchorage laboratory and confirmed in nine specimens in Dallas. Good correlation was obtained in nine cases of diarrheal disease studied by fluorescent antibody staining of rectal swab specimens for enteropathogenic serotypes of Escherichia coli.

5092

Nikitin, V.M. 1964. The use of immunofluorescent paper disks for the rapid detection of pathogenic microbes. VoennoMed. Zh. 11:55-58. In Russian.

Instead of the liquid form of labeled gamma globulins, strips or disks of various kinds of paper saturated with fluorescent conjugates were prepared. Best results were obtained with filter paper disks. One drop of fluorescent immune serum of certain specificity was applied to each side of the disks measuring 1 cm in diameter. The disks were dried and stored at 4 C. Trials on cultures of Flexner's bacillus, Escherichia coli, tularemia vaccine, and anthrax vaccine were carried out both by the direct and the indirect methods. The disks kept their immunological capacity for specific staining up to 15 months, although exposure time had to be increased as storage time was increased. The use of these disks simplifies storage, application, and transportation of the fluorescent antibodies and facilitates their standardization. The recommendation of their use in field conditions is justified.

5093

Ocklitz, H.W.; Boigk, J.; Hahn, M. 1965. The clinical picture of pertussis diagnosed with immunofluorescence and culture methods. Z. Kinderheilk. 92:306-323. In German.

The aim of the present work was to register statistically, as far as possible, clinical data of bacteriologically affirmed cases of pertussis and to relate them with the results obtained in cultivation on the one hand and in FA on the other. Data have been collected of 478 bacteriologically positive children, 403 of whom were treated. Age and sex of the patients, and the type and frequency of complications, are quoted, the duration and intensity of cough are analyzed, and the bacteriological findings are shown. The distribution of the former over the whole course of the disease, and possible relations to the process of cough and the therapy are discussed in detail. Consideration is given to the questions of infectivity and carriership.

5094

Ocklitz, H.W.; Weppe, C.-M. 1964. The bacteriological diagnosis of pertussis; flourescence serology and culturing compared: II. The fluorochromatization of antibodies of Bordetella pertussis. Zentralbl. Bakteriol. 194:103-114. In German.

A report is made on personal experiences with the preparation of anti-pertussis serum suitable for fluorochromatization of antibodies, and also on the concentration, coupling, purification, and testing of this serum. Finally, the preparation and critical examination of the microscopical preparations are described.

5095

Ocklitz, H.W.; Weppe, C.-M.; Hahn, M. 1964. The bacteriological diagnosis of pertussis; fluorescence serology and culturing compared: III. Results of the comparison of both methods. Zentralbl. Bakteriol. 194:212-221. In German.

A report is given on the investigation of 2,660 mucus samples from 828 children. The results are broken down according to the age and origin of the test patients, and discussed especially with reference to the discordant findings. The fluorescent serological method produces on the whole more positive results than the culture method. The culturing gives positive findings more frequently at the beginning of the illness, but the antibody fluorochromatization gives positive findings more often at the end. There is no contradiction in this, as becomes clear in an in vitro test. Germs killed with antibiotics can, of course, no longer be cultured, but are as demonstrable as living ones by fluorescence serology. Germs killed by heat, on the other hand, can no longer be detected by either method.

5096

Pittman, B.; Cherry, W.B. 1965. Study of factors which affect the identification of Bordetella pertussis obtained by nasopharyngeal swabs. Bacteriol. Proc. M73:51.

Factors that affect the satisfactory cultural isolation or fluorescent antibody identification, or both, of B. pertussis from nasopharyngeal specimens were compared. These factors included the use of cotton swabs prepared on braided trolling wire, Saran-coated trolling wire, and on very small Teflon tubing. Swabs inoculated with an appropriate dilution of a pure culture of B. pertussis were held as dry swabs, placed in Casamino Acids solution or Stuart transport medium and stored at refrigerator, room, and 37 C temperatures. Cultural and FA tests were made at various intervals up to 2 weeks. At the end of this period, B. pertussis could be detected in all specimens by FA tests. This information served as the basis of a presumptive positive report until cultural isolation was completed. Cultural isolations could be made at the end of 2 weeks only from Teflon swabs kept at refrigerator temperature. It is felt that the use of Teflon swabs to obtain nasopharyngeal specimens can enhance the number of cultural isolations of B. pertussis.

5097

Quan, S.F.; Goldenberg, M.I.; Hudson, B.W.; Kartman, L.; Prince, F.M. 1964. A gram-negative bacterium from Alaska related to Pasteurella pestis and P. pseudotuberculosis. Bacteriol. Proc. M212:84.

A gram-negative bacterium isolated from a hare in Alaska was asserted to be P. pestis. Studies showed this Alaskan bacterium to differ from P. pestis culturally, biochemically, and pathologically. However,

serological studies showed a definite relationship of this organism to both P. pestis and P. pseudotuberculosis. Tissue smears from mice dying a day or more after being inoculated with large numbers of the Alaskan bacteria have been stained repeatedly with specific fluorescent antiplague conjugate. Antigens from a fresh agar culture were agglutinated with antiplague serum to full titer. Antigenic studies by agar diffusion showed at least five precipitation bands with antiplague serum. All or most of these bands merged with those formed by P. pseudotuberculosis. Furthermore, many cultural and biochemical reactions were very similar to those of P. pseudotuberculosis. Nevertheless, this Alaskan bacterium also differed from P. pseudotuberculosis in being nonmotile, negative to flagella staining, and not being lysed by P. pseudotuberculosis bacteriophage. Present data indicate that the Alaskan bacterium definitely is not P. pestis, but is a species of Pasteurella.

5098

Quan, S.F.; Knapp, W.; Goldenberg, M.I.; Hudson, B.W.; Lawton, W.D.; Chen, T.H.; Kartman, L. 1965. Isolation of a strain of Pasteurella pseudotuberculosis from Alaska identified as Pasteurella pestis: An immunofluorescent false positive. Amer. J. Trop. Med. Hyg. 14:424-432.

A gram-negative, bipolar-staining rod, isolated from a snowshoe hare in Alaska, was identified as Pasteurella pestis. This identification was of particular importance because plague is under international quarantine and has never been reported from Alaska. Subsequent work has established that the organism is a strain of P. pseudotuberculosis type I B that possesses an antigenic substance very closely related to the Fraction I antigen of the plague bacterium. The presence of this antigen resulted in the fluorescent antibody test yielding a false-positive finding, and has raised the question as to the current emphasis in differentiating between these two bacterial species on the basis of the presence or absence of Fraction I. The determination of P. pestis must include other testing procedures, such as the isolation of the organism or the demonstration of its presence in tissues of test animals.

5099

Redmond, D.L.; Kotcher, E. 1963. Cultural and serological studies on Haemophilus vaginalis. J. Gen. Microbiol. 33:77-87.

When several strains of Haemophilus vaginalis were grown on Casman rabbit blood agar, individual morphological and cultural differences were noted between the Amies strains that formed pleomorphic and filamentous organisms and large umbonate colonies, and the Dukes, Edmunds, King, and U-L strains, which were microscopically coccobacillary to bacillary, nonfilamentous, and formed minute convex smooth colonies. Dukes, Edmunds, King, and U-L strains required whole blood for main-

tenance; a whole-blood derivative was sufficient to maintain the Amies strains. Serological studies by tube agglutination, direct, indirect, and inhibition immunofluorescent methods showed that Dukes, Edmunds, King, and U-L strains reacted in a homologous manner with H. vaginalis antisera. Amies strains did not react with these antisera. However, Amies cross-reacted with H. aegyptius antiserum, but the Dukes, Edmunds, King, and U-L strains did not.

5100

Redmond, D.L.; Kotcher, E. 1963. Comparison of cultural and immunofluorescent procedures in the identification of Haemophilus vaginalis. J. Gen. Microbiol. 33:89-94.

The application of immunofluorescent techniques for the detection and identification of Haemophilus vaginalis in vaginal secretions by using fluorescent H. vaginalis antiglobulin was as specific and sensitive as cultural methods and had the advantage of being simple and rapid.

5101

Richardson, M.; Holt, J.N. 1964. Multiplication of Brucella in cultured lymphoid and nonlymphoid cells. J. Bacteriol. 88:1163-1168.

Growth curves were established for the multiplication of Brucella abortus in cultured bovine cells. The number of viable brucellae was determined by colony count after lysis of the parasitized tissue cells. The number of brucellae dropped during the first 3 to 6 hours. This was followed by intracellular growth. Brucellae multiplied in uterine mucosal and fetal skin cells at an exponential rate with a 4-hour generation time. In contrast, only limited multiplication occurred in spleen cell cultures, usually approaching the stationary phase by 20 to 30 hours. Prelimary results indicated an average generation time of 8 hours in calf spleen cells. Differences were apparent in the ability of spleen cells from individual calves to support intracellular growth. This suggests that a relationship may exist between the establishment of intracellular pathogens in vitro and the natural resistance of the animal. By the use of fluorescein-labeled anti sera, some insight was gained into the fate of brucellae in lymphoid cells. Fluorescent antisera stained intact brucellae and also revealed soluble antigen in the cytoplasm of reticular-like cells.

5102

Sapozhnikov, I.I.; Sattarov, I.S. 1965. Indirect fluorescent method for early detection of pertussis and parapertussis antibodies. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:7:103-108. In Russian.

Fluorescent detection of so-called incomplete antibodies was confirmed. Experiments were carried out on rabbits immunized with various associated pertussis-containing antigens and infected with pertussis and

parapertussis cultures. The sera of children who sustained parapertussis and were vaccinated against pertussis were investigated as well. The FA method was 10 to 30 times more sensitive in experiments and 20 to 50 times in examination of children than the agglutination reaction. However, it was impossible to differentiate pertussis from parapertussis serologically with this method. Further investigations are required to clarify the functional role of incomplete antibodies in pertussis and their connection with the protective properties of the sera.

5103

Schmidt, J. 1965. Studies on fluorescence serologic detection of Pasteurella pseudotuberculosis antibodies. Pathol. Microbiol. 28:21-25.

The indirect fluorescent antibody method was found to be equal in specificity (Types I, III, and V antibody) and titer values to the Widal agglutination test, but allowed an additional determination of incomplete antibodies. Since results are obtained within 2 or 3 hours, the reaction can eventually be used for rapid diagnosis.

5104

Schmidt, J. 1965. Comparative studies on the detection of Pasteurella pseudotuberculosis antibodies by indirect fluorescent serological methods and by the Widal reaction. Arch. Hyg. Bakteriol. 149:2:154-160. In German.

Investigations are reported that have been carried out o. 41 sick persons, including seven confirmed cases of infection by Pasteurella pseudotuberculosis, and healthy control persons for the purpose of comparing the reaction of Widal with indirect FA for proof of the antibodies against P. pseudotuberculosis. FA revealed the same specificity against the serological Types I, III, and V as the agglutination test. The titer of antibody was almost equal with both of the reactions FA detected prozones, including incomplete antibodies. Results are available 2 to 3 hours after the test, and the reaction may therefore be used for a rapid diagnosis.

5105

Sell, S.H.W.; Cheatham, W.J.; Young, B.; Welch, K. 1963. Hemophilus influenzae in respiratory infections. Amer. J. Dis. Children 105:466-469.

Fluorescein-tagged type-specific globulins were prepared from antisera to Hemophilus influenzae, Types a, b, c, d, e, and f. The fluorescence produced in bacteria by direct staining with the conjugates was type-specific and could be inhibited by both rabbit and chick homologous-type unlabeled antisera. Therefore, the method was applicable to typing of H. influenzae. Strains of H. influenzae recently

isolated from 73 patients were typed by agglutination at room temperature and direct staining by fluorescein-tagged antibody. When the results were compared, 31 could be typed by the latter but only 16 by the former method. Indirect fluorescent antibody methods were not type-specific.

5106

Sell, S.H.W.; Sanders, R.S.; Cheatham, W.J. 1963. Hemophilus influenzae in respiratory infections: II. Specific serological antibodies identified by agglutination and immunofluorescent techniques. Amer. J. Dis. Children 105:470-474.

A total of 39 children, ill with acute upper respiratory infection, were studied to determine whether specific serologic antibodies were developed to homologous strains of Hemophilus influenzae isolated as the predominating bacterial species in nasopharyngeal cultures. Results of agglutinations were compared with inhibition of specific immunofluorescence in acute and convalescent serum samples. Of these, 35 patients had agglutinating antibodies and of these, 18 had strains that could be typed by direct immunofluorescent techniques. All developed antibodies that could inhibit the fluorescence. Titers rose in 13, fell in four, and remained high in one. The results compared favorably with those of agglutination. Paired serum samples from 19 children, whose strains of H. influenzae could not be typed, were tested for inhibition of fluorescence of the six type-specific stock strains. The results of 15 compared favorably with agglutinations: 12 had type-specific antibodies, three had none in either serum sample. The remaining four had agglutinating antibodies that could not be identified by the six types tested. This raised the question of additional types of H. influenzae. H. influenzae acted as a pathogen in most of these young children during the acute respiratory infection.

5107

Smith, H.; Gallop, R.C.; Tozer, B.T. 1964. The production of specific rabbit antibodies by injecting individual antigen-antibody complexes separated from mixed antigens. Immunology 7:111-117.

Injection of a few hundred micrograms of antigen-antibody precipitates of fluorescent ovalbumin, I-131 - labeled human serum albumin, lysozyme, antigen 3 of Pasteurella pestis, and myoglobin into rabbits produced a 10-fold to 100-fold increase in antibody compared with that injected in the precipitates. Before injection the precipitates had been separated from either I-131 - labeled human serum albumin serological precipitate or fluorescent ovalbumin serological precipitate after the reaction of mixed antigens with mixed antibodies. The antibodies produced by this method precipitated only their homologous antigen from a mixture of it with either I-131 - labeled human serum albumin or fluorescent ovalbumin. If secondary precipitates formed from antibody produced in this way were injected into rabbits in larger quantities, a further 8-fold to 35-fold increase in specific antibody was obtained.

5108

Staak, C. 1961. Studies on staining brucellae with the aid of fluorescent antibodies. Inaugural Dissertation. Institut fur Veterinar-Hygiene, Freien Univ. Berlin. Journal No. 357. 59p. In German.

Anti-Brucella antisera were used in a study of conjugation procedures. Both RB 200 and FITC were used. When amounts of dye in reaction mixtures increased, protein conjugation was increased. Excess dye conjugation did not result in better staining. Non-specific fluorescence made it impossible to distinguish brucellae in mixtures of brucellae and guinea pig blood. Culture of Brucella on agar containing fluorochrome revealed that acridine orange, 1:10,000, was the best combination. The bacteria took up the dye. Mixture of the stained brucellae with guinea pig blood resulted in increased fluorescence of lymphocytes, granulocytes, and other blood components.

5109

Suchkov, Yu.G. 1964. Serological investigations in plague: XIV. Trial of some serological reactions in epizootologic examination in the natural plague foci. Zh. Mikrobiol. Epidemiol. i Immunobiol. 41:4:135-141. In Russian.

A study was made of the use of passive hemagglutination for retrospective diagnosis of plague in wild rodents and antibody neutralization for detection of a specific plague antigen. Formalinized hen and sheep erythrocytes sensitized with the capsular antigen, Fraction 1A after Baker, of P. pestis were used. In the Volga-Urals focus the last plague epizootic had occurred in 1952. Of 1,635 sera of the blood of Meriones meridianus Pall., only one agglutinated the sensitized erythrocytes. In another focus 382 sera were investigated. Specific antibodies were revealed in 167 animals, but P. pestis was isolated in only three cases. Antigen neutralization reaction makes it possible to detect plague antigen in decayed carcasses. FA was employed to confirm results.

5110

van Drimmelen, G.C.; Botes, H.J.W.; Claassen, N.; Ross, W.F.; Viljoen, M. 1963. Fluorescent antibody for the diagnosis of Br. ovigenitalium infection in sheep semen smears. S. African Med. J. 37:216.

Genital brucellosis in rams is best diagnosed by microscopic examination of semen smears. When stained by conventional methods the smears from some known infected animals occasionally fail to show infection owing to the small number or the atypical appearance of Brucella organisms. FA has shown an advantage over the fuchsin staining by aiding the detection of infrequent organisms and by providing additional

proof of the identity of Brucella organisms. Indirect tests promise to yield even better results, although the elaborate facilities demanded may limit the wide application of this technique.

5111

White, J.D.; McGavran, M.H. 1965. Identification of Pasteurella tularensis by immunofluorescence. J. Amer. Med. Ass. 194:294-296.

Pasteurella tularensis, the causative organism of tularemia, was readily and positively identified in formalin-fixed and paraffin-embedded human tissues from eight of nine cases examined.

5112

White, J.D.; Rooney, J.R.; Prickett, P.A.; Derrenbacher, E.B.; Beard, C.W.; Griffith, W.R. 1964. Pathogenesis of experimental respiratory tularemia in monkeys. J. Infect. Dis. 114:277-283.

Rhesus monkeys were exposed to the SCHU S-4 strain of Pasteurella tularensis in aerosols consisting of particles either 1 or 8 microns in diameter. Intracellular P. tularensis was demonstrated in respiratory bronchioles by fluorescent antibody staining of tissues obtained 20 minutes after exposure to aerosols of 1-micron particles. The initial lesion seen at 24 hours in the 1-micron group and at 48 hours in the 8-micron group was a respiratory bronchiolitis. This bronchiolitis appeared to expand into adjacent pulmonary tissue and involve the alveolar spaces. P. tularensis was readily identified in the bronchiolar and bronchopneumonic lesions.

5113

White, L.A.; Deacon, W.E. 1964. Identification of Haemophilus ducreyi by the fluorescent antibody technique. Bacteriol. Proc. M71:56.

Living suspensions of five laboratory strains of H. ducreyi were used in preparing immune serum in rabbits. Agglutination titers were demonstrated by the latex particle agglutination procedure. The globulin fraction of each antiserum was tagged with fluorescein isothiocyanate. Culture smears were examined by direct and indirect fluorescent antibody methods. Cross-reactions were observed with the five strains of H. ducreyi in both the agglutination and the fluorescent antibody reactions. Cross-reactions with other microorganisms did not occur. Specimens from 47 cases of clinically diagnosed chancroid were examined by direct smears and culture. Positive fluorescence was observed in 12 (25 per cent) of the smears examined by direct FA, and in 16 (34 per cent) of the smears examined by the FA inhibition technique. Isolations were obtained from 8.5 per cent of the specimens. The results suggest that the FA procedure provides a rapid, specific test for the demonstration of H. ducreyi in direct smears.

5114

Zak, K.; Vesnik, Zd. 1963. The possibilities of detection of Brucella antigens by fluorescent antibodies in gynecology. Zentralbl. Gynakol. 85:1058-1063. In German.

Fluorescent antibodies were used to detect Brucella in various tissues of pregnant and non-pregnant guinea pigs. The Brucella antigen was found intra- and extracellularly. The inhibition test and the examination of noninfected material have proved the specificity of the method. It can be assumed that this method may be used to detect Brucella as well as other infectious agents in the genitalia and can thus contribute to the accuracy and rapidity of diagnosis.

V. CORYNEBACTERIACEAE

5115

Allen, J.C.; Cluff, L.E. 1963. Identification of toxinogenic C. diphtheriae with fluorescent antitoxin: Demonstration of its nonspecificity. Proc. Soc. Exp. Biol. Med. 112:194-199.

Immunofluorescent staining of various corynebacteria and other microorganisms with fluorescein-labeled diphtheria antitoxin resulted in staining of toxinogenic and atoxinogenic strains of C. diphtheriae as well as of certain other corynebacteria and unrelated microorganisms. Specificity of this staining for identification of toxinogenic C. diphtheriae could not be improved by alterations in conditions of staining, or by chemical manipulation of antitoxin solutions. This lack of specificity is felt to be due to multiple non-antitoxic (accessory) antibodies in the antitoxin preparations that cross-react with a variety of bacterial antigens. It is suggested that stainable diphtheria toxin may not be present at or on the surface of viable, intact diphtheria organisms, and specific identification of toxinogenic C. diphtheriae by fluorescent antitoxin may not be possible. On the basis of data presented here, this technique as currently described seems to offer little more than a Gram stain to the diagnostic bacteriology of diphtheria.

5116

Biegeleisen, J.Z., Jr. 1963. Fluorescent antibody studies on Listeria monocytogenes, p. 183-185. In Second Symposium on Listeric Infection. Veterinary Research Laboratory, Montana State College, Bozeman, Montana.

FA studies were not entirely successful. Satisfactory staining of specimens from a fatal human case was obtained using a conjugate against a serotype 4a strain of Listeria.

5117

Biegeleisen, J.Z., Jr. 1964. Immunofluorescence techniques in retrospective diagnosis of human listeriosis. J. Bacteriol. 87:1257-1258.

Conjugates were prepared from globulins of antisera to Listeria monocytogenes, Serotypes I, II, III, IVA, and IVB. These conjugates did not stain heterologous bacteria, but cross-reactions were noted amongst Types I, II, and III. Serotypes IVA and IVB stained specifically. These conjugates should be useful in presumptive diagnosis of listeriosis in women and in retrospect from fetuses.

5118

Biegeleisen, J.Z., Jr.; Mitchell, M.S.; Marcus, B.B.; Rhoden, D.L.; Blumberg, R.W. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: I. Clinical evaluation of conjugates on smears prepared directly from cerebrospinal fluid sediments. J. Lab. Clin. Med. 65:976-989.

Techniques were described for the cultivation and immunofluorescent identification of Hemophilus influenzae, Diplococcus pneumoniae, Neisseria meningitidis, and eight less common pathogens in specimens of cerebrospinal fluid from 100 patients with bacterial meningitis. A comparison of the results obtained by conventional methods and by immunofluorescent staining indicated that the latter method was fully as sensitive as the former and was more accurate in treated cases. Some of the dangers involved in the use of the Gram stain of the sediment as a tool for presumptive diagnosis were discussed, as were shortcomings of fluorescent antibody staining, particularly in infections caused by uncommon gram-negative organisms. The immunofluorescent staining technique was recommended for the rapid screening of spinal fluid specimens, as well as of cultural isolates.

5119

Cummins, C.S. 1965. Chemical and antigenic studies on cell walls of mycobacteria, corynebacteria, and nocardias. Amer. Rev. Resp. Dis. 92(Suppl.): 63-72.

As a portion of this study, indirect FA was used to detect the uptake of anti-smegma serum by various bacteria strains. In general FA results and agglutination results agreed. Negative FA results were seen against M. avium, M. fortuitum, M. smegmatis (fluorescent dots at surface), and Nocardia brasiliensis. It was felt that the negative FA results where positives were expected resulted from a lack of accessibility of the antigen to the serum. Purified cell walls of M. fortuitum and M. smegmatis gave strong FA reactions.

5120

Dacres, W.G. 1963. Fluorescein-labeled antibody technique for identification of leptospiral serotypes: Refixation of formalin-fixed organisms with osmic acid vapor. Amer. J. Vet. Res. 24:1321-1323.

Leptospires fixed with osmic acid vapor were stained specifically with fluorescein-labeled antibody conjugates. Leptospires fixed with formalin underwent a great degree of cross-staining. Those fixed with formalin and subsequently treated with osmic acid vapor stained specifically.

5121

Eveland, W.C. 1963. Fluorescent antibody studies on Listeria monocytogenes, p. 186-189. In Second Symposium on Listeric Infection. Veterinary Research Laboratory, Montana State College, Bozeman, Montana.

Preliminary studies pointing toward diagnostic applications are reported. Preparation of a polyvalent serum by rabbit inoculation with multiple serogroups is described. Conjugates were purified by DEAE cellulose chromatography. Comments on listeriosis in abortion cases are included.

5122

Eveland, W.C. 1963. Demonstration of Listeria monocytogenes in direct examination of spinal fluid by fluorescent antibody technique. J. Bacteriol. 85:1448-1450.

Polyvalent rabbit sera were prepared and FITC-conjugated. This material specifically stained the organism in spinal fluid and pure culture. Identification was culturally confirmed.

5123

Gulmezoglu, E.; Sayre, J.W. 1964. The use of fluorescent labelled diphtheria antitoxin for the diagnosis of diphtheria cases. Turkish J. Pediat. 6:1-7.

With the fluorescent technique used in this study it was possible to demonstrate the presence of fluorescence in diphtheria bacilli in the throat of two of eight cases that had positive cultures by examination of direct smears. Fluorescence was also demonstrated on smears taken from cultures in five cases. Halo phenomenon was seen in one of the latter. The results of this study indicate that the degree of fluorescence is not sufficient or consistent enough to allow positive identification of diphtheria organisms in suspected cases. Possible causes of the lack of fluorescence are discussed and alternative techniques suggested.

5124

Jones, W.L.; Foster, J.W.; Lewis, V.J. 1965. Effect of bacterial cell wall components on immunofluorescence staining. Bacteriol. Proc. M131:61.

Fluorescein-labeled antisera for Corynebacterium diphtheriae also stained strains of cocci encountered frequently in clinical specimens. Improvement of the specificity of the conjugate was attempted through investigation of the chemical composition of the cellular sites of cocci and C. diphtheriae responsible for immunofluorescence staining. Washed suspensions of cells were subjected to acid hydrolysis. Bacteria removed from samples taken at intervals during hydrolysis were tested

for staining with the conjugate. The bacteria-free supernatant liquids from the samples were examined by thin-layer chromatography for identification of cellular material. Removal of glutamic acid from the cocci and a hexoseamine from the diphtheria bacteria accompanied loss of stainability. Compounds that were detected in acid hydrolyzates of cell walls from C. diphtheriae, staphylococci, or streptococci were tested for their ability to inhibit staining upon addition to conjugates for the diphtheria organism. Addition of glutamic acid eliminated staining of diphtheria bacteria and of the cocci. Addition of glycine or certain hexoses prevented staining of cocci without inhibiting the staining of the diphtheria bacteria.

5125

Lewis, V.J.; Brooks, J.B. 1964. Comparison of fluorochromes for the preparation of fluorescent antibody reagents. J. Bacteriol. 88:1520-1521.

Conjugates of anti-Corynebacterium diphtheriae and anti-Escherichia coli antisera were prepared using four dyes, FITC, RB 200, DANS, and tetramethylrhodamine isothiocyanate. When titers were compared, FITC was superior.

5126

Lewis, V.J.; Jones, W.L.; Brooks, J.B.; Cherry, W.B. 1964. Technical considerations in the preparation of fluorescent antibody conjugates. Appl. Microbiol. 12:343-348.

Ammonium sulfate, hydrochloric acid, Ethodin, and ethanol were compared for fractionation of rabbit antiserum prior to conjugation with fluorescein isothiocyanate. Fractionation with the salt was the method of choice from the standpoints of simplicity and recovery of antibody effective in conjugates prepared from the fractions. Effects of pH, temperature, dye-protein ratio, and molarity and type of buffer upon conjugation were studied. These technical factors were adjusted to produce conjugates for Corynebacterium diphtheriae that possessed higher specific titers than did reagents obtained by previously employed techniques.

5127

Miller, J.K.; Muraschi, T. 1963. Relation of Listeria monocytogenes in vaginal flora as detected by immunofluorescence and the interruption of pregnancy, p. 325-331. In Second Symposium on Listeric Infection. Veterinary Research Laboratory, Montana State College, Bozeman, Montana.

The vaginal secretion of 496 pregnant women in one upstate and two Albany hospitals was examined for the presence of Listeria monocytogenes by means of immunofluorescence. Of these, 22 showed fluorescing organisms one or more times. The majority of positive reactions was against

Type 1 antiserum. In no instance was L. monocytogenes detected by culture or other serologic methods. The significance of these findings for the relation of L. monocytogenes to interruption of pregnancy has not been determined.

5128

Moody, M.D.; Jones, W.L. 1963. Identification of Corynebacterium diphtheriae with fluorescent antibacterial reagents. J. Bacteriol. 86:285-293.

Conditions are described in which fluorescent antibody reagents can be prepared and used to identify Corynebacterium diphtheriae in pure and mixed cultures and in clinical materials. The use of O and OK antigens for immunization of rabbits to prepare the antibody was compared. The most satisfactory reagents were those made from serum of rabbits injected with live OK suspensions of C. diphtheriae. Such fluorescent reagents were used successfully in direct and indirect fluorescent antibody tests to identify both toxinogenic and atoxinogenic C. diphtheriae but not to differentiate the two kinds of organisms.

5129

Navarrete-Reyna, A.; Rosenstein, D.L.; Sonnenwirth, A.C. 1965. Bacterial aortic aneurysm due to Listeria monocytogenes: First report of an aneurysm caused by Listeria. Amer. J. Clin. Pathol. 43:438-444.

This is the first report of a bacterial aneurysm due to Listeria monocytogenes. The patient was a 79-year-old diabetic woman with intermittent, low-grade fever and paralysis of the left vocal cord. Her death was caused by rupture of the bacterial aortic aneurysm into the left main stem bronchus. L. monocytogenes was cultured from blood obtained at autopsy. Typical, pleomorphic, gram-positive rods were seen in the tissues microscopically and verified by immunofluorescence. Incidentally, this also seems to be the first case of listeriosis treated solely by erythromycin, albeit in ignorance of the etiologic agent.

5130

Nelson, J.D.; Shelton, S. 1963. Immunofluorescent studies of Listeria monocytogenes and Erysipelothrix insidiosa: Application to clinical diagnosis. J. Lab. Clin. Med. 62:935-942.

A study of FA adapted to rapid identification of suspected organisms from cultures or from clinical specimens was undertaken. Serotypes 1, 2, and 3 share a common, thermostable, somatic antigen, Factor II, and Type 4 organisms have common somatic antigens, Factors V and/or VI.

Antisera to a Type 4b organism and to a Type 3 organism were conjugated with FITC. The labeled 4b antiserum gave specific fluorescent staining of Type 4b organisms when five strains were tested. Type 4a organisms of two strains did not stain other serotypes or Erysipelothrix insidiosa. The labeled Type 3 antiserum stained organisms of eight strains of Type 1, two strains of Type 2 and four strains of Type 3, but not Types 4a, 4b, or E. insidiosa. The clinical and pathologic features of a fatal case of the meningoseptic form of listeriosis of the newborn are presented. Identification of L. monocytogenes Type 4b from the blood and cerebrospinal fluid cultures was made by FA and confirmed by serologic and biochemical methods. FA is suggested for screening gram-positive bacilli from blood or cerebrospinal fluid cultures for L. monocytogenes infections.

5131

Pernis, B.; Cohen, M.W.; Thorbecke, G.J. 1963. Specificity of reaction to antigenic stimulation in lymph nodes of immature rabbits: I. Morphologic changes and gamma globulin production following stimulation with diphtheria toxoid and silica. J. Immunol. 91:541-552.

Experiments were conducted to test whether antigenic substances rather than nonspecific stimuli are necessary for induction of gamma globulin formation and associated morphologic changes in lymphoid tissue. Diphtheria toxoid was used as the antigen, crystalline silica as the nonantigenic adjuvant. Neonatal rabbits were used as the experimental animals because of their lack of pre-existing stimulation. Stimulated lymph nodes were studied with regard to secondary nodule and plasma cell formation and, in addition, to gamma globulin production in vitro by means of autoradiography of immunoelectrophoretic patterns. Immunofluorescence studies were performed to determine presence of antibody and gamma globulin in lymph node cells. Results indicate that antigenic stimulation is needed for the induction of the specific histologic changes associated with gamma globulin formation. Gamma globulin synthesis was more readily demonstrable than antibody formation in immature rabbits. Several possible reasons for this phenomenon are discussed.

5132

Pillot, J.; d'Azambuja, S. 1963. Indirect immunofluorescence and complement fixation reaction carried out with Leptospira icteronaemorraghiae. Ann. Inst. Pasteur Paris 104:137-141. In French.

Indirect immunofluorescence is rather difficult to carry out: the organism is frail and its structure does not respond well to fixation, contact with antibody, and washings. A good complement fixation can be obtained with an antigen constituted of centrifuged, non-washed cultures, the anticomplementary capacity of which (because of protein impurities of the medium) is eliminated by heating in a boiling waterbath.

These two reactions, like agglutination lysis, particularly demonstrate serotype-specific antibodies.

5133

Scarpa, B. 1963. Immunofluorescent reaction in the diagnosis of diphtheria: II. Practical applications. Rass. Med. Sarda 65:693-697. In Italian.

The immunofluorescent test employed for identification of Corynebacterium diphtheriae appears very delicate and therefore cannot be relied on for laboratory routine.

5134

Schoenberg, M.D.; Stavitsky, A.B.; Moore, R.D.; Freeman, M.J. 1965. Cellular sites of synthesis of rabbit immunoglobulins during primary response to diphtheria toxoid - Freund's adjuvant. J. Exp. Med. 121:577-590.

The present studies are based on previous observations that the intravenous injection of diphtheria toxoid and complete Freund's adjuvant into rabbits resulted in an increased proliferation of cells associated with antibody synthesis; an accelerated, enhanced, and prolonged synthesis of antibody; and a lengthened interval between the appearance of gamma-M and gamma-G hemagglutinating antibodies in the circulation. The molecular species of antibodes that were synthesized by fragments of the spleens were determined by the incorporation of labeled amino acid into antibody and by binding of radioactive antigen by antibody. These studies were paralleled by determination of the presence and type of antibody within the cell by immunofluorescence. Evidence was obtained that non-phagocytic mononuclear cells in the walls of the sinusoids of the red pulp of the spleen are a major source of 19S gamma-M antibody and that plasma cells in the non-follicular white pulp are a major source of gamma-G antibody. It was hypothesized that the 19S and 7S antibody responses evolved independently with the development of at least two different cell types, a mononuclear cell with capacity for 19S immunoglobulin synthesis and a plasma cell with capacity for 7S immunoglobulin synthesis.

5135

Smith, C.W.; Metzer, J.F. 1963. Identification of Listeria monocytogenes in experimentally infected animal tissue by immunofluorescence, p. 179-182. In Second Symposium on Listeric Infection. Veterinary Research Laboratory, Montana State College, Bozeman, Montana.

FA is useful for identifying Listeria in surgical and autopsy materials. The rapid results possible by FA contrast to tedious cultural methods for these organisms. Immune responses to Listeria are unpredictable. Preparation and testing of conjugates is the only way to assure a good FA reagent. Counterstaining was especially useful in these studies.

5136

Villella, R.L.; Halling, L.W.; Biegeleisen, J.Z., Jr. 1963. A case of listeriosis of the newborn with fluorescent antibody histologic studies. Amer. J. Clin. Pathol. 40:151-156.

A case of listeric infection of the newborn is described, and the pertinent clinical and pathologic features of this form of infection are reviewed. Morphologic diagnosis and the role of methods with fluorescent antibody are discussed.

5137

Watson, B.B.; Eveland, W.C. 1965. The application of the phage-fluorescent antiphage staining system in the specific identification of Listeria monocytogenes: I. Species-specificity and immunofluorescent sensitivity of Listeria monocytogenes phage observed in smear preparations. J. Infect. Dis. 115:363-369.

A procedure has been described and results presented on the application of the phage-fluorescent antiphage staining system as an indirect technique in the identification of Listeria monocytogenes. Within the limits of tests performed on 63 strains of L. monocytogenes, staining reactions appeared to be both sensitive and specific. Both phage-sensitive and to a lesser degree phage-resistant strains showed fluorescence. All heterologous species of bacteria tested were negative. The staining pattern was distinct from that of ordinary methods of fluorescent staining by antibacterial serum in that organisms appeared irregular and often bizarre. A method of indirectly observing phage attachment by dark-field illumination is discussed.

5138

Watson, B.B.; Eveland, W.C. 1965. The application of phage-fluorescent antiphage staining in the specific detection of Listeria monocytogenes in experimentally infected animal tissues. Bacteriol. Proc. M132:61.

Phage-fluorescent antiphage staining utilizes species-specific L. monocytogenes phages combined with the sensitivity of the immunofluorescent reaction in the detection of phage after attachment. This is an indirect technique in that bacteria are outlined by staining. Previous studies had shown this staining, with the use of smear preparations, to be highly specific and sensitive. These studies were extended to include its possible application in identification of L. monocytogenes in infected tissues. Infected and normal tissue sections were exposed for 3 hours to 100 million plaque-forming units per 0.1 ml of phage, washed with buffered saline, stained with conjugated phage-specific antiserum, and observed. Staining with conjugated listerial antiserum and two histochemical stains were also used to detect organisms in serial sections. A high correlation was found between phage-fluorescent antiphage and conjugated

listerial antiserum, although fewer tissue-associated organisms were observed to fluoresce after staining by the former.

5139

White, R.G. 1963. The applications of fluorescent antibody techniques in bacteriology and virology; the fluorescent antibody technique in the detection of localization of bacterial antigens: Application to mycobacteria, Nocardia, and corynebacteria. Proc. Roy. Soc. Med. 56:474-478.

The use of a double-layer fluorescent antibody technique with rabbit antiserum to Mycobacterium smegmatis results in a pattern of staining of corynebacteria, nocardias, and some mycobacteria species that conforms with previously reported results of cell-wall analyses for sugars, amino sugars, and amino acids and of cell wall agglutination tests. It presumably depends upon the existence of a common antigenic determinant identical with that present in the glycopeptide moiety of wax D of human strains of M. tuberculosis. The failure of the fluorescent antibody method to stain M. smegmatis, M. avium, M. lepraemurium, and a strain of M. phlei is attributed to the inaccessibility of this antigen to antibody at the surface of these organisms. Antigen is accessible in cell-wall preparations. The widespread occurrence of this antigen throughout mycobacteria, corynebacteria, and nocardia species is of importance in the specific diagnosis of antigens or bacteria in these genera by the fluorescent antibody method.

VI. ENTEROBACTERIACEAE

5140

Abele, D.C.; Tobie, J.E.; Hill, G.J.; Contacos, P.G.; Hornick, R.B. 1964. Alterations in serum proteins and antibody production during human infections. Federation Proc. 23:1475:347.

Certain alterations in plasma protein immunoelectrophoretic patterns were observed in sera of volunteers infected with Plasmodium vivax. No significant changes were noted in volunteers infected with Salmonella typhi. Among the immunoelectrophoretic changes observed during the course of the malarial infections there was a consistent, marked increase in the beta-2 macroglobulins. This increase appeared temporally related to the first appearance of antimalarial antibodies as detected by the indirect fluorescent antibody technique and persisted in some cases as long as 60 days. A number of sera have been separated by Sephadex G-200 filtration and the fractions tested for antibody activity. The results indicate that the increase in beta-2 macroglobulin is partially due to the presence of antimalarial antibodies in this serum component. Data also suggest that early antimalarial antibody is predominantly a beta-2 macroglobulin and that later antibody is predominantly a 7S gamma globulin. Complete article.

5141

Anonymous. 1964. Salmonellas in meat. Lancet 2:999-1000.

As a portion of this general discussion of a public health problem, FA was mentioned as possibly giving rapid screening results.

5142

Bauer, I. 1965. Fluorescence-serological detection of Shigella sonnei. Acta Biol. Med. Ger. 14:2:167-174. In German.

FA experiments were performed on Shigella strains from various sources. Conjugates were prepared using either FITC or DANS. The fluorescence-serological detection was completely successful. The intensity of fluorescence indicated a dependence on the degree of agglutination. Cross-reactions with other Enterobacteriaceae could not be observed.

5143

Beumer-Jochmans, M.P. 1963. Characterization of pools of protein in cells of Shigella flexneri F6S infected with phage H-Sh. Nature 198:506-507.

Lesions in S. flexneri F6S following infection by phage H-Sh were studied by staining with acridine orange, coriphosphine, FA anti-bacterial

serum, and FA anti-phage serum. The RNA and DNA patterns are described. Comparisons of the developmental progression of staining by the two FA reagents are made. Anti-bacterial staining is total and immediate. Anti-phage staining is spotty and progressive with time until burst of the bacteria.

5144

Biegeleisen, J.Z., Jr.; Mitchell, M.S.; Marcus, S.B.; Rhoden, D.L., Blumberg, R.W. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: I. Clinical evaluation of conjugates on smears prepared directly from cerebrospinal fluid sediments. J. Lab. Clin. Med. 65:976-989.

Techniques were described for the cultivation and immunofluorescent identification of Hemophilus influenzae, Diplococcus pneumoniae, Neisseria meningitidis, and eight less common pathogens in specimens of cerebrospinal fluid from 100 patients with bacterial meningitis. A comparison of the results obtained by conventional methods and by immunofluorescent staining indicated that the latter method was fully as sensitive as the former and was more accurate in treated cases. Some of the dangers involved in the use of the Gram stain of the sediment as a tool for presumptive diagnosis were discussed, as were shortcomings of fluorescent antibody staining, particularly in infections caused by uncommon gram-negative organisms. The immunofluorescent staining technique was recommended for the rapid screening of spinal fluid specimens, as well as of cultural isolates.

5145

Boris, M.; Thomason, B.M.; Hines, V.D.; Montague, T.S.; Sellers, T.F., Jr. 1964. A community epidemic of enteropathogenic Escherichia coli 0126:B16:NM gastroenteritis associated with asymptomatic respiratory infection. Pediatrics 33:18-29.

A 15-month survey was conducted at a metropolitan hospital for detection of enteropathogenic Escherichia coli (EEC). Of the 383 admissions, 151 cases of gastroenteritis (39.6 per cent) were due to EEC as determined by the fluorescent antibody method. 0126:B16:NM strain was unusually prevalent having been detected in 47.5 per cent of EEC enteritis. The outbreak was localized in three geographically distinct areas. In three selected hospital populations 6 of 270 surveyed infants were asymptomatically infected with EEC, an incidence of 2.2 per cent. In a community investigation, 50 per cent index households had one or more asymptomatic pharyngeal carriers of strain 0126:B16:NM; next-door neighbor households and distant community control households had pharyngeal carrier rates of 33 and 0 per cent respectively. Intestinal carriage rates in these three groups were 18, 3, and 0 per cent respectively. Follow-up studies in houses with pharyngeal but no intestinal carriers revealed new infections. A respiratory transmission of EEC is indicated.

5146

Bradstreet, C.M.P. 1965. Immunofluorescence in the diagnosis of enteropathogenic Escherichia coli infections. Lancet 1:319.

FA detects antigens, but it does not differentiate between organisms having antigens in common. Care must be exercised in choosing serum for use in FA studies.

5147

Brooks, J.B.; Lewis, V.J.; Pittman, B. 1965. Separation of fluorescent antibody conjugates into 7S and 19S globulin components by gel filtration. Proc. Soc. Exp. Biol. Med. 119:748-751.

Gel filtration proved to be a simple, effective method for separation of fluorescein-labeled antiglobulin for E. coli into 7S and 19S fractions. Both fractions were found to contribute considerable nonspecific staining, but specific staining was associated predominantly with the 7S globulin. The ratio of specific staining titer to nonspecific staining activity was threefold greater with a pool of fractions in which only 7S globulin was detected than with the unfractionated conjugate when the two preparations were tested at the same protein concentration.

5148

Caldwell, W.J.; Stulberg, C.S. 1964. Characterization of Salmonella antigens by immunofluorescence. Bacteriol. Proc. M134:69.

To further analyze antigenic relationships of the Salmonella by fluorescent antibody, a modification of the technique was developed whereby both somatic and flagellar reactions could be readily determined, permitting a comparative study of FA and conventional serological reactions. O-H antisera were prepared for 22 serotypes representing major antigenic complexes of the Salmonella, and each was characterized by conventional serological procedures. The fluorescein isothiocyanate - labeled globulin fractions were comparatively studied by agglutination and by fluorescent reactions; in the latter, both somatic and flagellar staining were accomplished by treating formalinized organisms in suspension with labeled antibody. Flagella were specifically stained by labeled antibody whenever homologous or related antigens were present. Similarly, specific somatic staining by labeled antibody occurred in homologous systems as well as in heterologous systems of related antigens. The latter relationships were observed as percentage reactions in which the staining intensity was maximal but fluorescence was observed only in a certain constant percentage of the organisms in a given population. These were confirmed by inhibition or blocking tests. The data illustrate the general parallelism between FA reactions and agglutination, and the significant differences between the two, and emphasize antigenic relationships not readily apparent by conventional procedures.

5149

Carlson, V.S.; Welker, C. 1963. Fluorescence serology studies on the cause of the toxic effect of antibiotics on guinea pigs. Arch. Hyg. Bakteriol. 147:201-217. In German.

Twenty guinea pigs were fed with antibiotics and another ten with a suspension of E. coli. Five animals were injected with lipopolysaccharides extracted from E. coli. Fluorescein-tagged antibodies revealed E. coli lipopolysaccharides in the sections of organs of all of the animals. Antigen-antibody complexes were found as precipitates, especially in the intestine, liver, spleen, and kidney. The feeding of antibiotics to guinea pigs leads to a massive multiplication of E. coli, with a migration into the lower regions of the small intestine. This in turn leads to a release and resorption of E. coli lipopolysaccharides from the intestine, which then damage the organism as a whole as endotoxins; most cause the death of the animals.

5150

Chadwick, P.; Abbott, L. 1964. Specificity and sensitivity of a microcolony technique for fluorescent antibody identification of pathogenic Escherichia coli serotypes. Can. J. Microbiol. 10:853-859.

The specificity and sensitivity of a fluorescent antibody technique applied to growing microcolonies has been investigated, using serotypes of Escherichia coli responsible for infective enteritis as a model. Microcolonies of ten E. coli serotypes showed bright fluorescence when treated with homologous conjugated antiserum but no fluorescence when treated with heterologous conjugated antisera. Microcolonies of Enterobacteriaceae strains of other genera or of E. coli strains not associated with infective enteritis showed no fluorescence when treated with conjugated antisera prepared against the enteritis serotypes. Experiments with artificially infected fecal suspensions showed that the sensitivity of the microcolony technique was approximately 100 times greater than that of the direct smear method. A number of other advantages and possible disadvantages of the microcolony technique are discussed and its usefulness in epidemiological work is suggested.

5151

Chung, K.L.; Hawirko, R.Z.; Isaac, P.K. 1964. Cell wall replication: II. Cell wall growth and cross wall formation of Escherichia coli and Streptococcus faecalis. Can. J. Microbiol. 10:473-482.

Cell wall replication in E. coli and S. faecalis was studied by differential labeling of living cells with FA and non-fluorescent antibody. In E. coli the initial step in cell division was the formation of a cross wall at the cell equator, followed by the appearance of new cell wall on either side of the cross wall. The process was repeated in sequence at subsequent sites in the polar, the subcentral, and the subpolar areas. Constriction

occurred at random so that the divided parent cells were composed of several daughter cells. A polar type of unidirectional cell wall growth and elongation was also observed in E. coli. It was initiated by the synthesis of a ring of new cell wall material around the polar tip. A second ring was then formed at the subpolar area during the rapid enlargement of the first ring in a single direction. Evidence shows that cell wall synthesis is independent of cell division and that in E. coli, it is initiated at multiple but specific sites within the cell and not by diffuse intercalation of old and new walls. Contrary to the synthesis of cell wall at multiple sites in E. coli, S. faecalis replicated new cell wall at only one site per coccus. The new wall segment was initiated and enlarged at the coccal equator, and was followed by the formation of a cross wall, centripetal growth and constriction to separate the daughter cells.

5152

Cole, R.M. 1963. Cell wall replication in Salmonella typhosa, followed by immunofluorescence. Bacteriol. Proc. G13:26.

To follow cell wall growth, O antigen (9 and 12) was labeled with fluorescein-conjugated homologous antibody globulin (FAG). Cells of Salmonella typhosa, first incubated 1 to 2 hours in FAG, were washed and transferred to Penassay broth. Alternatively, instead of washing, an excess of the same globulin, unlabeled, was added with a little broth. Samples were taken at zero time and at 60-minute intervals during incubation with shaking at 37 C. After a lag of 1 hour, growth was exponential. Samples were centrifuged and washed and smears made. Several strains of S. typhosa and appropriate antibody globulins gave similar results. Findings show progressive and generalized decrease of intensity of cell-wall fluorescence with time of incubation after removal or block of excess FAG at zero time. The reverse technique of prior growth in unlabeled antibody, wash, reincubation in broth, and FAG staining after smearing shows increasing cell-wall fluorescence with time. The lack of discrete and microscopically resolvable areas of old cell-wall fluorescence is opposed to previously reported findings for Streptococcus pyogenes, in which old labeled wall remains discrete, resolvable, and continuously distinguishable from new. The results are compatible with an hypothesis of cell-wall replication in S. typhosa by diffuse or generalized ultramicroscopic intercalation of new cell-wall material. At least two different modes of cell-wall replication have been distinguished among unrelated bacteria.

5153

Cole, R.M. 1964. Cell wall replication in Salmonella typhosa. Science 143:820-822.

Changes in the fluorescence of the cell wall of Salmonella typhosa were studied during growth after direct labeling with fluorescein-conjugated homologous or anti-O globulins. Fluorescence decreased evenly with culture growth and cell division, but the addition of chloramphenicol resulted in large, nondividing cells that showed increasing interruption of fluorescence of the wall marker. The process thus differs from the equatorial origin and discrete hemispherical addition of new wall previously described in Streptococcus pyogenes. These findings, in addition to demonstrating the formation of new wall in the presence of chloramphenicol, appear consistent only with the concept that wall replication in the salmonellas occurs by diffuse intercalation of new materials among old.

5154

Cole, R.M. 1965. Symposium on the fine structure and replication of bacteria and their parts: III. Bacterial cell wall replication followed by immunofluorescence. Bacteriol. Rev. 29:326-344.

This is an interpretive and critical report. The author urges further application of FA to the study of surface-antigen replication in walled microorganisms. Confirmation or denial of controversial points in this study area will follow only from such further study. FA has clear advantages over any other method for cell wall study. The chief advantage is the ability to apply a specific label to the wall of a living cell.

5155

Cotran, R.S. 1963. Retrograde Proteus pyelonephritis in rats: Localization of antigen and antibody in treated sterile pyelonephritic kidneys. J. Exp. Med. 117:813-822.

Rats with retrograde Proteus pyelonephritis were treated with antibiotics until their kidneys became sterile. Using FA, specific P. mirabilis antigen was found in some sterile pyelonephritic kidneys 20 weeks after cessation of treatment and presumed renal sterilization. Persistent antigen was associated with interstitial chronic inflammation but not with acute inflammation or progressive scarring. Rat gamma globulin and Proteus antibody were localized in plasma cells of the renal inflammatory infiltrates. Persistent antigen in chronic pyelonephritis may lead to the continued local appearance of antibody-producing cells.

5156

Cotran, R.S.; Thrupp, L.D.; Hajj, S.N.; Zangwill, D.P.; Vivaldi, E.; Kass, E.H. 1963. Retrograde E. coli pyelonephritis in the rat: A bacteriologic, pathologic, and fluorescent antibody study. J. Lab. Clin. Med. 61:987-1004.

Retrograde infections of the urinary tract in rats can be established by inoculation of E. coli into the bladder. In contrast to Proteus as the infecting organism, renal lesions produced with E. coli are generally mild and limited to pyelitis. A small number of animals develop pyelonephritis. The organisms are initially present in the kidneys but disappear during subsequent weeks. Kidneys are sterile by the 14th week and free of bacterial antigen as determined by FA. When glass beads are placed in the infected bladder, bacteriuria and positive cultures of the kidney persist for at least 14 weeks. Bacteriuria may be present when the kidneys have become sterile, and in the absence of apparent renal lesions. In retrograde infections of the urinary tract, pyelonephritis of wide ranges of severity may be produced by varying the infective organism. In infections with a relatively avirulent organism, persistence of bacteria in the urinary tract may not be necessarily associated with significant renal lesions. It is suggested that a comparable situation in man may account for incidences of persistent bacteriuria in the absence of a demonstrable renal lesion.

5157

Cotran, R.S.; Vivaldi, E.; Zangwill, D.; Kass, E. 1963. Retrograde Proteus pyelonephritis in rats. Amer. J. Pathol. 43:1-31.

Retrograde pyelonephritis has been produced in rats by the inoculation of P. mirabilis into the bladder. The pathogenesis and course of pyelonephritis by this route have been studied in an attempt to correlate the morphologic and bacteriologic changes. It was found that organisms appeared in the kidneys by 24 to 48 hours after injection into the bladder, that they first invaded the pelvis, and that the infection involved the medulla and the cortex by continuity through the interstitium and the tubules. The infection was usually bilateral and unequal in both kidneys. The surviving animals developed chronic active pyelonephritis with persistence of bacteria and of morphologic evidence of pyelonephritis for at least 13 months after initiation of infection. Fluorescent antibody studies indicated that bacterial antigen persisted in the renal parenchyma following the initial bacterial proliferation. After the acute stage, recognizable bacterial bodies were limited to the pelvis and occasional abscesses; however, variable amounts of amorphous bacterial antigen were present within some renal scars for periods up to 13 months. After hematogenous infection the initial localization was in cortical and medullary blood vessels, followed by passage into the interstitium and production of scattered abscesses.

5158

Cowart, G.S.; Thomason, B.M. 1965. Immunofluorescent detection of Escherichia coli: Incidence of certain serogroups suspected of being pathogenic. Amer. J. Dis. Children 110:131-136.

This study was conducted to determine the relative importance of a variety of E. coli serogroups in diarrheal disease. Of 1,021 specimens, 9.0 per cent gave positive results with FA and 3.4 per cent were isolated and confirmed as E. coli of the groups sought. E. coli serogroup 06:K13 was the group most frequently detected, constituting 68.6 per cent of the isolates. Four different serotypes of 06:K13 were present in what appeared, on the basis of serogrouping alone, to be an outbreak in a hospital nursery due to a single organism. The possible relationship of 06 organisms present in the intestine to urinary tract and other extraintestinal infections and a nosocomial flora was discussed. The authors conclude that these serogroups did not account for a significant proportion of the diarrheal cases studied.

5159

Danielsson, D. 1965. A membrane filter method for the demonstration of bacteria by the fluorescent antibody technique: 1. A methodological study. Acta Pathol. Microbiol. Scand. 63:597-603.

Bacteria suspended in tap water or cultured in broth, and then trapped on non-fluorescent membrane filters, could be identified within one hour by means of the fluorescent antibody method. For this purpose the fluorescence microscope was equipped for incident illumination. The technique described allowed a quantitative determination of the bacteria identified serologically.

5160

Danielsson, D.; Laurell, G. 1963. Rapid detection of small numbers of bacteria in water by means of fluorescent antibodies. Acta Pathol. Microbiol. Scand. 58:159.

FA technique combined with membrane filter technique has been used to study bacterial contamination of water. Known concentrations of the test bacteria, enteropathogenic E. coli, were added to fixed volumes of water. As a rule 1 liter was filtered through a membrane filter (Millipore HAWG, 47 mu). At bacterial densities of 500 to 1,000 bacteria per liter identification was accomplished in 2 hours. At lower concentrations the membrane filters were incubated for various periods in broth and subsequently centrifuged to concentrate the bacteria. A concentration of 50 bacteria per liter could be detected in 5 to 6 hours, 15 to 20 in 8 to 10 hours, and 2 to 5 in 12 to 16 hours, respectively. The technique was also quantitative at different ratios (0.2 to 100) between concentration of contaminating bacteria and test

bacteria. By conventional methods, diagnosis could be made after 48 hours at the earliest. At a high ratio of contaminating bacteria to test bacteria, the latter could often not be isolated. Promising attempts have been made to detect bacteria directly on nonfluorescent membrane filters (Millipore HABG). Samples of the water of a river in central Sweden were examined for enteropathogenic E. coli. By the use of FA ten different strains of these bacteria were detected. Six of these were also isolated by conventional tests. Complete article.

5161

Danielsson, D.; Laurell, G. 1964. Detection of enteropathogenic Escherichia coli in a Swedish watercourse, the river Fyris, by means of fluorescent antibodies and by conventional methods. Acta Paediat. Scand. 53:49-54.

During a 6-month period a minor watercourse in Sweden has been investigated by the fluorescent antibody technique and by conventional methods on the occurrence of Escherichia coli serotypes associated with infantile diarrhea. Forty-eight strains belonging to ten different O groups were identified by fluorescent antibody. By conventional techniques nine strains belonging to six O groups were isolated. The origin of the strains is discussed.

5162

Danielsson, D.; Laurell, G. 1965. A membrane filter method for the demonstration of bacteria by the fluorescent antibody technique: 2. The application of the method for detection of small numbers of bacteria in water. Acta Pathol. Microbiol. Scand. 63:604-608.

The limit of sensitivity for demonstration of small quantities of bacteria in water by the fluorescent antibody method in combination with the membrane filter technique was investigated. After elution of bacteria from white membrane filters the lower limit of sensitivity for direct fluorescence microscopy was about 5,000 per liter of water. By direct staining of nonfluorescent membrane filters, the lower limit could be reduced to 1,000 bacteria per liter, and by combining this technique with an enrichment procedure, it was possible to demonstrate bacteria present in a concentration of 2 to 50 per liter within 4 to 6 hours.

5163

Danielsson, D.; Laurell, G.; Sjolin, S. 1965. An outbreak of diarrhea due to enteropathogenic Escherichia coli studied by means of fluorescent antibody identification and conventional bacteriological culture. Acta Paediat. Scand. 54:432-438.

An outbreak of diarrhea due to enteropathogenic Escherichia coli (EEC) at an infants' home was studied by fluorescent antibody identi-

fication and conventional bacteriological culture. Forty-three infants were included in the study. EEC were demonstrated in 64 specimens from 31 infants. Thirty-three specimens were positive on culture of 64 in FA tests. The epidemic was dominated by serotypes 026:B6 and 055:B5, but sporadic cases of serotypes 0126:B6, 0125:B15, and 0128:B12 occurred. Fifty-one adults, mothers and staff of the infants' home, were included in the study, and in two adults three EEC serotypes were demonstrated by culture and FA test. EEC belonging to ten different serotypes were identified by the FA method in an additional 91 specimens from 41 adults. Two or more serotypes were demonstrated in 31 cases. The occurrence of suspected false-positive results by the FA method in adults is discussed.

5164

Davis, B.R.; Ewing, W.H. 1963. Serologic relations that may lead to erroneous diagnoses of Escherichia coli infections by means of fluorescent antibody technics. Amer. J. Clin. Pathol. 39:198-202.

Insofar as the Enterobacteriaceae are involved, no particular type can be identified by means of FA techniques alone, any more than it can be identified by slide agglutination tests alone. FA techniques are not a substitute for isolation and definitive serologic and biochemical study of the microorganisms, but once the etiologic agent in an outbreak has been established through complete characterization by means of conventional methods, then the FA technique becomes a valuable tool for rapid examination of fecal specimens.

5165

de la Vaissiere, C.; Goiffon, B. 1963. Diagnosis of toxic gastroenteritis due to pathogenic colibacilli in infants by means of immunofluorescence. Concours Med. 85:185-188. In French.

The immunofluorescent method supplements the conventional methods of biochemical and serological isolation and identification by permitting results in a much shorter time. The procedures of direct and indirect examination are described. The latter is preferred. When a very large number of Escherichia coli bacilli from stool samples has been rendered fluorescent, the assumption is a diagnosis of infantile gastroenteritis coli bacilli diarrhea. Treatment can then be initiated immediately, while conventional studies, requiring more time, can be completed.

5166

Demissie, A. 1964. The isolation of enteropathogenic salmonellae from the River Fyris and their detection by the fluorescent antibody method. Acta Pathol. Microbiol. Scand. 60:299-300.

In an investigation aimed at isolating enteropathogenic salmonellae from the River Fyris, a modified swab technique and filtration through membrane filters, supercel, and Seitz filters were compared. Negative results were obtained when impure river water was filtered through membrane and Seitz filters. Nine salmonellae strains were isolated. One, S. typhimurium, NS phage type, was isolated by the supercel method. Swabs were made from strips of gauze compactly rolled around rectangular wire frames, 15 by 20 cm and about 5 to 7 inches thick. These swabs immersed in the river for 48 to 96 hours were effective traps for salmonellae. One strain of S. paratyphi B, 3A1 var. 2, one of S. blockley, and one of S. enteritidis were isolated by this method.

5167

Demissie, A. 1965. Immunofluorescence identification of Salmonella in fecal specimens. Acta Pathol. Microbiol. Scand. 65:271-286.

An investigation into the usefulness of the FA method in detecting salmonellae in feces and river water was undertaken. Cross serological reactivity was a serious handicap. All the isolated strains belonged to the family Enterobacteriaceae. Absorption techniques were employed to minimize cross-reactivity, and the results indicate that this may be the solution of the problem. Sera of satisfactory specificity were produced for the Salmonella Groups B, C, and D. Absorbed sera for Group E still gave some cross-reaction.

5168

Eveland, W.C. 1964. Use of a fluorescein-labeled sonically disrupted bacterial antigen to demonstrate antibody-producing cells. J. Bacteriol. 88.1476-1481.

Cells obtained by primary tissue culture of the spleens of chickens immunized with sonically disrupted Escherichia coli 0111 organisms were stained with a fluorescein-labeled homologous antigen by use of direct immunofluorescent methods. Brilliant staining of the cytoplasm in cells from immunized birds appeared to be diffuse in certain cells and rather globular in others. In contrast, cells from nonimmunized birds showed no staining at all. The cells involved in the specific reaction appeared to be those of the lymphocyte-monocyte-plasma cell types, as shown when stained by the May-Grunwald-Giemsa method. Preparations stained by the methyl green-pyronin technique revealed an increase in the pyrinophilic cells in the preparations from the immunized birds, thus demonstrating increased amounts of ribonucleic acid in these cells,

which in turn is consistent with the presence of antibody globulin. Specificity of the reaction was confirmed also by staining antibody-coated E. coli 0111 organisms with the conjugate, precipitin reaction with specific antibody, and specific agglutination with circulating antibody from the immunized birds.

5169

Eveland, W.C.; Griffes, J.W. 1964. Use of a fluorescein-labeled sonic-disrupted bacterial antigen to demonstrate antibody-producing cells. Bacteriol. Proc. M109:64.

Cells obtained by primary culture of the spleen of a chicken that had been immunized with sonic-disrupted Escherichia coli 0111 organisms were stained by direct immunofluorescent methods. Sonic-disrupted organisms were centrifuged at 5,000 revolutions per minute for 15 minutes, and the supernatant was conjugated with fluorescein isothiocyanate by the method of Clark and Shepard. After conjugation, the material was recentrifuged to remove any particulate matter. The conjugate was then applied directly to acetone-fixed cover slip preparations of the primary spleen tissue cultures. Examination of the stained preparations, taken at various periods of time, by fluorescence microscopy revealed brilliant fluorescence in the cytoplasm of cells that appeared to be of the lymphocyte-monocyte-plasma cell series. The reaction ranged from diffuse staining of entire cytoplasm to concentrated spheres dispersed throughout the cytoplasm of the cell. No nuclear staining was apparent. Controls included a duplicate set of cover slip preparations from an uninoculated bird. Specificity of the reaction had been previously demonstrated by positive precipitin reactions of the conjugated antigen with a known E. coli 0111 antiserum and by the use of an indirect sandwich technique on E. coli organisms.

5170

Formal, S.B.; LaBrec, E.H.; Kent, T.H.; Falkow, S. 1965. Abortive intestinal infection with an Escherichia coli - Shigella flexneri hybrid strain. J. Bacteriol. 89:1374-1382.

The mechanism of the apparent loss of virulence of an Escherichia coli - Shigella flexneri hybrid strain was studied. The parent Shigella strain caused a fatal enteric infection when fed to starved guinea pigs, and signs of dysentery followed its oral administration to monkeys. The hybrid strain failed to produce any apparent symptoms when fed to either of these species. The parent strain was shown to invade the intestinal mucosa of starved guinea pigs. This caused a severe inflammatory reaction in the lamina propria, which progressed to ulceration of the intestinal epithelium and resulted in death of the animal. The hybrid strain also invaded the intestinal mucosa and produced an inflammatory reaction. In this case, the inflammatory reaction subsided, the intestine returned to normal within 4 days after

challenge, and the animal survived. Both fluorescent antibody techniques and in vivo growth studies have shown that the hybrid strain cannot maintain itself in the intestinal mucosa. Preliminary studies have indicated that a similar situation also exists in the monkey. It is concluded that the virulence of dysentery bacilli rests not only in the capacity to reach the lamina propria, but also in the ability to multiply in this region.

5171

Formal, S.B.; LaBrec, E.H.; Schneider, H. 1965. Pathogenesis of bacillary dysentery in laboratory animals. Federation Proc. 24:29-34.

FA was used principaliy to determine the morphologic relationship of Shigella to intestinal lesions. FA demonstrated that virulent dysentery bacilli can cross the intestinal epithelial barrier, passing through the epithelial cell. A postulation of events leading to the ulcerative lesion is: entry into the epithelial cell and multiplication, accumulation of metabolites and endotoxin, hypoxia and death of the epithelium resulting in an ulcer. Symptom severity could depend on the number of ulcer sites. Bowel motility may be a defense mechanism. Results seen in guinea pigs were also noted in a natural host, the monkey. These results probably can be extended to man.

5172

Freid, M.A.; Lepper, M. 1965. Endemicity of enteropathogenic Escherichia coli: Studies of screening procedures. Arch. Environ. Health 10:742-746.

Stool specimens from pediatric admissions to and discharges from three hospitals were screened for enteropathogenic Escherichia coli by the fluorescent antibody method, and positives were subjected to cultural techniques for confirmation. The FA-positive, culture-negative results were separated into two groups, those due to nonspecific causes such as reactions with other organisms or laboratory error in interpretation and those due to the greater sensitivity of the FA technique, representing EEC actually present in the specimens. When these groups were expressed quantitatively, the latter was found to account for the majority of unconfirmed positive results. Unconfirmed FA positives correlated very highly with diarrhea. These data are presented as evidence for the adequacy of the FA technique as a screening device for EEC during endemic periods.

5173

Geck, P.; Gago, G.; Kovacs, S. 1965. Immunofluorescent tests and their significance in controlling dyspepsia coli. Orv. Hetilap 106:1171-1172. In Hungarian.

A total of 114 infants under 1 year old showing diarrhea was examined for 'dyspepsia coli' infection, using both the traditional bacteriological

and the immunofluorescence methods. Of 641 samples processed, the latter method gave 78.8 per cent positive results, compared to 22.8 per cent with the bacteriological test. Comparison of the two methods according to age groups showed that up to the age of 1 year the infants were approximately equally susceptible to infection. Non-symptomatic bacteria dischargers were indicated with the immunofluorescent test. The test also reduced the necessary time for diagnosis to 2 or 3 hours.

5174

Geck, P.; Osvath, P.; Voltay, B.; Backhausz, R.; Losonczy, G.; Vigh, G.; Bognar, S. 1963. Immunofluorescence and passive haemagglutination in infantile enterocolitis. Acta Microbiol. Acad. Sci. Hung. 10:1-6.

In order to detect Shigella excreters, 252 hospitalized children, one to 14 years of age, have been examined. The examination of feces and rectal samples was performed by cultural, FA, and passive hemagglutination methods. Shigellae were cultured in 40.5 per cent from rectal samples taken on the day of admission, but only in 12.7 per cent from feces obtained next day. Cultural methods were compared with FA in 187 cases. By the latter, shigellae were detected three times more frequently than by culturing. The combination of the three methods gave positive results in 50.6 per cent. The rise in the passive hemagglutination titer in 18 paired sera confirmed the positive results of the former methods. Rising titers were observed also in some patients who, although they excreted bloody and mucous stools, yielded no evidence of Shigella infection by any other examination methods.

5175

Geck, P.; Szanto, R. 1964. Comparative examination of chronic typhoid carriers with immunofluorescent and cultural methods. Acta Microbiol. Acad. Sci. Hung. 11:211-214.

Fecal samples from 200 typhoid carriers have been examined in order to compare immunofluorescent tracing with the conventional enrichment cultivation technique. The presence of S. typhi was confirmed in 42 per cent with cultivation and in 69.5 per cent by FA. Only two specimens giving positive cultures were negative with FA. Positive results increased by 27.5 per cent using FA. Between FA positivity obtained within one hour and cultivation there was a 95 per cent agreement. The specific fluorescence of S. typhi was highly increased when the fecal samples were alkalinized.

5176

Georgala, D.L.; Boothroyd, M. 1964. A rapid immunofluorescence technique for detecting salmonellae in raw meat. J. Hyg. 62:319-327.

A rapid 18-hour technique has been developed for detecting Salmonella-contaminated carcass and boneless meats. It is based on 43 C selenite enrichment of samples, followed by immunofluorescent detection of Salmonella cells in the enrichment. In tests with 286 meat samples, the rapid and conventional techniques agreed in the detection of 93 positive and 149 negative samples. The two tests failed to agree for the remaining 44 samples. The rapid technique thus lacks precision, but could be used as a rapid presumptive Salmonella test, so that contaminated material could be prevented from reaching the processing lines of food factories.

5177

Georgala, D.L.; Boothroyd, M. 1965. Preparation of fluorescent polyvalent Salmonella antisera. Nature 205:521-522.

The conjugated polyvalent antisera described here are being assessed in routine immunofluorescence Salmonella tests on raw meat. Experience so far confirms that the direct technique may have worthwhile advantages over the indirect technique used in our earlier investigations. If necessary, a series of such conjugated polyvalent antisera could be prepared, using Group O antigens from all existing Salmonella groups.

5178

Georgala, D.L.; Boothroyd, M.; Hayes, P.R. 1965. Further evaluation of a rapid immunofluorescence technique for detecting salmonellae in meat and poultry. J. Appl. Bacteriol. 28:421-425.

A rapid 18 to 24 hour FA technique detected 14 of 15 positive samples in tests on 706 routine samples, which included 656 home produced raw beef samples. The rapid technique also recorded 49 false positive results, i.e. samples that proved negative in subsequent cultural tests. FA could be used as a presumptive screening test aimed at the rapid detection of negative samples. In this way Salmonella-free raw materials should usually be cleared for production within one day of sampling.

5179

Green, G.M.; Kass, E.H. 1964. The role of the alveolar macrophage in the clearance of bacteria from the lung. J. Exp. Med. 119:167-176.

Pulmonary clearance of bacteria was studied by histologic, bacteriologic, and radiotracer methods. When mice were exposed to an aerosol of radioactive Staphylococcus aureus or Proteus mirabilis, and the rate of disappearance of viable bacteria was compared with the rate of their mechanical removal, bacterial viability declined 80 to 90 per cent in 4 hours, although radioactivity declined only 14 to 20 per cent. The marked disparity in these rates indicated that mechanical removal comprised a relatively small fraction of the total clearing process. The in situ bactericidal action of the lung predominated over the mechanical removal process in achieving clearance of the inhaled bacteria. By immunofluorescent methods, the inhaled bacteria were found to be localized in the alveolar spaces and within alveolar macrophages. These observations suggest that the bactericidal action of the bronchopulmonary tree is due primarily to the phagocytic activity of the alveolar macrophages, and that the action of the mucociliary stream, in relation to bacterial particles, may be largely related to the transport from the lung of phagocytes containing material of bacterial origin.

5180

Guardiola-Rotger, A.; Lopez, V.A. 1964. An outbreak of diarrhea at the San Juan City Hospital Department of Pediatrics. Bol. Asoc. Med. Puerto Rico 56:161-174.

Two surveys for E. coli O111:B4 were conducted. Twenty-two children in the diarrhea ward were studied as well as 63 nasopharyngeal washings and 15 stool specimens collected from the attending personnel. In a second survey 65 children were studied. The laboratory data showed that an outbreak due to E. coli O111:B4 occurred at the diarrhea ward. Strains were resistant to antibiotics and especially to neomycin, the drug of choice at the hospital. Three sensitivity patterns were obtained for the isolated strains. The possible respiratory transmission of E. coli serotype infections is discussed. Fluorescent antibody methods yielded more positive findings than conventional culture methods. This was especially noted in persons undergoing antibiotic treatment.

5181

Gustafson, A.A.; Hundley, J.B. 1962. Fluorescence microscopy: A new diagnostic tool in North Dakota. Proc. N. Dakota Acad. Sci. 16:75-78.

The method has greatly facilitated diagnosis in streptococcal cases but has not been sufficiently tested for rabies and enteropathic Escherichia coli. BA-44-7351.

5182

Haglund, J.R.; Ayers, J.C.; Paton, A.M.; Kraft, A.A.; Quinn, L.Y. 1964. Detection of Salmonella in eggs and egg products with fluorescent antibody. Appl. Microbiol. 12:447-450.

Organisms of the genus Salmonella are detected in eggs and egg products within 24 hours in the presence of Pseudomonadaceae and other Enterobacteriaceae by combining selective cultural methods with fluorescent antibody techniques. These techniques are specific for Salmonella when H antibodies are used. Absorption techniques are necessary before the O antibodies give specific reactions for Salmonella. No cross-reactions appear when H antiserum is used. Absorption and interference techniques indicate the test is specific for Salmonella.

5183

Haglund, J.R.; Ayres, J.C.; Paton, A.M.; Kraft, A.A.; Quinn, L.Y. 1964. The detection of Samonella in eggs and egg products using fluorescent antibody. Poultry Sci. 43:1324-1325.

Salmonella may be detected in eggs and egg products within 24 hours in the presence of Pseudomonadaceae and other Enterobacteriaceae by combining selective cultural methods with FA. These techniques are specific for Salmonella when H antibodies are used. Absorption is necessary before the O antibodies give specific reactions. A method for commercial application is proposed consisting of an 8-hour incubation in selective enrichment broths followed by an 8- to 16-hour incubation in nonselective broth prior to fluorescent antibody staining. Slides are then prepared using the cultures obtained by these enrichments, stained by indirect FA and viewed. The method suggested has the advantage of saving at least 2 days over present techniques in obtaining results.

5184

Haglund, J.R. 1965. A fluorescent antibody method for detecting salmonellae in egg and poultry products and in processing plants. Diss. Abstr. 26:30-31.

FA satisfied all of the requirements of specificity. Pure cultures were not necessary for detection of salmonellae in egg and poultry products and in processing equipment. Bacteria isolated from egg and poultry products and identified by cultural methods and a comprehensive selection of other possible contaminants were stained by indirect FA to detect cross-reactions. Absorption of the sera with five cross-reacting E. coli strains was necessary before FA was specific. Although indirect FA was longer than direct FA, the indirect method was preferred as it produced greater staining intensity. The direct test was specific, but elimination of nonspecific staining was more difficult. Salmonellae were detected in egg and poultry products and in processing

equipment within 24 hours when combinations of cultural and FA methods were used. Results obtained by FA were in agreement with findings using the IAPI and Montford and Thatcher methods. When S. cerro was present in the samples, the FA test did not always detect the presence of this species because the necessary somatic antibody was not included in the sera. A test that combined cultural enrichments and FA was suggested for rapid and specific detection of salmonellae. The procedure enabled detection of salmonellae within 24 hours, a saving of 2 to 3 days over conventional methods. Suitable commercial salmonellae antisera were not available, and this was a limitation of the test.

5185

Harris, T.N.; Dray, S.; Ellsworth, B.; Harris, S. 1963. Rabbit gamma globulin allotypes as genetic markers for the source of antibody produced in recipients of Shigella-incubated lymph node cells. Immunology 6:169-178.

Rabbits homozygous for each of an allelic pair of allotypes of gamma globulin A4 and A5 were used as donors and recipients of transferred antigen-incubated lymph node cells, the cells of donors of one allotype being in each case transferred to recipients of the other. When agglutinins to Shigella appeared in the sera of the recipient animals, the allotype of the agglutinin was determined by adsorbing it to Shigella on a glass slide, then treating the preparations with fluorescein-conjugated rabbit anti-A5 gamma globulin and anti-A4 gamma globulin antisera, respectively. In each case the reactions of the recipients' sera were positive for gamma globulin of the donor allotype but not of their own. Positive reactions were given only by sera above a certain range of agglutinin titer. After a sufficient decline of the agglutinin level in the sera of the recipients, these animals were actively immunized with Shigella. The agglutinins that now appeared in these rabbits gave positive reactions with the fluorescent antibody against their own allotype. These data indicate that in moderately X-irradiated rabbits given antigen-incubated rabbit lymph node cells, the antibody that subsequently appears in the recipient's serum has been synthesized by the donor's cells.

5186

Harris, T.N.; Dray, S.; Ellsworth, B.; Harris, S. 1963. Rabbit gamma globul. allotypes as genetic markers for the source of antibody produced in recipients of Shigella-incubat d lymph node cells. Federation Proc. 22:636:266.

Rabbits homozygous for each of an allelic pair of allotypes of gamma globulin, A4 and A5, were used as donors and recipients of lymph node cells incubated with Shigella antigen, the cells of donors of one allotype being in each case transferred to recipients of the other. When agglutinins to Shigella appeared in the sera of the recipient

animals, the allotype of the agglutinin was determined by adsorbing it to Shigella on a glass slide, then treating the preparations with fluorescein-conjugated rabbit anti-A5 gamma globulin and anti-A4 gamma globulin, respectively. In each case the reactions of the recipient sera were positive for gamma globulin of the donor allotype but not of their own. Positive reactions were given only by sera above a certain range of agglutinin titer. Later, the former recipients were actively immunized with Shigella. The agglutinins that then appeared in these rabbits gave positive reactions with the fluorescent antibody against their own allotype. These data indicate that in moderately X-irradiated rabbits given antigen-incubated rabbit lymph node cells, the antibody that subsequently appears in the recipient serum had been synthesized by the donor cells. Complete article.

5187

Hirsch, J.G. 1964. Demonstration by fluorescence microscopy of adsorption onto bacteria of a heat-labile factor from guinea pig serum. J. Immunol. 92:155-158.

On incubation of staphylococci or salmonellae with fresh guinea pig serum, a heat-labile factor was adsorbed onto the bacteria. This adsorption did not take place at 0 C, and was also blocked by high salt concentrations in the medium. Divalent cations were not required for adsorption. Heat-labile guinea pig serum factor bound to the bacterial surface was altered or destroyed on exposure to 56 C, to trypsin, or to hydrazine. Incubation of fresh guinea pig serum with concentrated suspensions of Staphylococcus albus, followed by removal of the microbes by centrifugation, led to reduction or elimination of the content of heat-labile factor as estimated by immunofluorescence tests employing the homologous staphylococcus or an unrelated organism, Salmonella typhimurium. Fluorescence techniques were devised that permitted estimation of adsorption to the same bacterial cells of both heat-labile serum factor and specific antibody. Adsorption to staphylococci of either of these two serum components did not detectably augment or diminish subsequent binding of the other.

5188

Hoffman, E.; Correa, P.; Lujan, R. 1963. Immunofluorescence in rhinoscleroma. Rev. Lat. Amer. Anat. Pat. 7:67-76. In Spanish.

Use of the fluorescent antibody technique has shown the usefulness of the method in the diagnosis of scleroma in paraffin and frozen sections; the presence of antibodies against Klebsiella rhinoscleromatis in the serum of patients suffering with the disease; and that, in the scleroma tissues, etiological agents other than the K. rhinoscleromatis do not exist or at least are not demonstrable by immunofluorescence.

5189

Horowitz, R.E.; Bauer, H.; Paronetto, F.; Abrams, G.D.; Watkins, K.C.; Popper, H. 1964. The response of the lymphatic tissue to bacterial antigen: Studies in germfree mice. Amer. J. Pathol. 44:747-761.

Lymph nodes from 76 germfree and 76 conventional mice were examined by histologic, histochemical, autoradiographic, and immunoctyochemical techniques, at intervals of 2 hours to 14 days after foot pad injection with killed E. coli organisms. All nodes draining the injection site showed transient initial acute lymphadenitis and persistent weight gain. Particulate antigen appeared in sinus macrophages 2 hours after injection in both germfree and conventional nodes but disintegrated more rapidly in the latter. Immunoblasts proliferated first in the intermediate zone of the cortex and later at the corticomedullary junction. This was followed by the appearance of plasma cells, and gamma globulin-containing cells at the corticomedullary junction and in the medullary cords in both germfree and conventional lymph nodes. Circulating antibody developed in all mice after 4 days. The lymphatic tissue of the germfree animal is capable of responding to antigenic stimulation. Previous experience with a microbial flora confers only minor advantages upon conventional animals.

5190

Hornung, J.E. 1965. Immunofluorescent studies of Shigella in infants and young children. Amer. J. Med. Technol. 31:239-255.

The use of the immunofluorescent technique to give a preliminary or presumptive report in infections with Shigella flexneri or Shigella sonnei appears to be feasible if its limitations are rigidly observed. Positive cases are diagnosed quickly and with a fair degree of accuracy. Cases negative by bacteriologic methods may be expected to give false-positive staining in approximately 20 per cent of the cases. Completely negative staining appears to be in good agreement with the bacteriologic findings. In treated cases the correlation between fluorescent antibody staining and bacteriologic studies appears variable and would have to be analyzed on an individual case basis.

5191

Hornung, J.; Weiner, L.M. 1964. Diagnosis of human shigella infections by fluorescent antibody. Bacteriol. Proc. M66:55.

Stool samples from young children suspected of having bacillary dysentery have been studied by the fluorescent antibody technique and the results compared with the findings of the routine diagnostic laboratory. Of 17 cases culturally diagnosed as Shigella sonnei, 15 reacted only with fluorescein-tagged sonnei antiserum. The remaining two stained with S. flexneri polyvalent antiserum as well as with the sonnei anti-

serum. Of four cases diagnosed as salmonellosis, none gave positive fluorescence with any of the Shigella antisera. In all, 65 specimens were found to be bacteriologically negative for enteric pathogens. Of this group, 74 per cent were also negative by the fluorescent antibody technique. Of the remaining 17, eight stained with antipolyvalent S. flexneri serum, five with anti-S. sonnei serum, and four with both antisera. In addition, studies were performed on single-colony isolates from such false-positive stool specimens to indicate the extent and nature of the cross-reactions and the possibility that the fluorescent antibody technique picked up some Shigella infections overlooked by routine procedures.

5192

Hovhanian, H.P.; Brennan, T.A.; Botan, E.A. 1964. Quantitative rapid immunofluorescence microscopy. J. Bacteriol. 87:473-476.

An elaborate electronic-optical instrument for detection and measurement of FA stain reactions is described. It consists of a UV source, UV monochromator, UV filter system, bright-field fluorescence microscope, secondary filter system, a UV television camera, a microspot scanner, a quantitative light-reading device, television monitor, and an oscilloscope. Satisfactory tests were made with FA systems for E. coli, S. lutea, and B. globigii.

5193

Ivanova, S.P.; Bochorishvili, V.G. 1963. Accelerated diagnosis of typhoid-paratyphoid infections. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:1:61-65. In Russian.

A study was made of the possibility of detecting typhoid-paratyphoid bacilli with fluorescent antibodies in the blood smears, as well as in liquid nutritive medium during the first hours of hemoculture development. The sensitivity of the method was increased in experiments with artificial infection during investigation of mixtures of bacteria with blood, as well as in hemocultures from patients. The sensitivity and specificity of the FA method was tested in detection of typhoid-paratyphoid bacilli in material obtained from patients. The possibility of identification of typhoid-paratyphoid bacteria in preparations from hemocultures and bile on enriched Rapoport medium was demonstrated by using type-specific adsorbed agglutinating sera and indirect FA. The use of fluorescent antibodies permits detection of typhoid-paratyphoid bacilli in samples of blood and bile from patients more rapidly than classical methods of cultivation.

5194

Khomenko, N.A.; Olshevskaya, T.R. 1965. Increase of fluorescent globulin specificity for the direct method of Shigella staining. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:7:33-36. In Russian.

The specificity of fluorescent antibodies for the detection of Shigella dysenteriae could be increased by their preparation from specific sera after meticulous sorption of heterologous agglutinins. For the same purpose fluorescent antibodies should be used in high dilutions, 1:32 to 1:64. To produce a more intensive fluorescence of bacteria, stain the smears for a more prolonged period of time, 1.5 to 2 hours at 37 C in a humid chamber.

5195

Kramar, R. 1965. Attempt to use the method of immunofluorescence in the identification of enteropathogenic E. coli. Cesk. Epidemiol. Mikrobiol. Immunol. 14:225-228. In Czech.

The method of indirect immunofluorescence was used for the rapid detection of enteropathogenic E. coli in stools. Definite advantages were displayed by the procedure involving precultivation and the use of diagnostic agglutination sera and anti-rabbit conjugates. The results were compared with standard cultivation and agglutination tests. It was found that FA was sufficiently specific, with the results available much earlier than with the standard procedure of testing.

5196

LaBrec, E.H.; Schneider, H.; Magnani, T.J.; Formal, S.B. 1964. Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery. J. Bacteriol. 88:1503-1518.

A parent strain of Shigella flexneri 2a and a colonial mutant derived from it were studied in three animal models. Both strains were equally virulent for mice when living cells suspended in hog gastric mucin were injected by the intraperitoneal route. Feeding the parent strain to starved guinea pigs, followed by the intraperitoneal injection of opium, resulted in the formation of ulcerative lesions in the intestinal tract and in the death of these animals. When the colonial variant was fed, the animals survived, and the intestinal tract remained normal. The parent produced diarrheal symptoms and intestinal lesions after its oral administration to rhesus monkeys; the variant caused neither symptoms nor pathology. Neither serological studies nor growth studies conducted both in vitro and in vivo offered a clue to explain this difference. The virulent parent penetrated the bowel epithelium and entered the lamina propria; the avirulent mutant did not. Entrance to the lamina propria was by way of the epithelial cell of the mucosa. The avirulent mutant did not penetrate this cell. The virulent parent

possesses the ability to infect and multiply within HeLa cells. The organisms are able to penetrate epithelial cells of the guinea pig cornea, causing ulcerative lesions. The avirulent variant possesses neither of these capacities. Epithelial cell penetration is a major factor in determining the pathogenicity of dysentery bacilli. FA was used to study progress of the infections.

5197

Laurell, G. 1963. Serologic typing of E. coli. Nord. Med. 69:178-180. In Norwegian.

Fluorescent antibody is a practicial method for typing E. coli, useful in epidemiology of diarrhea of the newborn. The test is easily performed in the clinical laboratory.

5198

Lewis, V.J.; Brooks, J.B. 1964. Comparison of fluorochromes for the preparation of fluorescent antibody reagents. J. Bacteriol. 88:1520-1521.

Conjugates of anti-Corynebacterium diphtheriae and anti-Escherichia coli antisera were prepared using four dyes, FITC, RB 200, DANS, and tetramethylrhodamine isothiocyanate. When titers were compared, FITC was superior.

5199

Linz, R.; Lejour, M. 1964. Diagnosis of enteropathogenic Escherichia coli by immunofluorescence. Acta Clin. Belg. 19:237-247. In French.

Enteropathogenic E. coli were sought in 300 feces simultaneously by two methods, culture and indirect FA. The results of both methods were concordant for 273 feces; 225 of these were negative and 46 were positive. Among 55 feces with a positive culture, 46 were positive on IF staining. In one case, a positive IF result served to correct an erroneous and hasty conclusion. Among 240 feces with negative cultures, the results of IF staining were negative 225 times, but positive 15 times. Therefore, IF staining of enteropathogenic E. coli in smears of feces cannot be considered a perfect method, but it appears useful if a quick presumptive diagnosis is desired and if a culture control is initiated at the same time.

5200

Maiztegui, J.I.; Biegeleisen, J.Z., Jr.; Cherry, W.B.; Kass, E.H. 1965. Bacteremia due to Gram-negative rods: A clinical, bacteriologic, serologic, and immunofluorescent study. New Engl. J. Med. 272:222-229.

One hundred patients with bacteremia due to Gram-negative bacilli were studied at a large municipal hospital. The source of bacteremia was identified in all but seven cases, and was found most commonly to be the urinary tract, followed by the skin. The over-all mortality was 55 per cent.

5201

Mancini, L.; Beni, G.; Itri, G.B. 1964. Immunofluorescent anti-somatic and anti-ciliary antibodies after typhoid vaccination. Ann. Med. Nav. 69:17-20. In Italian.

In a new series of studies on the serological changes in men inoculated with typhoid vaccine, it was found that immunofluorescent antibodies were present and that they reacted with both antigens. It was concluded that specific vaccination in man is effective in provoking an immunological response toward both antigens of S. typhi.

5202

Marsden, H.B.; Hyde, W.; Bracegirdle, E. 1965. Immunofluorescence in the diagnosis of enteropathogenic Escherichia coli infections. Lancet 1:189-191.

The fluorescent antibody technique, using materials that are readily available, is compared with cultural methods in the diagnosis of enteropathogenic Escherichia coli gastroenteritis. Cross-reactions have been few, although care should be taken in identifying serotype 0127 by fluorescence alone. The greater sensitivity and rapidity of the fluorescent antibody technique make it a most useful procedure for routine investigation in hospitals for children.

5203

Marsden, H.B.; Hyde, W.A.; Bracegirdle, E. 1965. Immunofluorescence in the diagnosis of enteropathogenic Escherichia coli infections. Lancet 1:606.

This letter answers objections raised by Bradstreet to use of routine diagnostic antisera for FA identification of bacteria.

5204

Martin, A.J.; O'Brien, M. 1965. Detection of enteropathogenic Escherichia coli in fecal cultures by use of a modified fluorescent antibody technique. J. Bacteriol. 89:570-573.

The application of the fluorescent antibody technique to cultures of feces on blood-agar plates for the detection of enteropathogenic Escherichia coli serotypes is described. The results of a study of 364 fecal specimens, examined by both this modified technique and conventional cultural methods, are reported; the findings by the two methods are compared. The modified FA method offers several advantages over other techniques. The results of the examination can be reported sooner; more serotypes are discovered; it is simpler, easier, and quicker to prepare and examine growth from the blood plate than to examine fecal material; and the results are more definite. The results obtained by use of this modified FA procedure have shown that infection with multiple serotypes occurs much more frequently than cultural examinations alone indicate. Intensive cultural studies suggest that the inability to confirm serotypes that have been found by the FA method is probably due to inefficiencies in the cultural method.

5205

Mayorova, G.F.; Korn, M.Ya. 1963. A study of the antipertussis fluorescent serum specificity. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:9:42-48. In Russian.

In studies on the specificity of fluorescent serological examination of H. pertussis with the aid of antipertussis fluorescent serum, it was impossible to detect H. pertussis with the aid of heterologous sera. Antipertussis fluorescent serum stained some species of microorganisms nonspecifically: P. pestis, E. coli, Bacillus brucellosis, P. tularensis, and others. Staphylococci and streptococci were stained specifically. The method of fixation also influenced the results of the investigation. Rough fixation by flame provoked microbial staining of H. pertussis with heterologous fluorescent sera. Identification of H. pertussis in practical conditions by FA at present is fraught with some difficulties, since the Staphylococcus and Streptococcus may be stained simultaneously. It was impossible to distinguish H. parapertussis from H. pertussis by the indirect method.

5206

Mikhailov, I.F.; Pers, I.F. 1963. Fluorescent antibody method applied for the detection of antigenic relationships among bacteria of the intestinal group. Zh. Mikrobiol. Epidemiol. i Immunobiol. 42:5:97-103. In Russian.

An indirect fluorescent antibody method was employed to detect antigenic relationships among bacteria of the Enterobacteriaceae. Precipi-

tating sera against the antigens, obtained by disintegration of bacterial cells, were used at the first stage of processing the ultrasonic preparations. Bacterial membrane impermeability for antibodies was established when comparing the results obtained in the reaction with fluorescent antibodies and with the aid of the precipitation reaction. Therefore, serological relationships caused by deeply located antigenic complexes were undetectable by the methods applied; antigenic relationships between some of the bacteria studied were revealed only at the expense of their superficial antigens.

5207

Mikhailov, I.F.; Pers, I.F. 1964. Isolation of antibodies from antigen-antibody complex by ultrasound. Zh. Mikrobiol. Epidemiol. i Immunobiol. 41:112-119. In Russian.

Antibodies were separated from their specific complex with somatic antigens by ultrasound and then eluted in saline. Antibody preparations were free of nonspecific protein, heterologous antibody, and the antigen. Antibodies retained their immunologic specificity. An anti-flexneri conjugate labeled with fluorescein isocyanate was among the sera used.

5208

Mikhailov, I.F.; Stanislavsky, E.S. 1963. Staining of isolated bacterial structures with fluorescent antibodies. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:6:74-79. In Russian.

Antigenic interrelationships between individual structural elements of intestinal bacteria were studied with the aid of FA. S. paratyphi B and E. coli O111 were used. Staining peculiarities of isolated structural elements of the bacterial cell with fluorescent antibodies were also established. Antigens common to the two species were located in the cell membrane and cytoplasmic membrane. Cytoplasm contained no O antigen. H antigen was present only in flagellae. An even specific fluorescence of the stained structures was noted in studying the antigens of individual structural elements of the microbial cell. Isolated membranes were characterized by brighter fluorescence along the periphery.

5209

Nelson, J.D.; Hempstead, B.; Tanaka, R.; Pauls, F.P. 1964. Fluorescent antibody diagnosis of infections. J. Amer. Med. Ass. 188:1121-1124.

In areas without bacteriology laboratory facilities, certain infections can be diagnosed by the fluorescent antibody technique from specimens mailed to a central laboratory. An outbreak of respiratory illness occurred in a remote area of Alaska. Nasopharyngeal swab

specimens applied to microscope slides were sent to laboratories in Anchorage, Alaska, and Dallas, Texas. Positive fluorescent antibody identification of Bordetella pertussis was made in 14 specimens by the Anchorage laboratory and confirmed in nine specimens in Dallas. Good correlation was obtained in nine cases of diarrheal disease studied by fluorescent antibody staining of rectal swab specimens for enteropathogenic serotypes of Escherichia coli.

5210

Nikitin, V.M. 1964. The use of immunofluorescent paper disks for the rapid detection of pathogenic microbes. VoennoMed. Zh. 11:55-58. In Russian.

Instead of the liquid form of labeled gamma globulins, strips or disks of various kinds of paper saturated with fluorescent conjugates were prepared. Best results were obtained with filter paper disks. One drop of fluorescent immune serum of certain specificity was applied to each side of the disks measuring 1 cm in diameter. The disks were dried and stored at 4 C. Trials on cultures of Flexner's bacillus, Escherichia coli, tularemia vaccine, and anthrax vaccine were carried out both by the direct and the indirect methods. The disks kept their immunological capacity for specific staining up to 15 months, although exposure time had o be increased as storage time was increased. The use of these disks simplifies storage, application, and transportation of the fluorescent antibodies and facilitates their standardization. The recommendation of their use in field conditions is justified.

5211

Ogawa, H.; Takahashi, R.; Honjo, S.; Takasaka, M.; Fujiwara, T.; Ando, K.; Nakagawa, M.; Muto, T.; Imaizumi, K. 1964. Shigellosis in cynomolgus monkeys (Macaca irus): III. Histopathological studies on natural and experimental shigellosis. Jap. J. Med. Sci. Biol. 17:321-332.

A histopathological survey was made on 61 cases of natural shigellosis among monkeys kept in quarantine. By oral inoculation of Shigella flexneri 2a isolated from infected monkeys, a diarrheal disease was induced in monkeys, which were examined sequentially. The same colitis as observed in natural shigellosis appeared within 1 week after the infection, although formation of ulcerative lesions was not observed. Organisms distributed on the intestinal wall were observed by the fluorescent antibody technique. Both in natural and experimental shigellosis, organisms were detected on the surface of the mucous membrane of the inflamed part of the large intestine, on lamina propria, and rarely in lymphatic nodules where severe inflammation was extended. No organism was found in submucosa even when inflammation was severe.

5212

Olarte, J.; Ramos-Alvarez, M; Galindo, E. 1964. The use of fluorescent antibodies in the study of some infectious diseases: IV. Results obtained with the fluorescent antibodies method and with cultures in the study of enteropathogenic Escherichia coli infections. Gac. Med. Mex. 94:319-324. In Spanish.

FA was used in one epidemic of diarrhea in premature children, caused by E. coli 0126:B16, as well as in ten cadavers of children dead of infections by diverse serotypes of pathogenic E. coli. Of 33 rectal swabs taken from the prematures, 13 were negative both to culture and to the fluorescence methods. In the other 20 swabs the presence of E. coli 0126:B16 was demonstrated in 12 by the two procedures, only by fluorescence in seven, and in one by culture alone. Of 60 samples taken at different levels of the digestive tract of the ten dead children, 19 were negative by both. Thirty-seven were positive to both procedures, having identified E. coli 0111:B4 in three cases, E. coli 0126:B16 in three cases, E. coli 086:B7 in two and E. coli 0125:B5 in the other two cases. In the other four samples the culture was positive and fluorescence negative. FA is an important help in the rapid diagnosis of enteropathogenic E. coli infections, but cannot be considered as a culture substitute. BA-46-95152.

5213

Opferkuch, W.; Huth, E. 1963. Diagnosis of enteropathogenic Escherichia coli by fluorescent antibody. Pediatrie 18:703-707. In French.

The authors discuss an identification method for samples of enteropathogenic colibacilli by optical fluorescence. From a study of 39 cases, they showed that the sensitivity of this process is not inferior to that of the agglutination method. The identification of each sample can be made with certainty, and there are no cross reactions. Since FA is easier and faster, it is recommended in practice.

5214

Pittman, B.; Hebert, G.A.; Cherry, W.B.; Taylor, G.C. 1964. Quantitative measurements of nonspecific staining by fluorescein isothiocyanate - labeled globulin employing mammalian cell cultures. Bacteriol. Proc. M110:64.

This paper describes a quantitative procedure for measuring fluorescence emission from cultured mammalian cells stained with FITC-labeled globulins. Cells in a monolayer of 60 to 120 per high dry microscopic field were stained with high-titered rabbit antiglobulins for Escherichia coli labeled with FITC. Labeling conditions were varied to give conjugates having ratios of fluorescein to protein ranging from approximately 1 to 20. Cell preparations were stained with the conjugates; photometric measurements of fluorescence emission and cell count were

made on each field. Results were expressed as photometric units per tissue cell and served as a measure of nonspecific staining. Conjugates of E. coli applied to noninfected mammalian culture cells provided a completely heterologous antigen-antibody system; any fluorescence beyond that given by the unstained cells was considered nonspecific. When the fluorescein-to-protein ratios of conjugates were plotted against photometric units per cell, a linear relationship was demonstrated. Conjugates within a certain range of ratios gave maximal specific staining titers for E. coli but nonspecific staining of intermediate values as measured on tissue cells. It is not desirable to label globulins under conditions resulting in high ratios of fluorescein to protein because nonspecific staining is increased without increase in the specific titer. Precise quantitative measurement of nonspecific staining furnishes a sound basis for the evaluation of the quality of conjugates.

5215

Reimers, E. 1965. Demonstration of the antigens of enteropathogenic Escherichia coli by fluorescence microscopy. Z. Kinderheilk. 93:85-90. In German.

This is a report of a different and spotted reaction of enteropathogenic Escherichia coli with fluorescent antibodies; some microphotographs are given for several findings. A minor reaction was found, sometimes in fresh cultures of feces stored for several days in a refrigerator after normal incubation, sometimes in the beginning of an outbreak of EEC, during antibiotic therapy, or with older control cultures. It appears to be necessary to distinguish between agglutinating and adherent antibodies. It is proposed to use only fresh isolated EEC or freshly seeded refrigerant-dried cultures of EEC for immunizing and controlling purposes with the fluorescent antibody method.

5216

Schaffer, J.; Lewis, V.; Nelson, J.; Walcher, D. 1963. Antepartum survey for enteropathogenic Escherichia coli: Detection by cultural and fluorescent antibody methods. Amer. J. Dis. Children 106:170-173.

A sporadic outbreak of diarrhea associated with enteropathogenic Escherichia coli of serotype O111:B4 occurred. The isolation of the same serotype from both the infant and mother suggested the possibility of reservoir of organisms in the antepartum mother. Seventy-two of the 657 prenatal women (11 per cent) screened by a single swab examination harbored various serotypes of enteropathogenic E. coli in their gastrointestinal tracts. Enteropathogenic E. coli was also detected in six of 149 antepartum women (4 per cent) attending the prenatal clinic of a university hospital. Screening of asymptomatic adults for enteropathogenic E. coli is feasible with the fluorescent antibody technique. The immunofluorescent and standard bacteriologic methods were compared on 241 of the total of 806 swab specimens. An agreement of 88 per

cent was observed between the immunofluorescent and agglutination methods. Nine strains of O111:B4 did not show neomycin resistance beyond 5 ug per ml by the serial tube dilution method.

5217

Schimmelpfennig, H. 1964. Fluorescence serology in bacteriological diagnosis: VII. Studies on Salmonella. Zentralbl. Veterinaermed. Reihe B 11:7:633-643. In German.

Demonstration of Salmonella using indirect FA succeeded with agglutinating multivalent absorbed serum with Salmonella OH sera, and with monofactor serum 09. By using various dilutions it was possible to use the method to examine in detail the antigenic structure of the strains. To demonstrate Salmonella, particularly S. typhi, in feces, water, and meat, FA is not satisfactory. It will not detect small numbers of organisms with sufficient certainty. Nonspecific reactions caused by other bacteria cannot be entirely prevented. BA-47-83126.

5218

Shingu, K. 1964. On the detection of dysentery bacilli in feces by the fluorescent antibody technique. J. Jap. Ass. Infect. Dis. 38:193-202. In Japanese.

The fluorescent antibody technique was used to examine dysentery bacilli in feces, resulting in the following findings: The commercial Shigella typing serum, rabbit euglobulin, used in clinical laboratories could be used to detect dysentery bacilli by this technique. The rate of dysentery bacilli detection in feces from patients did not notably vary according to the method used, the routine culture method or the present technique. In the fluorescent antibody technique a cross-reaction was occasionally observed between dysentery antiserum and a few cultured strains of Enterobacteriaceae, especially E. coli. Several cases were noted of patients infected with E. coli that reacted positively with the dysentery antibody. In spite of this cross-reaction, this technique is believed to have several advantages: In the majority of the cases of dysentery, the technique is able to quickly identify the group specificity of dysentery bacilli directly in feces. Furthermore, the possible presence of Shigella boydii in feces, which is difficult to identify by the ordinary culture method because of its K antigen and the presence of some other strains of dysentery bacilli similar to E. coli in biological characteristics, can be rather easily recognized by the present technique.

5219

Strachiloff, D.; Mohr, J. 1965. The use of the immunofluorescence method for antigen analysis of Salmonella. Zentrabl. Bakteriol. Parasitenk. Infektionskrankh. Hyg. 196:1:29-33. In German.

The application of the direct and indirect FA technique in the demonstration of the flagellum system of salmonellae is described. BA-46-99196.

5220

Stulberg, C.S.; Caldwell, W.J.; Kennedy, D.W.; Page, R.H. 1964. Application of immunofluorescence to an epidemic due to Salmonella typhimurium. Bacteriol. Proc. M68:56.

A hospital outbreak of S. typhimurium in infants afforded an opportunity to study the epidemiological applicability of an FA procedure for identification of Salmonella. Consecutive daily specimens from involved infants, of whom 18 were symptomatic and 22 asymptomatic, and surveys of 74 adult nursery personnel provided material. The FA technique, based on identification of both somatic and flagellar antigens, was performed by formalinizing broth cultures of fecal specimens and staining the organisms in suspension with specific labeled antibody. FA reactions with infant specimens were uniquely specific in that only 2 of 445 specimens exhibited somatic but not flagellar cross-staining with nonpathogens; 30 of 74 adult specimens revealed somatic but not flagellar cross-staining with normal flora, indicating the over-all specificity of the combined reaction. Comparative sensitivity of FA and culture was judged from paired results on 91 infant specimens in which correlation was obtained with 73, of which 35 were positive and 38 negative. Sixteen were FA-positive but culturally negative, and only two were culturally positive but FA-negative. Thus, the feasibility of rapid presumptive identification permitted current consecutive observations on each infant, including changes in characteristics of the enteropathogens, shedding, effects of therapy, and absence of cross infections.

5221

Sutter, L.S.; Ulrich, E.W. 1964. H, O, and fluorescent antibody titers in salmonellosis. Amer. J. Gastroenterol. 42:626-632.

Serologic findings in 32 different patients suffering from salmonellosis are presented. The cases representing these patients are classified into four different categories as follows: food poisoning, gastroenteritis, gastroenteritis with bacteremia, and localized infection with or without bacteremia. The data are composed of H, O, and fluorescent antibody titers, all of which have been determined by the use of antigens prepared from homologous infecting species. Fluorescent antibody titers were

determined by the indirect method. The FA titer was in general similar to that of the H. The O titers were much lower than the H, the figure being zero in the food poisoning category.

5222

Taylor, C.E.D.; Heimer, G.V. 1964. Rapid diagnosis of Sonne dysentery by means of immunofluorescence. Brit. Med. J. 2:165-166.

A fluorescent antibody technique for the detection of S. sonnei in specimens of feces from cases of acute diarrhea is described and compared with a cultural method. The two procedures agreed in 95.6 per cent of 388 specimens examined; 1.8 per cent of the results are regarded as false positive by the fluorescence technique and 2.6 per cent are regarded as false negative. The technique enables a provisional report to be telephoned within one hour after a specimen arrives in the laboratory, and is regarded as a useful procedure for the rapid diagnosis of Sonne dysentery, provided that it is combined with a cultural method.

5223

Taylor, C.E.D.; Heimer, G.V.; Lea, D.J.; Tomlinson, A.J.H. 1964. A comparison of a fluorescent antibody technique with a cultural method in the detection of infections with Shigella sonnei. J. Clin. Pathol. 17:225-230.

A comparison has been made of a fluorescent antibody technique with a cultural method for the detection of S. sonnei in feces. The two methods were in agreement in 73 per cent of the 394 specimens examined; 14.5 per cent of the specimens were positive by culture only; 13.2 per cent gave positive results by fluorescence microscopy that were not confirmed by culture. Most of the latter are thought to be false-positive results. The value and usefulness of fluorescence microscopy in the diagnosis of Sonne dysentery are discussed.

5224

Taylor, C.E.D.; Lea, D.J.; Heimer, G.V.; Tomlinson, A.J.H. 1963. Fluorescent antibody techniques in diagnostic bacteriology. Proc. Roy. Soc. Med. 56:478.

The fluorescent antibody technique in the rapid diagnosis of Sonne dysentery appears to have certain definite limitations. Applied to outbreaks of diarrhea caused by S. sonnei, it should be possible to determine the causative organism within 2 hours after receiving specimens from half a dozen representative cases. In our hands, the technique is not sufficiently reliable for the detection of symptomless carriers or the surveillance of convalescent cases.

5225

Thomason, B.M.; Boris, M.; Hines, V.D.; Cowart, G.S.; Davis, B.E.; Dixon, O.P. 1963. Fluorescent antibody detection of respiratory carriers of enteropathogenic Escherichia coli 0126:B16:NM during a community epidemic. Bacteriol. Proc. M138:89.

Surveillance of all cases of infantile diarrhea admitted to a large municipal hospital revealed an unusually high incidence of enteropathogenic E. coli (EEC) 0126:B16:NM from July 1962 through October 1962. Fecal specimens from 664 consecutive infants with acute gastroenteritis were examined by fluorescent antibody techniques for nine EEC serogroups. FA-positive specimens were cultured and isolates serotyped. The patients consisted of 383 hospitalized cases and 281 infants seen in the outpatient clinics. The incidence of all EEC infections in children under 10 months of age was 48 per cent in those hospitalized and 30 per cent in outpatients. Of the EEC detected by FA, 40 per cent were confirmed as serotype 0126:B16:NM. A survey using FA techniques was undertaken to study the mode of spread of this organism. Rectal and pharyngeal swabs were obtained from family members of 28 cases of EEC diarrhea due to 0126:B16:NM. The adjoining household and a randomly selected family in the neighborhood were sampled simultaneously. Fifty per cent of the families of the index case and 33 per cent of the adjacent families had one or more asymptomatic members carrying EEC 0126:B16:NM in the pharynx. Fecal specimens were positive in only 18 per cent of the index families and 4 per cent of the adjacent families. Of the specimens positive for EEC 0126:B16 by FA, 57 per cent were isolated and all confirmed as nonmotile. The data suggest respiratory spread of this organism in the community.

5226

Thomason, B.M.; Cowart, G.S.; Cherry, W.B. 1965. Current status of immunofluorescence techniques for rapid detection of shigellae in fecal specimens. Appl. Microbiol. 13:605-613.

Polyvalent Shigella conjugates were prepared for Shigella groups A, B, C, and D. After preliminary testing with pure cultures of both homologous and heterologous organisms, these reagents were used in three evaluation studies. Fecal specimens from patients hospitalized with diarrhea, from children involved in an institutional outbreak of dysentery due to S. sonnei, and from patients with diarrhea in Arizona were screened by fluorescent antibody tests and were cultured. Specimens were examined at various periods of time after collection and after incubation in broth and saline. Shigellae were detected most frequently when specimens were cultured immediately after collection. The S. sonnei conjugate gave the most reliable results of any of the Shigella FA reagents used in these investigations. It proved to be both sensitive and specific. FA tests revealed more positive results when the specimens were incubated in either saline or broth than when they were examined immediately after collection.

5227

Thomason, B.M.; Cowart, G.S.; Cherry, W.B. 1965. Fluorescent antibody detection of Shigella sonnei in fecal specimens. Bacteriol. Proc. M127:60.

In the past, attempts to utilize fluorescent antibody techniques for rapid detection of shigellae in fecal specimens have met with limited success. This report describes the preparation and testing of a fluorescein-labeled Shigella sonnei reagent. Preliminary testing with pure cultures of various Enterobacteriaceae indicated that few cross-reactions would occur with other common enteric organisms. Fecal specimens from 542 individuals suspected of having shigellosis were screened by FA and cultural procedures. Of these, 36 were positive for S. sonnei by the FA test. Isolations of S. sonnei were obtained from 66.7 per cent of the FA-positive specimens. Fecal specimens were cultured and examined by FA shortly after collection. FA and cultural examinations were repeated after incubation of the specimens in broth or saline or both. More isolations were obtained from immediate plating; the FA test revealed more positives from the broth-enriched specimens. Results indicate that FA procedures can be used to advantage in rapid detection of cases of dysentery due to S. sonnei.

5228

Thomason, B.M.; McWhorter, A.C. 1965. Rapid detection of typhoid carriers by means of fluorescent antibody techniques. Bull. WHO 33:681-685.

FA techniques have proved advantageous for rapid detection of enteropathogenic Escherichia coli. Previous attempts to adapt these procedures to the rapid identification of Salmonella typhi have been limited by the number of cross-reactions obtained when a polyvalent Salmonella or a S. typhi O conjugate was used to stain fecal smears. The results obtained using a purified Vi conjugate and a S. typhi O, Vi conjugate to screen fecal smears from a number of typhoid carriers are described in this report. Essentially the same number of carriers were found positive by FA as were found by conventional bacteriological methods, although each technique missed a number of positive specimens that were detected by the other. The FA technique has the advantage of being more rapid and economical to perform than cultural procedures.

5229

Thomason, B.M.; McWhorter, A.C.; Sanders, E. 1964. Rapid detection of typhoid carriers by means of fluorescent antibody techniques. Bacteriol. Proc. M67:56.

Fecal specimens from 130 typhoid carriers were examined by both fluorescent antibody and cultural techniques. Specimens from normal individuals were examined in the same manner. Two fluorescein-labeled antibody solutions were utilized, purified Vi antibody and an anti-

body against O and Vi antigens of S typhosa. Duplicate smears were made from fecal suspensions and stained with the two labeled reagents. Cultural procedures consisted of preparing 10 to 20 per cent suspensions of feces in either saline or broth MacConkey, SS, and bismuth sulfite agar plates were inoculated from the suspensions Selenite broth was used for enrichment of the fecal specimens. The FA results were obtained by one direct examination. Sixty-eight per cent were positive for S. typhosa by FA; 69.2 per cent were positive by culture. Six carriers that were negative by culture were positive by FA tests; cultural methods yielded positive results on seven carriers that were negative by FA. The FA results using purified Vi antibody gave more positives than those obtained with the O, Vi reagent. All control specimens were negative by both tests. The results indicated the feasibility of using FA procedures for the rapid detection of typhoid carriers. The combined use of FA and cultural methods permitted detection of more carriers than either method alone.

5230

Trabulsi, L.R.; Camargo, M.E. 1965 Comparative study between immunofluorescence and coproculture in the diagnosis of intestinal infections by enteropathogenic Escherichia coli. Rev. Inst Med. Trop. Sao Paulo 7:65-71.

The fluorescent antibody and the classical coproculture methods were compared in the research of enteropathogenic E. coli in stool specimens obtained from 147 children suffering from acute diarrhea. Both methods were agreed in 84.3 per cent of the cases. In 18 cases only immunofluorescence yielded positive results; coproculture alone was positive an another five cases. A greater sensitivity of the technique and three instances of false-positive reactions accounted for the higher number of positive results obtained with the fluorescent antibody method.

5231

Voino-Yasenetsky, M.V.; Khavkin, T.N. 1964. A study of intra-epithelial localization of dysentery causative agents with the aid of fluorescent antibodies. Zh. Mikrobiol. Epidemiol i Immunobiol. 41:98-100.

With the aid of specific fluorescent antibodies it was shown that Shigella is localized in the epithelial intestinal cells of monkeys suffering from dysentery. Shigella antigenic substances are well preserved by fixation of pieces of tissues with formalin and embedding in paraffin.

VII. LACTOBACILLACEAE

5232

Angelino, P.F.; Vacca, G. 1965. Immunofluorescence with streptococcic antigens in chronic rheumatic myocarditis. Minerva Med. 56:2349-2352. In Italian.

In 14 of 26 patients studied, gamma globulin on amputated auricles reacted with fluorochrome-labeled streptococcic antigens (O-streptolysi , streptokinase, and streptohyaluronidase). The immunofluorescent reacti ns were localized in the interstitial tissue and vessel walls and, in two cases, in the myofibers. These findings seem to support the assumption of an evolutionary rheumatic pathology of the organ.

5233

Ayoub, E.M.; Wannamaker, L.W. 1964. Identification of Group A streptococci: Evaluation of the use of the fluorescent antibody technique. J. Amer. Med. Ass. 187:908-913.

The fluorescent antibody technique for identifying Group A streptococci was compared with a standard precipitin method. When employed after isolation on blood agar, definite agreement between the two techniques was obtained in 174 of 203 instances. Evaluation of primary identification by the fluorescent antibody technique showed agreement in 189 of 205 instances, but analysis of the 33 specimen pairs in which Group A streptococci were identified by one or both techniques revealed agreement in only 17 instances. Primary isolation on a blood agar plate, followed by group identification by one of several available techniques, has the advantages of an easy method of screening out negative specimens and of providing quantitative information on positive specimens. These advantages seem to outweigh those of the more rapid but probably less definitive method of primary identification by the fluorescent antibody technique.

5234

Biegeleisen, J.Z., Jr.; Mitchell, M.S.; Marcus, B.B.; Rhoden, D.L.; Blumberg, R.W. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: I. Clinical evaluation of conjugates on smears prepared directly from cerebrospinal fluid sediments. J. Lab. Clin. Med. 65:976-989.

Techniques were described for the cultivation and immunofluorescent identification of Hemophilus influenzae, Diplococcus pneumoniae, Neisseria meningitidis, and eight less common pathogens in specimens of cerebrospinal fluid from 100 patients with bacterial meningitis. A comparison of the results obtained by conventional methods an by

immunofluorescent staining indicated that the latter method was fully as sensitive as the former and was more accurate in treated cases. Some of the dangers involved in the use of the Gram stain of the sediment as a tool for presumptive diagnosis were discussed, as were shortcomings of fluorescent antibody staining, particularly in infections caused by uncommon gram-negative organisms. The immunofluorescent staining technique was recommended for the rapid screening of spinal fluid specimens, as well as of cultural isolates.

5235

Chung, K.L.; Hawirko, R.Z.; Isaac, P.K. 1964 Cell wall replication: II. Cell wall growth and cross wall formation of Escherichia coli and Streptococcus faecalis. Can. J. Microbiol. 10:473-482.

Cell wall replication in E. coli and S. faecalis was studied by differential labeling of living cells with FA and non-fluorescent antibody. In E. coli the initial step in cell division was the formation of a cross wall at the cell equator, followed by the appearance of new cell wall on either side of the cross wall. The process was repeated in sequence at subsequent sites in the polar, the subcentral, and the subpolar areas. Constriction occurred at random so that the divided parent cells were composed of several daughter cells. A polar type of unidirectional cell wall growth and elongation was also observed in E. coli. It was initiated by the synthesis of a ring of new cell wall material around the polar tip. A second ring was then formed at the subpolar area during the rapid enlargement of the first ring in a single direction. Evidence shows that cell wall synthesis is independent of cell division and that in E. coli, it is initiated at multiple but specific sites within the cell and not by diffuse intercalation of old and new walls. Contrary to the synthesis of cell wall at multiple sites in E. coli, S. faecalis replicated new cell wall at only one site per coccus. The new wall segment was initiated and enlarged at the coccal equator, and was followed by the formation of a cross wall, centripetal growth and constriction to separate the daughter cells.

5236

Cole, R.M. 1964. Cell wall replication in Salmonella typhosa. Science 143:820-822.

Changes in the fluorescence of the cell wall of Salmonella typhosa were studied during growth after direct labeling with fluorescein-conjugated homologous or anti-O globulins. Fluorescence decreased evenly with culture growth and cell division, but the addition of chloramphenicol resulted in large, nondividing cells that showed increasing interruption of fluorescence of the wall marker. The process thus differs from the equatorial origin and discrete hemispherical addition of new wall previously described in Streptococcus pyogenes. These findings, in addition to demonstrating the formation of new wall

in the presence of chloramphenicol, appear consistent only with the concept that wall replication in the salmonellas occurs by diffuse intercalation of new materials among old.

5237

Cole, R.M. 1965. Symposium on the fine structure and replication of bacteria and their parts: III. Bacterial cell wall replication followed by immunofluorescence. Bacteriol. Rev. 29:326-344.

This is an interpretive and critical report. The author urges further application of FA to the study of surface-antigen replication in walled microorganisms. Confirmation or denial of controversial points in this study area will follow only from such further study. FA has clear advantages over any other method for cell wall study. The chief advantage is the ability to apply a specific label to the wall of a living cell

5238

Danilova, T.A.; Korn, T Ya. 1964. The possibility of elimination of cross-reactions between streptococci of various groups and staphylococci in applying the fluorescent antibody method. Zh. Mikrobiol. Epidemiol. i Immunobiol. 41:13-16.

In the direct method of fluorescent antibodies the fluorescent serum to Group A Streptococcus stained not only strains of the homologous group, but also cultures of C and G groups and some Staphylococcus strains. The sorption of a labeled serum with the live Streptococcus Group C culture eliminated the specific staining of strains of the Groups C and G; however, the sorbed serum retained its capacity to stain Staphylococcus. Treatment of fixed smears in a trypsin solution has made it possible to eliminate the Staphylococcus staining without disturbing the specific fluorescence of the Group A Streptococcus.

5239

Domingue, G.J.; Pierce, W.A., Jr. 1965. Effect of partially purified streptococcal M protein on the in vitro phagocytosis of Streptococcus pyogenes. J. Bacteriol. 89:583-588.

M protein from Streptococcus pyogenes Type 12 was adsorbed onto cells of a glossy mutant of this strain. Phagocytosis experiments were performed in vitro with rabbit peritoneal polymorphonuclear leukocytes. Direct microscopic counts suggest M protein-treated cells were more resistant to phagocytosis than were untreated cells. With the Cohn and Morse technique for evaluating quantitatively the fate of bacteria during phagocytosis, there was observed a more rapid drop in total and extracellular counts of treated, as compared with untreated, cells.

Experiments with radioactively labeled streptococci showed that M-treated cells were more readily destroyed within leukocytes than were nontreated glossy streptococci, which suggested a reason for the scarcity of treated streptococci in direct microscopic counts.

5240

Eisen, A.H.; Eidinger, D.; Rose, B.; Richter, M. 1964. Prolonged exposure to nephritogenic beta-hemolytic streptococcus in intraperitoneal diffusion chambers. Proc. Soc. Exp. Biol. Med. 115:367-369.

Diffusion chambers containing beta-hemolytic streptococci, nephritogenic in man, implanted in the peritoneal cavity of rats and rabbits for intervals ranging from 2 to 42 days did not produce definite glomerular lesions and, in particular, the lesion characteristic of glomerulonephritis. Tubular damage was noted in all animals. These results are somewhat at variance with previous reports of experiments performed with rats and mice, but similar to the results of others using mice. Indirect FA did not demonstrate kidney-specific gamma globulin in rats with intraperitoneal chambers.

5241

Estela, L.A.; Shuey, H.E. 1963. Comparison of fluorescent antibody, precipitin, and bacitracin disk methods in the identification of Group A streptococci. Amer. J. Clin. Pathol. 40:591-597.

During a 9-month period, a total of 6,574 throat swabs were cultured from patients at the Pediatric Clinic of Fitzsimons General Hospital. There were 2,138 isolates of beta hemolytic streptococci from these cultures. Of these strains of streptococci, 1,416 were identified as belonging to Group A by the precipitin method. All of the 1,416 Group A streptococci were also identified as such by the fluorescent antibody technique performed directly on smears of 2-hour broth cultures. An additional four cultures yielded positive results with both Group A-fluorescent and Group C-fluorescent antisera. They were subsequently identified as Group C by the precipitin method. There were no false-negative results by the fluorescent antibody method in this series. A comparison of the bacitracin disk method with the precipitin test demonstrated that the bacitracin method yielded 3.09 per cent false-positive test results and 7.06 per cent false-negative test results for an over-all error of 10.15 per cent.

5242

Francois, R.J. 1965. Beta-haemolytic streptococci and antistreptolysin-O titres in patients with rheumatoid arthritis and a matched control group. Ann. Rheum. Dis. 24:369-377.

Antistreptolysin-O titer was determined and a throat and a nose swab were taken monthly for 6 months in a group of patients with rheumatoid arthritis and a control group. At any moment of the study the prevalence of beta-hemolytic streptococci of Groups A, C, and G was significantly higher in the patients with rheumatoid arthritis than in the controls. This was due in part to a slightly higher acquisition rate, but mainly to reduced elimination rate. Equal numbers of raised antistreptolysin-O titers were found in both groups, but significantly more higher titers in the rheumatoid patients. This seems to agree with the lower elimination rate, as prolonged infection leads to higher antibody response

5243

Gill, F.A.; Cole, R.M. 1964. The behavior of immunofluorescent complexes on the surface of Group A streptococci after phagocytosis by macrophages. Federation Proc. 23:2445:509.

A method for studying the intracellular fate of antigen-antibody complexes on the surface of bacteria after phagocytosis by macrophages is described. M protein of living Group A Type 1 streptococci was labeled with specific fluorescein-tagged rabbit gamma globulin. The washed, labeled organisms were then exposed to unstimulated mouse peritoneal macrophages cultivated in vitro on glass cover slips. After phagocytosis, extracellular bacteria were washed off and the remaining macrophages containing phagocytized bacteria were observed throughout their life span by phase and ultraviolet microscopy. Two patterns of behavior were noted. An early, irregular loss of bacterial surface fluorescence was followed by diffuse, extrabacterial pooling fluorescence in macrophage vacuoles. Rare extracellular bacteria in the system served as controls, undergoing slow growth but no other change in surface fluorescence. These findings suggest that following partial degradation of an immunofluorescent complex on the surface of streptococci by mouse macrophages, part of the complex is retained within the cell in a diffuse form. Complete article.

5244

Gill, F.A.; Cole, R.M. 1965. The fate of a bacterial antigen, streptococcal M protein, after phagocytosis by macrophages. J. Immunol. 94:898-915.

A method is described for studying the postphagocytic fate of a bacterial surface antigen during degradation of the organism by macrophages. Observations of Group A, Type 1 streptococci after phago-

cytosis by unstimulated mouse peritoneal macrophages demonstrate cessation of bacterial growth, early partial loss of an immunofluorescent complex from the bacterial surface, and later extrabacterial pooling of part of the complex in macrophage vacuoles. Evidence is presented that suggests that the observed changes in fluorescence reflect the fate of M protein still bound to its fluorescent label. The observations indicate, therefore, that M protein is removed from the bacterial cell wall and pooled within the surrounding macrophage vacuole. The significance of a mechanism within the macrophage that modifies particulate antigen is discussed in relation to initiating antibody synthesis and explaining the action of agents that alter the immune response.

5245

Grover, A.A.; Spoerk, K.; Evans, A.S. 1965. Streptococcal infections: Observations from a public health laboratory. Amer. J. Public Health 55:1609-1621.

In a 3-year period approximately 100,000 throat cultures have been received, of which 22.7 per cent have been positive for beta hemolytic streptococcus. Of the hemolytic cultures, 90.6 per cent were identified as Group A by FA. A simple and effective agar transport medium has been developed and field-tested for shipment of streptococcal cultures through the mail. Age, seasonal, and familial aspects of streptococcal infections have been reviewed from data received in the laboratory. Questionnaires to physicians revealed acute rheumatic fever to be a continuing public health problem.

5246

Hahn, J.J.; Cole, R.M. 1963. Studies on the mechanism of the long-chain phenomenon of Group A streptococci. J. Exp. Med. 117:585-594.

The formation and destruction of long chains by growth of Group A streptococci in the presence of type-specific antibody have been studied by FA. Long-chain formation depends on the presence of free antibody during the growth of the bacteria. Destruction of long chains depends on the continued growth and division of the bacteria in the absence of free antibody. Univalent antibody fragments formed by proteolytic digestion of antibody globulin have the combining properties of untreated antibody but do not result in the production of long chains. A model involving end-to-end agglutination during growth of Group A streptococci explains the mechanism of production of long chains by growth of Group A streptococci in the presence of type-specific antibody.

5247

Hahn, J.J.; Cole, R.M. 1962. Streptococcal M antigen location and synthesis, studied by immunofluorescence. J. Exp. Med. 118:659-666.

Streptococcal M protein has been studied directly in the intact streptococcal cell by specific immunofluorescence. By this method, it can be seen to be concentrated in or on the cell wall, but cannot be detected in the capsule. The lack of type-specific immunofluorescence after trypsinization, and the inhibition of group-specifc immunofluorescence by unlabeled type-specific antibody, are observations most compatible with a location of the M antigen determinants on the cell surface superficial to the group antigen. M antigen is not resynthesized after trypsinization of living cells, but appears anew only at sites of new cell-wall growth. A limited amount of such growth, leading sometimes to detectable amounts of M in the gross, can take place in deficient m[illegible]d without detectable increases in optical density of the cell population.

5248

Hammond, B.F.; Rosan, B.; Williams, N.B. 1964. Encapsulated lactobacilli: II. Specific capsular reaction of Lactobacillus casei. J. Bacteriol. 88:1807-1808.

An indirect Quellung reaction was used to identify strains of L. casei. Capsular swelling was demonstrated. The procedure was useful for quantitation of capsular material, and it aided in study of decapsulation methods and factors in capsule production.

5249

Hosty, T.S.; Johnson, M.B.; Freeur, M.A.; Gaddy, R.E.; Hunter, F.R. 1964. Evaluation of the efficiency of four different types of swabs in the recovery of Group A streptococci. Health Lab. Sci. 1:163-169.

This report describes the preservation of Group A streptococci on four swab types used as carrying media. From each type container paired swabs were cultured at 4- and 72-hour periods after the patient's throat had been swabbed. A total of 2,802 paired specimens were examined and it was found that both Dacron and cotton swabs with silica gel gave satisfactory results. The best results on 72-hour cultures were obtained from the Dacron swabs with silica gel. The streak plate was the most efficient culture method. FA used for grouping decreased reporting time by one day. FA results confirmed results by other methods.

5250

Hovnanian, H.P.; Botan, E.A.; Brennan, T.A.; Graff, W.P. 1964. Quantitative rapid immunofluorescence microscopy: II. Studies on streptococci of Group A. Health Lab. Sci. 1:170-178.

Fine distinctions between degrees of immunofluorescence are not reliable when based upon visual impressions alone. This is an attempt to define a quantitative basis for brightness in FA preparations.

5251

Hsu, K.C.; Rifkind, R.A.; Zabriskie, J.B. 1964. Immunochemical studies with fluorescein and ferritin doubly labeled antibodies. J. Histochem. Cytochem. 12:7.

A technique is described for the double labeling of antibody with fluorescein and ferritin that makes it possible to employ the same conjugate for both fluorescence and electron microscopy. Studies with microorganisms and tissues demonstrate that the specificity of the antibody is not affected by the double labeling. Pneumococci, Types 2 and 18, were treated with homologous ferritin-conjugated antibody or fluorescein and ferritin-conjugated antibody and examined under the electron microscope; capsular swelling with penetration of ferritin granules through the capsular material up to the cell wall was seen. The same doubly labeled antisera used with fluorescence microscopy demonstrated specific 'staining' of the same organisms. Control experiments included blocking of the reaction with unlabeled antibody. Both the ferritin conjugates and the doubly labeled conjugates presented the same immunologic pattern of activities by Ouchterlony agar-gel diffusion and immunoelectrophoretic analysis. Complete article.

5252

Jablon, J.M.; Brust, B. 1963. Suppression of cross-reactions in fluorescent antibody identification of Group A streptococci. Bacteriol. Proc. M137:88.

Cross-reactions may occur when the fluorescent antibody technique is used for the rapid identification of Group A streptococci. In this study these reactions were suppressed in two ways. Unconjugated Group C streptococcal antiserum was added to fluorescein-conjugated Group A antiserum. With this technique no cross-reactions occurred in 1,145 throat cultures taken from school children with respiratory infections in Dade County, Fla., during the 1961-1962 school year. Of 271 hemolytic isolates studied by fluorescent antibody and conventional techniques, 177 were Group A and 94 were in Groups B, C, D, F, G, or nongroupable streptococci or staphylococci. The suppression of cross-reactions by this method probably depends on the ability of Group C antiserum to block the reaction of nonspecific fractions with fluorescein-conjugated

Group A antiserum. Cross-reactions were also eliminated or suppressed by treating suspensions of Groups C and G streptococci and staphylococci with trypsin (1:250) for 30 minutes before staining with fluorescein-conjugated Group A antiserum. Fluorescence of Group A streptococci remained unaffected by this treatment. Similar results were obtained with pepsin. Cross-reactions, therefore, may be due at least in part to nonspecific protein fractions on the surface of the cells.

5253

Jablon, J.M.; Brust, B ; Saslaw, M.S. 1965. Beta-hemolytic streptococci with Group A and Type II carbohydrate antigens. J. Bacteriol. 89:529-534.

Ten strains of beta-hemolytic streptococci with unusual somatic antigens were isolated from excised tonsils or throat cultures or both. Acid extracts of these strains reacted with commercial Group A and Group F antisera, but gave no reaction when tested with 35 type-specific Group A antisera. Serum cross-absorption and agar-gel diffusion studies established the identity of the reactive antigens as the specific carbohydrates of Group A and of Type II. The latter antigen has been found in many strains of Group F streptococci. The organisms do not possess the group-specific carbohydrate of Group F, and the reaction with the commercial Group F antiserum is due to the presence of Type II antibody in the antiserum. The organisms give a stronger reaction to fluorescein-conjugated Type II antiserum than to Group A. However, the organisms have only one group-specific carbohydrate, that of Group A, and, tentatively, should be classified as such.

5254

Jablon, J.M.; Saslaw, M.S.; Zinner, D.D.; Aran, A. 1965. Cellular antigens of human and animal strains of cariogenic streptococci. Bacteriol. Proc. M46:47.

Certain strains of streptococci have been used to produce dental caries in hamsters and rats. FITC conjugates were prepared with antisera against both the hamster and rat strains of streptococci. These conjugates were used to identify strains of streptococci isolated from human caries. Three strains of human caries streptococci were differentiated. One strain reacted with the anti-rat strain conjugate, one strain with the anti-hamster strain conjugate, and one human strain reacted with both conjugates. Precipitin studies using acid-extracted antigens confirmed the FA findings. The double-reacting human strain of streptococcus could be made specific by cross-absorption experiments. Immunoelectrophoresis demonstrated an antigen common to all strains. This antigen migrated toward the cathode and was probably responsible for the cross-reactions seen.

5255

Johnson, S., Streamer, C.W.; Williams, P.M. 1964. An evaluation of a streptococcal control program. Amer. J. Public Health 54:487-500.

An evaluation study of a streptococcal control program that used intramuscular prophylactic penicillin for household contacts of streptococcal infections has been described. Culture methods used to isolate Group A beta hemolytic streptococci were described, including the use of FA. The prophylaxis program resulted in fewer streptococcal infections and streptococcal sequelae in the households receiving the prophylactic treatment. This effect lasted two months beyond the time when the penicillin blood levels had fallen to zero. Suggestions for the use of this information are given.

5256

Kagan, G.Y.; Ershov, F.I.; Koptelova, E.I.; Fedorova, G.I. 1965. Antigenic properties of hemolytic streptococci L-forms. Biul. Eksp. Biol. Med. 59:78-81. In Russian.

Employment of the direct fluorescent antibody method demonstrated a different localization of the antigens in bacterial L-forms of streptococci and parent. Superficially located antigenic components of the cell wall were detected in the former. In the latter, because of the biosynthesis block of the cellular walls, antigenic components located in the cytoplastic membrane were revealed.

5257

Kantor, F.S. 1964. Fate of streptococcal M protein after exposure to plasmin and human leukocytes. Yale J. Biol. Med. 36:259-267.

Partially purified Type 1 streptococcal M protein was destroyed by exposure to streptokinase-activated plasminogen or to viable human leukocyte suspensions. After phagocytosis of intact streptococci by human polymorphonuclear leukocytes and a short intracellular residence, the bacteria fail to stain with specific fluorescent anti-M conjugates.

5258

Kantor, F.S. 1965. Fibrinogen precipitation by streptococcal M protein: II. Renal lesions induced by intravenous injection of M protein into mice and rats. J. Exp. Med. 121:861-872.

Intravenous injection of Type 1 streptococcal M protein into mice and rats produced lesions confined to renal glomeruli. Thrombi of eosinophilic amorphous material, seen to occlude glomerular capillaries, were shown to contain M protein and fibrinogen. Gradual regression

of the morphological lesions was observed during the 3 weeks following injection. Initial abnormal proteinuria and azotemia returned to control levels by the end of the 1st week; a second rise in urinary protein excretion and urea retention was demonstrated in some rats coincident with appearance of anti-M antibodies. The mechanism of renal localization of streptococcal M protein by means of a complex with fibrinogen was suggested, which may comprise an initial phase in the pathogenesis of acute poststreptococcal glomerulonephritis.

5259

Kaplan, M.H. 1963. Immunologic relation of streptococcal and tissue antigens: I. Properties of an antigen in certain strains of Group A streptococci exhibiting an immunologic cross-reaction with human heart tissue. J. Immunol. 90:595-606.

Antisera prepared in rabbits against cell walls or M protein preparations of a Group A Type 5 strain of Streptococcus have been found reactive by immunofluorescence and complement fixation with human heart tissue. The reactant in heart tissue was localized to cardiac myofibers of all heart specimens tested and in smooth-muscle elements of vessel walls and endocardium of a proportion of heart specimens tested. Rheumatic and nonrheumatic hearts showed comparable reactivity. The reactant was also identified in human skeletal muscle and in heart and skeletal muscle of the rabbit. In the Streptococcus, the cross-reactive antigen was demonstrated in cell walls and in acid extracts of cell walls by immunofluorescent absorption tests. Following absorption of antisera with cell walls, antibody bound to cell walls could be eluted at pH 3.0 and shown to possess serologic reaction with heart. The immunologic relationship between streptococcal cell wall antigen and myofibers and smooth muscle of vessel walls is consistent with the hypothesis that bound gamma globulin observed in rheumatic hearts in these sites is derived from immune bodies.

5260

Kaplan, M.H. 1965. Induction of autoimmunity to heart in rheumatic fever by streptococcal antigens cross-reactive with heart. Federation Proc. 24:109-112.

Within the limits of current information, it is not apparent how the immunologic relationship of streptococcal cells and human tissue described can be related to the many varied clinical features of rheumatic fever. Induced autosensitization to myofibers and vessel walls may have significance for such manifestations as myocarditis, arteritis, erythema marginatum, and possibly even Aschoff lesions in the con text of the above proposition, but a relationship to other rheumatic manifestations, including valvular lesions, subcutaneous nodules, chorea, pneumonitis, or encephalitis is not obvious. Possibly other cross-

reactive antigens of the Streptococcus, that have recently been uncovered, and to which autoimmune mechanisms might be induced, may offer insight into these questions.

5261

Kaplan, M.H. 1965. Multiple nature of cross-reactive relationship between Group A streptococci and heart tissue. Federation Proc. 24:275:176.

The immunologic relationship of Group A streptococcal cells and mammalian heart tissue has been demonstrated by immunofluorescence of anti-streptococcal serum, precipitin reaction of anti-heart serum, and precipitin-absorption of human post-streptococcal serum with heart tissue. At least three different antigenic determinants in streptococcal preparations have been implicated in this cross-reactive relationship. Antigen 1 is present in cell walls and to a lesser extent in cell membranes of certain strains only; it is trypsin-sensitive, and has cathodal mobility. Antigen 3 is present in cell walls or membranes of all Group A strains, is trypsin-sensitive, and has anodal mobility. In the case of antigen 2, it has been possible to demonstrate that the precipitin line given with rabbit or chicken anti-streptococcal sera shows fusion with the precipitin line given with anti-heart serum. Complete article.

5262

Kaplan, M.H.; Bolande, R.; Rakita, L.; Blair, J. 1964. Presence of bound immunoglobulins and complement in the myocardium in acute rheumatic fever: Association with cardiac failure. New Engl. J. Med. 271:637-645

Clinical, electrocardiographic, pathological, and immunopathological findings in five children who died of acute rheumatic fever in cardiac failure are presented. In all five cases intensive deposits of gamma globulin and the beta-1A component of human complement were observed throughout the myocardial segments available for study. Variable staining for gamma-1A (beta-2A) and gamma-1M (beta-2M) globulins was also observed. These deposits were localized primarily in sarcolemma of cardiac myofibers and also in smooth muscle of vessel walls and endocardium, and in interstitial connective tissue. The possible relation of these findings to the concept of streptococcal-induced autoimmunity to heart is considered. An autoimmune mechanism of immune injury to myocardium may participate in the pathogenesis of rheumatic myocarditis.

5263

Kaplan, M.H.; Svec, K.H. 1964. Immunologic relation of streptococcal and tissue antigens: III. Presence in human sera of streptococcal antibody cross-reactive with heart tissue; association with streptococcal infection, rheumatic fever, and glomerulonephritis. J. Exp. Med. 119:651-666.

Sera from some patients with recent streptococcal infection or nonsuppurative sequelae exhibit a precipitin reaction with a partially purified streptococcal antigen that is immunologically related to human heart tissue. This precipitin could be absorbed from sera with normal human heart tissue homogenates but not with homogenates of other organs. Cross-reaction by heart absorption was dependent both upon the serologic properties of individual sera and the nature or purity of the streptococcal product employed as test antigen. Antigen was localized to cell walls and to a lesser extent to cell membranes of these strains. Reactive sera showed diminution or loss of serological activity following heat inactivation at 56 C or after prolonged storage at 4 C. Sera containing cross-reactive antibody exhibited FA reaction with sarcolemma of cardiac myofibers, which was inhibited by streptococcal cross-reactive antigen. Antibody to streptococcal cross-reactive antigen was observed in 24 per cent of patients with recent streptococcal infection and in the majority of patients with acute rheumatic fever, rheumatic heart disease, or acute glomerulonephritis. Induction of cross-reactive autoantibody to heart in certain individuals is associated with streptococcal infection.

5264

Kaplan, M.H.; Svec, K.H.; Kushner, I.; Arana-Sialer, J.; Suchy, M.L. 1963. Evidence in Group A streptococcal cells of cross-reactive antigens related to mammalian heart. Federation Proc. 22:340:217.

A cross-reaction between rabbit antiserum to Group A cell walls and mammalian heart has been demonstrated by complement fixation and immunofluorescence. The reverse cross-reaction between antisera to human or rabbit heart and streptococcal cells and cell walls may be shown by agar diffusion and immunoelectrophoresis. A single line of precipitation is observed with solubilized cell wall and three distinct lines with acid extracts or sonicates of whole cells. One of these latter antigens is related to cell wall, a second was present also in culture filtrates, and the third has been detected only in cell extracts. Absorption tests have confirmed the separate specificities of these antigens. Analysis of sera from patients with acute rheumatic fever frequently shows presence of antibody, with specificity directed to cross-reactive antigen of cell walls and heart. These data give direct support to the concept of the induction by streptococcal infection of autoimmunity to heart in rheumatic fever. Complete article.

5265

Karakawa, W.W., Borman, E.K ; McFarland C R 1964. Typing of Group A streptococci by immunofluorescence. I. Preparation and properties of Type I fluorescein-labeled antibody. J. Bacteriol. 87:1377-1382.

Unabsorbed fluorescein-labeled globulins derived from rabbits immunized with acid-extracted M protein of Type I streptococci, plus adjuvant, were found to have high fluorescent antibody staining titers and to be considerably more type-specific than were similar preparations derived from whole-cell immunization. Appropriate absorption rendered the anti-M reagent entirely type-specific without appreciable loss of titer; whole-cell reagent was appreciably weakened in FA titer by comparable absorption. Type specificity was confirmed by parallel bactericidal, long-chain, and precipitin studies. Removal of reactivity by absorption with homologous M protein was complete, confirming that the FA reaction was truly a manifestation of an M anti-M protein system. The data indicate that the development of FA reagents specific for the streptococcal types is feasible.

5266

Karakawa, W.W., Krause, R.M.; Borman, E.K. 1965. Immunochemical aspects of the cross-reactivity between Groups A and C streptococci as detected by the fluorescent antibody technique. J. Immunol. 94:282-288.

The cross-reactivity as detected by the fluorescent antibody method between Group A sera and Group C streptococci is dependent upon a rhamnose moiety that is shared by both Groups A and C carbohydrates and that, under certain circumstances may be serologically reactive. The cross-reactivity is inhibited by A-variant carbohydrate, and by a rhamnose disaccharide isolated from A-variant carbohydrate by the rhamnosidase of McCarty. Cross-reactivity between Groups A and C carbohydrates is dependent, in part, upon the fact that this rhamnose disaccharide is a structural feature common to both antigens.

5267

Karakawa, W.W.; Mageau, R.P., Borman, E.K. 1964. Immunochemical observations on the FA cross-reactivity between Groups A and C streptococci. Bacteriol. Proc M119.66.

Immunochemical studies support the hypothesis that the group carbohydrates of A and C streptococci contain a similar rhamnose moiety that, under certain circumstances, may be antigenic. Thus, carbohydrates of A and C streptococci may show a minor precipitin cross-reactivity with heterologous antiserum. Evidence presented here supports the view that this common rhamnose moiety is one of the factors responsible for the frequent and troublesome cross-reactivity between A and C streptococci observed by the fluorescent antibody technique

of identification. Trypsinized Group C cells treated with a rhamnosidase (McCarty's V enzyme) to remove the antigenically cross-reactive portion of the rhamnose moiety exhibited no significant staining reaction with a 1:10 dilution of Group A conjugate, but the control C cells gave strong fluorescence with a dilution of 1:160. A similar finding was noted with V enzyme-treated Group A cells and C conjugate. When the one-step inhibition method was employed with soluble A-variant carbohydrate, the heterologous cross-reaction was inhibited between Group C cells and Group A conjugate. Rhamnose oligosaccharide, obtained with the action of V enzyme on Group A-variant carbohydrate, strongly inhibited the heterologous staining reactions but did not appreciably inhibit the homologous staining reactions. These findings suggest an immunochemical basis for the FA cross-reactivity frequently observed between Groups A and C streptococci.

5268

Karakawa, W.W.; McFarland, C.R.; Borman, E.K. 1963. Typing of Group A streptococci by the FA method. Bacteriol. Proc. M136:88.

After sonic disruption and differential centrifugation of Type 1 streptococcal cells, M protein was extracted from the cell walls. This type-specific substance was used with mineral oil adjuvants to immunize rabbits. Resulting antisera after sorption with heterologous types as indicated by reaction patterns were subjected to bactericidal, long-chain, and precipitin tests for comparison with fluorescent antibody studies of fluorescein-labeled conjugates prepared from them. Although purified M protein had been reported to evoke only weak antibody response as judged by conventional detection methods, high FA titers were obtained readily. Sorbed Type 1 antisera and conjugates prepared from them were shown to be type-specific in tests against nine homologous and 42 heterologous types. Homologous FA titers of 1:1280 were obtained with heterologous staining inappreciable in 1:40 dilution. Sorption of these conjugates with purified homologous M protein removed all reactivity, confirming direct involvement of anti-M antibody in the FA reaction. The feasibility of obtaining specific conjugates for FA typing of Group A streptococci has been demonstrated.

5269

Karakawa, W.W.; Rotta, J.; Krause, R.M. 1965. Detection of M protein in colonies of streptococcal L forms by immunofluorescence. Proc. Soc. Exp. Biol. Med. 118:198-201.

M protein was detected in growing L-form colonies of Group A streptococci by an immunofluorescent technique that employs type-specific fluorescein-labeled antibody. The type-specificity of the FA-staining reactions exhibited by the L forms was confirmed by parallel precipitin studies. These data suggest the feasibility of identifying the L-form colonies of Group A streptococci by detecting the M protein with the FA method.

5270

Lazarus, J.M.; Sellers, D.P.; Marine, W.M 1965 Meningitis due to the Group B beta-hemolytic streptococcus. New Eng. J. Med. 272:146-147.

A case of meningitis due to Group B Streptococcus is reported. The epidemiology of contacts is included. FA was used to identify the organism in spinal fluid.

5271

Levine, S. 1963. Cerebral white matter: Selective spread of pneumococcal polysaccharides. Science 139:605-606.

Pneumococcal polysaccharides were implanted in rat brain and their distribution was studied by FA. The polysaccharides spread selectively in white matter, frequently extending from anterior to posterior poles. Selective localization of experimental and natural leukoencephalopathies may be related to an innate property of white matter that permits or facilitates spread of noxious agents.

5272

Lindberg, L.H.; Raffel, S.; Vosti, K.L. 1964. Streptococcal glomerulonephritis in rats. Federation Proc. 23:2446:509.

Experimental streptococcal glomerulonephritis was induced in rats by intraperitoneally implanting diffusion chambers containing broth cultures of hemolytic streptococci. The rats were placed in individual metabolism cages and urine was collected for protein determinations. Anti-streptolysin O, anti-kidney, and anti-M protein antibody titers were determined on individual sera obtained prior to insertion of the capsule and at the termination of the experiment. Sixty days after implantation of the capsules the rats were sacrificed, capsules were removed, and the chamber contents cultured. The kidneys were perfused with isotonic saline solution until grossly cleared of blood prior to removal, and portions were quick-frozen at -70 C. Sections were stained with fluorescein-labeled antisera against gamma globulin, appropriate streptococcal antigens, and complement. This model readily demonstrated the presence of tissue-bound gamma globulin, streptococcal antibody and antigen, and the fixation of complement on the glomerular basement membranes of kidneys from rats with proteinuria and elevated anti-M protein antibody titers. These observations support the concept that the pathogenesis of human post-streptococcal glomerulonephritis has an immunologic origin Complete article.

5273

Mardiney, M.R.; Shuler, S.E.; Feldman, J.D. 1965. Immunology and morphology of human post-streptococcal glomerulonephritis. Federation Proc. 24:3056:682.

Kidneys of a 10-year-old boy were biopsied 7 days and 7 months after the onset of post-streptococcal glomerulonephritis, PSGn. The acute lesion was characterized by large glomeruli in which capillaries were obstructed by swollen endothelial and mesangial cells, and by unobtrusive focal alterations of the basement membranes, BM. By fluorescence microscopy, gamma-2 globulin was distributed in or on the BM; beta-1C was similarly distributed, but in a segmental fashion; a few small foci of antigen, Group A, beta hemolytic streptococci, acid extract of walls, were deposited apparently randomly. After complete clinical recovery, the second biopsy showed patency of glomerular capillaries, an increased number of mesangial cells, some residual swollen endothelium, and focal alterations of the BM. By fluorescence microscopy, gamma-2 globulin was still distributed in or on the BM but with diminished intensity; beta-1C was found mostly in mesangial zones; antigen was not detected. In many details human PSGn was similar to serum sickness nephritis in the rabbit. However, there was still no firm evidence that complexes of antigen antibody and complement were responsible for the human lesion. Complete article.

5274

Markowitz, A.S.; Lange, C.F., Jr. 1965. Streptococcal-related glomerulonephritis: I. Isolation, immunochemistry, and comparative chemistry of soluble fractions from Type 12 nephritogenic streptococci and human glomeruli. J. Immunol. 92:565-575.

Soluble fractions obtained from pooled human glomeruli and the cell membrane of nephritogenic streptococci were shown to be immunologically cross-reactive. One of the components present in the glomerular extract was shown to be unrelated to the streptococcal cross-reactive material but related to a component present on all human red cells tested. Chemical and physical analysis of soluble fractions obtained from isolated human glomeruli and streptococcal cell membranes indicated that they are low-molecular-weight glycoproteins. The glomerular extract has approximately 78.9 per cent protein and 13 to 15 per cent carbohydrate; the streptococcal extract has 80 per cent protein and 7 per cent carbohydrate. The glomerular fraction contains 1.5 to 2 per cent sialic acid but no appreciable phosphorus; the streptococcal fraction has no sialic acid but 3.7 per cent phosphorus. FA was used to localize glomerular antigen at the basement membrane.

5275

Mayorova, G.F.; Korn, M.Ya. 1963. A study of the antipertussis fluorescent serum specificity. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:9:42-48. In Russian.

In studies on the specificity of fluorescent serological examination of H. pertussis with the aid of antipertussis fluorescent serum, it was impossible to detect H. pertussis with the aid of heterologous sera. Antipertussis fluorescent serum stained some species of microorganisms nonspecifically: P. pestis, E. coli, Bacillus brucellosis, P. tularensis, and others. Staphylococci and streptococci were stained specifically. The method of fixation also influenced the results of the investigation. Rough fixation by flame provoked microbial staining of H. pertussis with heterologous fluorescent sera. Identification of H. pertussis in practical conditions by FA at present is fraught with some difficulties, since the Staphylococcus and Streptococcus may be stained simultaneously. It was impossible to distinguish H. parapertussis from H. pertussis by the indirect method.

5276

Mitchell, M.S.; Biegeleisen, J.Z., Jr. 1965. The effect of penicillin on immunofluorescent staining of Diplococcus pneumoniae, Neisseria meningitidis, and Hemophilus influenzae in cerebrospinal fluid in vitro. J. Lab. Clin. Med. 66:53-63.

Several strains of Diplococcus pneumoniae, Neisseria meningitidis, and Hemophilus influenzae were exposed to a range of concentrations of penicillin G in pooled human cerebrospinal fluid at 37 C in vitro. Pneumococci were killed and lysed at bactericidal concentrations of penicillin, but a large percentage of these cells seemed to be unchanged in appearance from the untreated state on immunofluorescent staining. At subinhibitory concentrations, organisms of this species may have been slightly enlarged, but capsular staining was again totally unaffected. Neisseria meningitidis, Groups A and C, became enlarged and bloated, and lost most of their capsular staining at bactericidal concentrations of penicillin, but the only difference observed at subinhibitory concentrations was the enlargement of some cells. Neisseria meningitidis, Group B, seemed unchanged in size and staining characteristics at all concentrations of the antibiotic. Hemophilus influenzae, Type b, organisms lost all capsular staining, but were not disrupted at bactericidal concentrations of penicillin. At subinhibitory concentrations, generally little or no change in morphology and integrity of capsular antigen was observed, although 1 to 2 per cent of the cells were long forms with somewhat shaggy capsules.

5277

Mitchell, M.S.; Marcus, B.B.; Biegeleisen, J.Z., Jr. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: II. Growth, viability, and immunofluorescent staining of Hemophilus influenzae, Neisseria meningitidis, and Diplococcus pneumoniae in cerebrospinal fluid. J. Lab. Clin. Med. 65:990-1003.

H. influenzae, N. meningitidis, and D. pneumoniae were studied. H. influenzae, Type b, could be isolated in 7 to 9 days at 4 or 25 C, and intense FA staining of capsules was observed for as long as 2 weeks. At 37 C the organism could not be cultured beyond 2 to 3 days. The capsules appeared ragged, as judged by the FA staining reaction, and by 18 to 24 hours, fluorescence was considerably diminished. Ability to be stained was lost in most cases after 48 hours of incubation at 37 C. N. meningitidis, Group B, could be cultured from specimens of CSF for 7 to 8 days when stored at 4 or 25 C and an average of 15 days at 37 C. FA staining seemed equally bright indefinitely at all storage temperatures, although after 5 to 7 days the staining may well have been somatic rather than capsular. Pneumococci were cultured from CSF specimens for an average of 17 days at 4 or 37 C and an average of 21 days at 25 C. FA staining remained. In normal cerebrospinal fluid seeded with cultures of the above three species of organisms in vitro, immunofluorescent staining characteristics were very similar to those in clinical specimens, but viability was, in almost all instances, considerably shorter. All cultures tested multiplied consistently with the exception of meningococci of Groups A and C, which only rarely grew in CSF.

5278

Moody, M.D.; Lambert, M.A.; Baker, C. 1965. Identification of Group B streptococci by fluorescent antibody and precipitin tests. Bacteriol. Proc. M126:60.

The involvement of Group B streptococci in human infections, particularly in cases of neonatal sepsis and abortion, appears to be recognized more commonly. In the present study, 58 strains isolated from various clinical materials of human infections were characterized by comprehensive cultural, biochemical, and precipitin tests and found to be a highly homogeneous collection. Fluorescent antibody globulin prepared from grouping antisera was used to stain smears of each of the strains that had been cultured under a variety of conditions. Consistently brilliant staining reactions were demonstrated with six strains; 49 strains stained with variable intensity, and three failed to stain. FA reagents were made from antisera prepared with antigens of the three latter strains. Different patterns of staining reactions among the 58 strains were demonstrated with these reagents. However, it was possible to prepare a pool of five FA reagents with which brilliant

fluorescence reactions could be demonstrated for all 58 Group B strains. The results of extensive precipitin, absorption, inhibition, and FA tests with homologous and heterologous bacteria showed that type-specific variations among Group B strains were demonstrable by immunofluorescence that did not necessarily correspond with those demonstrable by precipitin tests.

5279

Moody, M.D.; Siegel, A.C.; Pittman, B.; Winter, C.C. 1963. Fluorescent antibody identification of Group A streptococci from throat swabs. Amer. J. Public Health 53:1083-1092.

The use of fluorescent antibody and cultural precipitin grouping procedures for identifying Group A streptococci from throat swabs was evaluated with paired throat swabs. The sensitivity of the fluorescent antibody technique was equivalent to or greater than that of cultural precipitin technique. The importance of a number of conditions and factors for successful use of fluorescent antibody tests on a routine basis is emphasized.

5280

Myerburg, R.J.; Jablon, J.M.; Mazzarella, J.A.; Saslaw, M.S. 1964. Evaluation of FA and conventional techniques for identifying Group A beta-hemolytic streptococci. Public Health Rep. 79:510-514.

To determine the most rapid and accurate method of identifying Group A beta-hemolytic streptococci, several modifications of the fluorescent antibody technique were compared with each other and with various conventional techniques. A total of 1,115 throat cultures were studied. Five fluorescent antibody technical modifications were evaluated. Best results were obtained when throat swabs were inoculated in Todd-Hewitt broth and incubated for 3 hours, followed by preparation of pour plates, which were then incubated for an additional 18 hours at 37 C. Hemolytic colonies were picked from the pour plates after incubation and placed in broth for 3 hours' further incubation. Fluorescent slides were prepared from broth suspensions. Of 956 cultures surveyed by this technique, all strains of Group A beta-hemolytic streptococci were identified. The other four fluorescent modifications met with varying degrees of success but none approached the accuracy of the above technique. The modified fluorescent antibody technique recommended provides a rapid and accurate method for the identification of Group A beta-hemolytic streptococci.

5281

Parish, W.E.; Rhodes, E.L. 1965. Investigation of nodular vasculitis by means of the fluorescent antibody technique. Brit. J. Dermatol. 77:529.

Biopsy specimens of nine cases of nodular vasculitis were examined for gamma globulin using FA. Gamma globulin was found in and around the vessel walls of two patients only, one of whom had developed her leg lesions 2 weeks after quinsy. The nine cases were then examined for streptococcal complexes with rabbit anti-streptococcal serum of both Group A and Group D streptococci, tracing any fixation of this serum with a fluorescent labeled goat anti-rabbit serum. Fluorescence with the streptococcal Group A antigen was found only in the section of the patient who had had quinsy and then in the areas where gamma globulin had been found previously. Sections were also examined for tubercle antigen using rabbit antihuman tubercle serum and tracing fixation with fluorescent goat anti-rabbit serum. Specific fluorescence indicating tubercle antigen was found in the sections from two patients, both of whom had active tuberculous glands in their necks. The lesions on the legs in all nine patients looked very similar.

5282

Petran, E.I. 1964. Comparison of the fluorescent antibody and the bacitracin disk methods for the identification of Group A streptococci. Tech. Bull. Regist. Med. Technol. 34:8-10.

The presumptive identification of Group A streptococci by means of the bacitracin 0.02 unit disk method is a satisfactory and practical method for the routine diagnostic laboratory. There was agreement between this method and the fluorescent antibody technique in 98 per cent of the 439 cultures of hemolytic streptococci tested. The disagreement resulted chiefly from over-sensitivity, inasmuch as strains of other groups occasionally yield zones that are comparable with those produced by Group A streptococci. By placing neomycin and Taxo A on the original plates, the presumptive grouping can be reported when the blood plate is examined for approximately two-thirds of the cultures that include hemolytic streptococci.

5283

Preston, J.A. 1964. The rapid identification of Group A streptococci in throat swabs: A comparative study of two methods. Michigan Med. 63:704-706.

A project was undertaken to make bacteriologic diagnoses in cases of sore throat more appealing to the clinician. Both conventional culture technique and FA were used in the study. A total of 627 throat swabs were submitted. Results were reported to the submitting physician

in from 6 to 18 hours after submission. Both methods proved to be acceptable. The project did in fact stimulate interest in making etiologic diagnosis in cases of sore throat.

5284

Rauch, H.C.; Rantz, L.A. 1963. Immunofluorescent identification of Group A streptococci in direct throat smears. J. Lab. Clin. Med. 61:529-536.

Swabs from 767 patients with nonpneumonic respiratory infection were examined by the usual bacteriologic methods and by an indirect immunofluorescent technique without enrichment. Group A streptococci were recovered from 121 swabs by culture methods. The direct smears were positive in 53 per cent when the number of hemolytic streptococci was small, and in 93 per cent when these organisms were present in the large numbers that were indicative of active infection. Fluorescent cocci were discovered in 24 smears prepared from swabs from which Group A streptococci were not isolated. Cross-reactions between strains of streptococci of Groups C and G or staphylococci were not an important problem. The method has value as a tool for the diagnosis of active hemolytic streptococcal respiratory infection but does not yet offer sufficient advantages so that it should supersede cultural study of the nasopharyngeal flora in clinical practice. Other techniques, including preliminary enrichment, may be more satisfactory if a maximum yield of Group A streptococci from nasopharyngeal materials is required.

5285

Redys, J.J.; Parzick, A.B.; Borman, E.K. 1963. Detection of Group A streptococci in throat cultures by immunofluorescence. Public Health Rep. 78:222-226.

The routine handling of large numbers of throat cultures by the Connecticut State Department of Health laboratories has been simplified by the development and application of a fluorescent antibody reagent highly specific for Group A streptococci and a modification of the laboratories' original culture procedures. Use of the FA reagent in a one-step inhibition technique has resulted in bright and distinct FA staining of Group A streptococci without interference from other streptococcal groups of staphylococci, when present. Sensitivity and specificity of the reagent have been routinely evidenced.

5286

Roberts, C.E., Jr.; Sherris, J.C. 1965. Direct fluorescent antibody staining of Group A streptococci in clinical material. Bacteriol. Proc. M126:60.

Fluorescent antibody methods utilizing a period of preincubation have been employed for identifying Group A streptococci in clinical material since 1958, but direct staining of clinical material has not been uniformly successful. This study is concerned with the presence in human sera of a substance that blocks fluorescent antibody staining of Group A streptococci. Of sera from hospitalized patients, 59 per cent produced this effect when incubated with the organisms. Development of blockade by hyaluronic acid was ruled out, but an adherent coating of globulin was demonstrable and apparently responsible for the inhibition of staining. This globulin apparently was not antibody against Group A polysaccharide. The organisms could readily be freed from the blocking substance without alteration of morphology or avidity for specific antibody by the use of strong alkali. Several clinical specimens from persons with Group A streptococcal infections that failed to show direct staining with Group A antibody conjugate showed good staining after treatment with 1 N sodium hydroxide. These findings may indicate a reliable way of rapidly detecting Group A streptococci directly in clinical material.

5287

Rotta, J.; Karakawa, W.W.; Krause, R.M. 1965. Isolation of L forms from Group A streptococci exposed to bacitracin. J. Bacteriol. 89: 1581-1585.

L forms were obtained from Group A streptococci by exposure to bacitracin on gradient plates, although novobiocin was ineffective in this respect. Subcultures of these L forms had morphological and bacteriological properties similar to those obtained with penicillin. M protein was detected in L-form colony smears by immunofluorescent staining with type-specific conjugate. The L forms were not stained with group-specific conjugate. Parallel precipitin tests performed with extracts from a heavy growth of L forms on agar confirmed these findings. Thus, the L forms obtained with bacitracin continue to produce M protein but are devoid of the group-specific carbohydrate that is a major component of cell-wall structure.

5288

Saslaw, M.S.; Jablon, J.M.; Mazzarella, J.A. 1963. Prevention of initial attacks of rheumatic fever. Public Health Rep. 78:207-221.

In Dade County, Florida, the fluorescent antibody technique was investigated as a means of rapidly identifying Group A streptococci in throat cultures of a random sample of school children in the elementary grades

in 12 schools. The correlation of results by both methods was good; 91 per cent of the over-all findings, both positive and negative, were in agreement. Only 2.4 per cent of the swabs positive by the conventional method were not recognized by the FA technique. The FA technique also indicated more Group A streptococci than did routine bacteriological techniques, and special emphasis is placed on its relationship to early diagnosis and effective treatment of Group A streptococcal infections. An epidemiologic description is given along with discussion of implications.

5289

Saslaw, M.S.; Viets, A.G. 1964. Prevention of rheumatic fever: Limitations. J. Pediat. 64:552-556.

Every episode of rheumatic fever is presumed to be heralded by an antecedent infection with Group A beta-hemolytic streptococci. Rheumatic fever can be prevented by adequate treatment of streptococcal infection. The fluorescent antibody technique for identification of Group A beta-hemolytic streptococci permits institution of treatment within 24 hours and obviates need for treatment of strains other than Group A. A concerted research effort to test the possibility of prevention of initial attacks of rheumatic fever is warranted and urged.

5290

Schimmelpfennig, H. 1964. The use of fluorescence serology in bacteriological diagnosis: II. Demonstration and differentiation of Streptococcus agalactiae by fluorescent antibody. Zentralbl. Veterinarmed. Reihe B 11:5:407-417. In German.

An anti-S. agalactiae serum marked with FITC was rendered specific after absorption with an antigen mixture of three streptococci strains in serological Groups C and G. With specific conjugates it was possible to differentiate S. agalactiae from other streptococci on blood plates. To demonstrate the organism in milk samples, FA was combined with an enrichment method using streptosel broth. With the help of both techniques, mastitis streptococci were detected in milk samples within 24 hours without solid media. Examination of 531 milk samples with the combined enrichment and fluorescence technique showed agreement with the results of control cultural and serological tests in 71 per cent of those positive to FA and 98.5 per cent of those that were negative to this test. The number of samples in which FA was positive, whereas cultural and serological ones for mastitis streptococci were negative, was 29 per cent.

5291

Seegal, B.C.; Andres, G.A.; Hsu, K.C.; Zabriskie, J.B. 1965. Studies on the pathogenesis of acute and progressive glomerulonephritis in man by immunofluorescein and immunoferritin techniques. Federation Proc. 24:100-108.

Findings are currently compatible with a hypothesis that in acute glomerulonephritis, antigen-antibody complexes may circulate in the blood, aggregate in glomerular capillaries, penetrate among proliferating cells, and form deposits beneath the basement membrane. Complement seems involved. Type 12 streptococcal products appear to be a source of antigen. In subacute nephritis, presence of gamma globulin and complement indicates a role in progression of this disease. FA has been most helpful in these studies, but we are only at the beginning of the problem.

5292

Shockman, G.D. 1965. Symposium on the fine structure and replication of bacteria and their parts: IV. Unbalanced cell-wall synthesis; autolysis and cell-wall thickening. Bacteriol. Rev. 29:345-358.

Biochemical and morphological studies have indicated that unbalanced growth of S. faecalis can result in autolysis. There are quantitative and perhaps qualitative differences between specific nutritional and inhibitory situations that result in the same general effect. These differences, which may have considerable significance, have not been emphasized in this paper. Other cellular components, such as DNA, RNA, ribosomes, protein, and membrane, are affected also by a change in environment. Our present state of knowledge allows us only to speculate about the biochemical mechanisms that regulate either balanced growth and cell division or the more specialized unbalanced states. A hypothetical scheme is presented in an attempt to correlate observations on cell-wall thickening and autolysis with those by FA studies.

5293

Smith, T.S. 1965. Clinical application of immunofluorescence: I. Grouping beta-hemolytic streptococci. J. Bacteriol. 89:198-204.

Procedures are described for the production of antistreptococcal serum in rabbits and for the preparation of group-specific conjugates for Lancefield Groups A, C, and G. A modification of the conventional technique of absorption and inhibition to prevent cross-reactions with common antigens was used with excellent results. In addition, a promising new approach to eliminating cross-reactions of Group A conjugate with antigens of Groups C and G by dilution with Group A-variant antiserum was tested. A complete method is introduced that enables the clinical laboratory to report whether Group A streptococci are present in a given throat culture well within 24 hours after the physician collects the sample.

5294

Truant, J.P.; Hadley, I.K.; Boyd, T.T 1965. A comparison of the immunofluorescence technique with conventional methods for the identification of Group A beta hemolytic streptococci. Henry Ford Hosp. Med. Bull. 13:357-375.

A relatively large percentage (31 per cent) of beta hemolytic streptococci isolated from throat swab specimens did not belong to the Lancefield Group A. Three- and 18-hour Todd-Hewitt broth cultures did not yield as many Group A streptococci as the sheep-blood-agar plates, using the FA Group A conjugates. Therefore, the sheep-blood-agar plate should also be used in conjunction with the FA broth procedure. The serological studies indicate that the streptococcal extracts to be used for Lancefield grouping procedures should be prepared by the HCl extraction procedure in order to avoid major cross-reactions between the various groups. The bacitracin disc test, which is used for the presumptive screening of Group A streptococci, may be in error 15 to 20 per cent of the time. Almost all beta hemolytic streptococci tested in this project, especially the Group A strains, were very susceptible to penicillin-G and erythromycin, but 15 to 30 per cent were resistant to tetracycline. Therefore, the clinician must be aware of the relatively high incidence of tetracycline-resistant beta streptococci, especially the Group A strains, that may be present in his patients.

5295

Wagner, M. 1964. Studies with fluorescent antibodies on growing bacteria: I. The new formation of the cell wall of Diplococcus pneumoniae. Zentralbl. Bakteriol. 195:87-93. In German.

Living pneumococcus cells were stained with fluorescent antibodies and re-incubated after removal of excess antibody. The growing chains mostly divided simultaneously at all individual cells. The dark zones that appear during division are the new sections of cell wall. Growth of the pneumococcus cell takes place in an equatorial zone.

5296

Whitehouse, F., Jr.; Kilduff, J.T.; Christian, W. 1964. Effect of pepsin-digested pneumococcal antibody on protection of mice injected with pneumococci. Bacteriol. Proc., M42:51.

Rabbit Type II pneumococcal antibody globulin (6.6S) was digested with pepsin to obtain 4.8S globulin (Porter Fraction I-I or II-II). Protection was assayed by mixing 1:1,000 to 1:100,000 dilutions of pneumococci plus 0.05 ml of 0.25 to 0.008 mg per ml dilutions of globulin and injecting into 16- to 18-gram male ICR mice. Immune globulin protected mice at all dilutions; digested globulin gave partial protection only at higher concentrations and with higher dilutions of organisms. Normal globulin gave no protection. Intravenous injection of immune

globulin protected mice injected intraperitoneally with digested globulin and pneumococcus complex; digested globulin and pneumococcus complex incubated with immune globulin and injected intraperitoneally did not kill mice; i.e., digested globulin did not block immune globulin. Immune globulin fixed complement at 0.004 mg per ml; digested globulin, at 0.5 mg per ml. Both globulins agglutinated pneumococci and when tagged with fluorescein produced equal fluorescence of pneumococci. Digested antibody did not clear pneumococci from blood in mice as well as immune globulin. Loss of Porter Fraction III, with decreased complement fixation, is correlated in vivo with decreased mouse protection. Also, trypsin-digested pneumococcus plus antibody complexes were not lethal for mice, and the digested antibody did not block protection by immune globulin.

5297

Zabriskie, J.B.; Freimer, E.H.; Seegal, B. 1964. An immunological relationship between streptococcal membranes and human heart tissue. Federation Proc. 23:1454:343.

Our immunological studies of streptococcal protoplast membranes reveal that they cross-react with human cardiac tissue. This property is present in all Group A strains tested as well as some Group C strains. In contrast, other streptococci and gram-positive bacteria are non-reactive. By means of the fluorescent antibody technique, this reactivity can be localized to the myofibers and vascular smooth muscle of both normal and rheumatic hearts. Fractionation experiments clearly demonstrate that the immunologically active material in the streptococcal cell resides in the cell membrane. Membranes, free of other cell fractions and antisera to these membranes, have been used. The activity in these antisera can be removed by prior absorption with purified membranes. In addition, those cell wall preparations that partially block the reaction are contaminated with membrane material. Extracts obtained by HCl or pepsin digestion of membranes also extinguish fluorescence, and the active substance appears to be a dialyzable polypeptide. Complete article.

5298

Zinner, D.D.; Jablon, J.M.; Aran, A.P.; Saslaw, M.S. 1965. Experimental caries induced in animals by streptococci of human origin. Proc. Soc. Exp. Biol. Med. 118:766-770.

Streptococci were isolated from human dental carious lesions that reacted with fluorescein-tagged antisera against rat or hamster cariogenic streptococci. The human strains fell into three different categories in fluorescence, morphology, microprecipitin, and gel diffusion studies. One category, similar to the hamster strain, produced caries when used to infect hamster. This occurred in 55 animals of 55 tested, and in

no controls. The other two categories of human isolates failed to produce hamster caries; one of these is being studied for possible cariogenicity in germfree rats.

5299

Zinner, D.D.; Jablon, J.M.; Haddox, C.H., Jr.; Aran, A.; Saslaw, M.S. 1965. Use of fluorescent antibody technique to identify experimental hamster and rat strains of cariogenic streptococci. J. Dent. Res. 44:471-475.

Cariogenic streptococci, rat and hamster strains, do not belong to known Group A through S as checked by standard grouping procedures. Cariogenic streptococcal antisera, rat and hamster, are immunologically specific, just as the organisms themselves are host-specific. The cariogenic streptococcal antisera were conjugated with fluorescein isothiocyanate. The conjugates were used to recognize unknown strains containing the same somatic antigen. Ratios of rhamnose to n-actyl glucosamine do not help in distinguishing cariogenic streptococci. Preliminary work with other sugars, however, indicates that this may be a more promising avenue for investigation.

VIII. MICROCOCCACEAE

Belli, C.; Tessari, L. 1964. Use of the immunofluoroscopic technique in the study of osteopathology. Arch. Sci. Med. 117:165-172. In Italian.

In preliminary tests of the use of immunofluoroscopic technology in osteological research, the antigen was the golden staphylococci that cause bone inflammation. The bone marrow inflammation was experimental in a rabbit and clinical in a man. The staphylococci were demonstrated by FA. The zones where the antibody-antigen reaction occurred were demonstrated microscopically with ultraviolet light because the green color on the blue fluorescent background of the usual bone tissue was difficult to see.

5301

Carda, G.; Martineau, B.; deRepentigny, J.; Sonea, S. 1964. Quantitative differentiation using fluorescent antibody against Staphylococcus isolates from serious clinical cases. Rev. Can. Biol. 23:455-459.

Pathogenic and non-pathogenic S. aureus strains recently isolated from patients could be differentiated with a quantitative FA method. There is a correlation between the severity of clinical staphylococal infections and the intensity of immunofluorescence on infective strains with conjugated Staphylococcus antitoxin or normal human gamma globulin. The difference between the individual strains as shown by FA intensities was more pronounced than those shown by immunodiffusion, but, as a whole, the results obtained with both methods agree. Fluorescent human 'normal' gamma globulin gives sometimes brighter fluorescence intensities than fluorescent Staphylococcus antitoxin.

5302

Cohen, J.O. 1963. Effect of culture medium on preparation of serological reagents for the n factor of Staphylococcus aureus. J. Bacteriol. 86:1118-1119.

Choice of culture medium was critical in preparation of serologic reagents. For absorption purposes in preparing n-factor FA conjugate, cells grown on nutrient agar were superior to those grown on trypticase soy agar.

5303

Cohen, J.O.; Newton, W.L.; Cherry, W.B.; Updyke, E.L. 1963. Normally occurring staphylococcal antibodies in germfree mice. J. Immunol. 90:358-367.

The sera of nonimmunized mice from both germfree and non-germfree colonies of similar genetic stock have been shown to have antibodies for staphylococci by serum-gel diffusion, agglutination, and fluorescent antibody tests. In general, agglutination titers were higher in conventionally reared animals than in germfree animals, and higher in 8-month-old animals than in 2-month-old animals. When pooled samples of serum from 8-month-old germfree mice were labeled with fluorescein isothiocyanate, some strains of staphylococci were stained in high dilutions of the labeled serum. The results indicate that several different antibodies for staphylococci were present in the sera of 8-month-old germfree and conventional mice. Two of the antibodies observed by reactions in gel-diffusion plates were related to previously reported activities. One of these substances is antibody to antigen A of Jensen, previously reported to occur normally in human sera. The other antibody reacts with the same soluble antigen, RL, with which pre-immune rabbit serum reacts. The source of or stimulus for the antibody formation has not been determined.

5304

Danilova, T.A.; Korn, T.Ya. 1964. The possibility of elimination of cross-reactions between streptococci of various groups and staphylococci in applying the fluorescent antibody method. Zh. Mikrobiol. Epidemiol. i Immunobiol. 41:13-16.

In the direct method of fluorescent antibodies the fluorescent serum to Group A Streptococcus stained not only strains of the homologous group, but also cultures of C and G groups and some Staphylococcus strains. The sorption of a labeled serum with the live Streptococcus Group C culture eliminated the specific staining of strains of the Groups C and G; however, the sorbed serum retained its capacity to stain Staphylococcus. Treatment of fixed smears in a trypsin solution has made it possible to eliminate the Staphylococcus staining without disturbing the specific fluorescence of the Group A Streptococcus.

5305

deRepentigny, J.; Sonea, S.; Frappier, A. 1963. Comparison of quantitative immunofluorescence and immunodiffusion for the evaluation of antigenic materials from Staphylococcus aureus. J. Bacteriol. 86:1348-1349.

Microphotometric measurements of FA-stained bacterial smears were made and compared with unstained smears. These results were compared with precipitin line results. Pathogenic and nonpathogenic S. aureus

strains were differentiated. Normal human gamma globulin was better for immunofluorescent differentiation than was rabbit antitoxin serum. The reverse was true for immunodiffusion.

5306

deRepentigny, J.; Sonea, S.; Frappier, A. 1964. Differentiation by immunodiffusion and by quantitative immunofluorescence between 5-fluorouracil-treated and normal cells from a toxinogenic Staphylococcus aureus strain. J. Bacteriol. 88:444-448.

Immunodiffusion and quantitative immunofluorescence can both detect antigenic changes produced by 5-fluorouracil (FU) in Staphylococcus aureus Wood 46 strain. When FU is added to the cultures in their logarithmic phase of growth, a number of bacterial antigens are no longer detectable by immunodiffusion and the intensity of the total immunofluorescence of bacteria is diminished; thus, these antigens are either profoundly modified or no longer synthesized. Uracil and, less effectively, thymine can reverse the FU inhibitory effect on the synthesis of antigens, and the number of precipitin lines remains closer to controls. The immunochemical approach provides a new way of obtaining information on the action of this pyrimidine analogue on metabolic processes in pathogenic bacteria. Microscopic quantitative immunofluorescence seems to be adaptable to give indirect information on changes in the metabolism or synthesis of antigens of a single bacterial cell.

5307

Friedman, M.E.; White, J.D. 1965. Immunofluorescent demonstration of cell-associated staphylococcal enterotoxin B. J. Bacteriol. 89:1155.

FA staining of cultured staphylococcal cells, strain S6, yielded brilliant peripheral staining. Washing the cells readily reduced fluorescence, indicating loose binding of enterotoxin that may be a cell-surface constituent.

5308

Green, G.M.; Kass, E.H. 1964. The role of the alveolar macrophage in the clearance of bacteria from the lung. J. Exp. Med. 119:167-176.

Pulmonary clearance of bacteria was studied by histologic, bacteriologic, and radiotracer methods. When mice were exposed to an aerosol of radioactive Staphylococcus aureus or Proteus mirabilis, and the rate of disappearance of viable bacteria was compared with the rate of their mechanical removal, bacterial viability declined 80 to 90 per cent in 4 hours, although radioactivity declined only 14 to 20 per cent. The marked disparity in these rates indicated that mechanical removal comprised a relatively small fraction of the total clearing process. The in situ bactericidal action of the lung predominated over the mechanical removal process in achieving clearance of the inhaled

bacteria. By immunofluorescent methods, the inhaled bacteria were found to be localized in the alveolar spaces and within alveolar macrophages. These observations suggest that the bactericidal action of the bronchopulmonary tree is due primarily to the phagocytic activity of the alveolar macrophages, and that the action of the mucociliary stream, in relation to bacterial particles, may be largely related to the transport from the lung of phagocytes containing material of bacterial origin.

5309

Hamashima, Y. 1963. Rapid diagnosis by fluorescent antibody. Jap. J. Clin. Pathol. 11:467-475. In Japanese.

Streptococcus, Staphylococcus, Treponema pallidum and Toxoplasma gondii can be specifically detected by the fluorescent antibody technique. This technique is applicable for clinical use. This technique, if some related problems are solved, can also be applied to the detection of other pathogenic bacteria.

5310

Hirsch, J.G. 1964. Demonstration by fluorescence microscopy of adsorption onto bacteria of a heat-labile factor from guinea pig serum. J. Immunol. 92:155-158.

On incubation of staphylococci or salmonellae with fresh guinea pig serum, a heat-labile factor was adsorbed onto the bacteria. This adsorption did not take place at 0 C, and was also blocked by high salt concentrations in the medium. Divalent cations were not required for adsorption. Heat-labile guinea pig serum factor bound to the bacterial surface was altered or destroyed on exposure to 56 C, to trypsin, or to hydrazine. Incubation of fresh guinea pig serum with concentrated suspensions of Staphylococcus albus, followed by removal of the microbes by centrifugation, led to reduction or elimination of the content of heat-labile factor as estimated by immunofluorescence tests employing the homologous staphylococcus or an unrelated organism, Salmonella typhimurium. Fluorescence techniques were devised that permitted estimation of adsorption to the same bacterial cells of both heat-labile serum factor and specific antibody. Adsorption to staphylococci of either of these two serum components did not detectably augment or diminish subsequent binding of the other.

5311

Hovnanian, H.P.; Brennan, T.A.; Botan, E.A. 1964. Quantitative rapid immunofluorescence microscopy. J. Bacteriol. 87:473-476.

An elaborate electronic-optical instrument for detection and measurement of FA stain reactions is described. It consists of a UV source,

UV monochromator, UV filter system, bright-field fluorescence microscope, secondary filter system, a UV television camera, a microspot scanner, a quantitative light-reading device, television monitor, and an oscilloscope. Satisfactory tests were made with FA systems for E. coli, S. lutea, and B. globigii.

5312

Klainer, A.S.; Madoff, M.A.; Cooper, L.Z.; Weinstein, L. 1964. Staphylococcal alpha-hemolysin: Detection on the erythrocyte membrane by immunofluorescence. Science 145:714-715.

Purified staphylococcal alpha-hemolysin was demonstrated on the surface of rabbit and human erythrocytes by immunofluorescence. This occurred during the period of maximal hemolysis and was a transient event. These findings have been analyzed in relation to previous data on the kinetics of leakage of both small and complex molecular constituents of the erythrocyte.

5313

Komninos, G.N.; Tompkins, V.N. 1963. A simple method of eliminating the cross-reaction of Staphylococcus in the fluorescent antibody technic. Amer. J. Clin. Pathol. 40:319-324.

Rabbit immune sera against various species of bacteria, when tested by the direct or indirect fluorescent antibody method, cross-react with certain staphylococci. When staphylococci on a slide were pretreated with papain, then anti-meningococcal, anti-streptococcal A, or anti-Escherichia coli immune serum failed to cross-react. When similarly treated slides were stained with anti-Haemophilus influenzae, anti-pneumococcal, or anti-Listeria monocytogenes immune serum, papain eliminated cross-reaction only after these sera were absorbed with a staphylococcal strain. The difference in the effectiveness of papain suggests that in the case of the first three immune sera the cross-reaction factor was solely a papain-sensitive substance and that in the latter three immune sera two factors were involved--a heterogenetic antigen requiring the corresponding absorption and the papain-sensitive substance. A practical application of these findings is discussed.

5314

Korn, M.Ya.; Mayorova, G.F. 1963. On some causes of Staphylococcus staining with heterologous fluorescent sera. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:11:51-56. In Russian.

Staphylococcus staining with heterologous fluorescent sera was studied. As demonstrated, the presence in the sera of the normal antibiotics to Staphylococcus served as one of the causes of this phenomenon.

5315

Mayorova, G.F.; Korn, M.Ya. 1963. A study of the antipertussis fluorescent serum specificity. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:9:42-48. In Russian.

In studies on the specificity of fluorescent serological examination of H. pertussis with the aid of antipertussis fluorescent serum, it was impossible to detect H. pertussis with the aid of heterologous sera. Antipertussis fluorescent serum stained some species of microorganisms nonspecifically: P. pestis, E. coli, Bacillus brucellosis, P. tularensis, and others. Staphylococci and streptococci were stained specifically. The method of fixation also influenced the results of the investigation. Rough fixation by flame provoked microbial staining of H. pertussis with heterologous fluorescent sera. Identification of H. pertussis in practical conditions by FA at present is fraught with some difficulties, since the Staphylococcus and Streptococcus may be stained simultaneously. It was impossible to distinguish H. parapertussis from H. pertussis by the indirect method.

5316

Parrini, L.; Tessari, L. 1963. Study of the localization of Staphylococcus and its relations to antibiotic therapy in human osteomyelitis. Arch. Ortop. 76:503-511. In Italian.

Immunofluorescence has been used to demonstrate the presence of Staphylococcus in bone cell lacunae and canaliculi of spongy bone tissue affected by osteomyelitis. In view of the barrier to the spread of antibacterial drugs afforded by bony tissue, microfluoroscopy was used to determine the circumstances in which tetracycline therapy may be effectively instituted in the treatment of osteomyelitis in man.

5317

Philpot, W.N. 1965. Identification of Staphylococcus aureus from the bovine udder by the fluorescent antibody technique. Diss. Abstr. 26:1292-1293.

This study was conducted to determine the practicability of staining and identifying S. aureus in mastitic milk using specific staphylococcal antiserum and commercial, fluorescein-labeled, sheep antiserum to rabbit globulin. A total of 100 cultures of S. aureus was isolated from dairy cows with clinical or subclinical staphylococcal mastitis. The cultures were characterized. Specific staphylococcal antisera were prepared in rabbits. Direct and indirect FA were compared. Five commercial, fluorescein-labeled sheep antisera to rabbit globulins were compared as to staining ability. Procedures were developed for preparing milk smears, and for FA study. Indirect staining technique

was superior to the direct staining technique because it stained the cultures more intensely and resulted in less background fluorescence. Approximately 90 per cent of all cultures agglutinated in, or stained with, the experimental antisera. S. aureus could be identified specifically in the presence of a variety of microorganisms commonly found in mastitic milk. Staphylococci could be concentrated, although the results were not entirely satisfactory. Attempts to stain S. aureus on membrane filters failed because the filters adsorbed dye, making it impossible to visualize fluorescing staphylococci. FA has great potential as a diagnostic tool in mastitis work.

5318

Rotter, J.; Alami, S.Y.; Kelly, F.C. 1964. Evidence for antibody-free staphylococcus-fibrinogen reactions. J. Bacteriol. 88:1810-1811.

Indirect FA was used in an attempt to detect staphylococcal antibodies in two brands of bovine fibrinogen, using 30 strains of staphylococci. No antibody was detected in either fibrinogen.

5319

Schmidt, J. 1964. Experiments on the fluorescence serological demonstration of Staphylococcus aureus. Zentralbl. Bakteriol. 195:190-201. In German.

Experiments relating to the fluorescence serological demonstration of S. aureus, compared with culture growth and Gram preparations, are reported. Some 275 samples of different origins (pus, wound swabs, biliary fluids, urine, fluids, sputa) were tested. In about 88 per cent of cases there was agreement between culture and fluorescence tests; the failure rate, in part due to nonspecific staining of coagulase-negative cocci, was at least 6 per cent. As compared with the Gram preparations, the fluorescence serological process was decidedly superior, especially in respect to the differentiation from streptococci and the majority of coagulase-negative staphylococci. There is a discussion of the advantages and disadvantages of the process.

IX. MYCOPLASMA (PPLO)

5320

Biberfeld, G.; Johnsson, T.; Johnsson, J. 1965. Studies on Mycoplasma pneumoniae infection in Sweden. Acta Pathol. Microbiol. Scand. 63:469-475.

Sera from 107 cases of pneumonia and 132 cases of milder respiratory infection were examined by the CF test against M. pneumoniae antigen. Thirty-five patients with pneumonia and two patients with bronchitis had serologic evidence of M. pneumoniae infection. All cases that had a significant antibody rise with the CF test also showed a corresponding rise with the fluorescent antibody test. M. pneumoniae was isolated from 10 of 18 serologically positive cases. Cold agglutinins were demonstrated in 17 of 37 cases with M. pneumoniae infection. M. pneumoniae infections occurred during all seasons of the year and were most common in older children and young adults. The clinical features of the cases with M. pneumoniae infection in this study resemble those described in similar investigations in other countries.

5321

Butler, M.; Leach, R.H. 1964. A mycoplasma which induces acidity and cytopathic effect in tissue culture. J. Gen. Microbiol. 34:285-294.

An agent that induced acidity and cytopathic effects in Hep-2 tissue cultures was investigated. The agent grew well in certain other tissue culture systems. Typical mycoplasma colonies were isolated from the contaminated Hep-2 cultures and on reinoculation into Hep-2 cultures produced effects indistinguishable from the original effects. There was no appreciable growth in tissue culture medium alone. The mycoplasma had biological properties similar to those of known mycoplasmas, including Mycoplasma hominis Type 1, but was serologically distinct from these. Fluorescent antibody and Giemsa-staining techniques showed extracellular forms. Other mycoplasmas were shown to grow in tissue culture; M. gallisepticum induced similar effects to the cytopathic agent but was distinct in serological and biological properties. The agent partially inhibited the growth of measles virus.

5322

Chanock, R.M.; Mufson, M.A.; Somerson, N.L.; Couch, R.B. 1963. Role of mycoplasma (PPLO) in human respiratory disease. Amer. Rev. Resp. Dis. 88:218-239.

This is a general presentation of the roll of Mycoplasma species in respiratory diseases and also possible implications of etiology as yet undetermined. FA is a valuable tool here to demonstrate evidence of infection.

5323

Clark, H.W.; Bailey, J.S.; Brown, T.M. 1964. Determination of Mycoplasma antibodies in humans. Bacteriol. Proc. M87:59.

Immunological studies of Mycoplasma were initiated to determine Mycoplasma antibodies in humans by agglutination, neutralization, complement fixation, fluorescent antibody, and immunodiffusion. Comparison of these methods revealed certain advantages to each. Neutralization uses viable native antigens but is limited by the presence of nonspecific inhibitors and difficulty in standardizing and interpreting results. Agglutination is easy and rapid but requires the production of stable and standardized Mycoplasma particles. FA techniques have the advantage of detecting Mycoplasma antibodies and antigens readily but are limited by nonspecific fluorescence. The CF test is sensitive and specific for either whole particles or purified antigen fractions. The standardization of these various antigen preparations is often a disadvantage. Immunodiffusion gives rapid identification of Mycoplasma antibodies, using solubilized particles or the purified antigens, but lacks the sensitivity to detect the presence of low antibody concentrations in human serum. The presence of Mycoplasma antibodies in human serum, urine, and joint fluid was investigated. Antibodies could be detected more readily by CF, and this was made more versatile by the use of a multiple antigen preparation. Positive CF serum also reacted in a sensitive indirect agglutination test using antihuman serum. The presence of at least three different types of Mycoplasma antibodies was determined in humans.

5324

Clark, H.W.; Bailey, J.C.; Fowler, R.C.; Brown, T.M. 1963. Identification of Mycoplasmataceae by the fluorescent antibody method. J. Bacteriol. 85:111-118.

The conditions of the fluorescent antibody reactions were studied in relation to their application to Mycoplasmataceae or pleuropneumonia-like organisms. Mycoplasma hominis Type 1 and 2 antigens and their homologous antisera were used to determine the activity and specificity of these and other strains. Fluorescein isothiocyanate - conjugated antiserum globulin preparations were used in both the direct and indirect fluorescent antibody methods. A direct tube technique was used for the detection and measurement of growth in broth cultures by the addition of conjugated antiserum. The specific fluorescent staining and recognition of M. hominis colonies fixed in hot water were presented as a suitable identification standard. The antigenic activity remained in the insoluble residue after exposure of M. hominis strains to sonic vibration, 9 kc for 30 minutes, and to centrifugation. Brief 2-minute exposures of tissue cells to vibration caused the disruption of tissues, with the release of viable and bound nonwashable strains that reacted specifically with fluorescent antibody. It is proposed to apply both the sonic vibration and the fluorescent antibody techniques for the identification of Mycoplasmataceae in human tissues.

5325

Clyde, W.A., Jr. 1963. Studies on Eaton agent in tissue culture. Amer. Rev. Resp. Dis. 88:Suppl.:212-217.

Experimental systems for working with Eaton agent have been expanded by the finding that the agent could be demonstrated directly in infected tissue cultures by means of Giemsa and fluorescent antibody techniques. The morphologic properties were those of the pleuropneumonia-like organisms. A method is described for quantitating the agent in tissue culture, permitting study of growth and inhibition of growth produced by antimicrobials and antisera. The agent was found to be susceptible in vitro to tetracycline and its derivatives, but was resistant to penicillin. Antisera produced reduction of growth in correlation with fluorescent-stainable antibody titers of the sera, confirming the work of Eaton.

5326

Clyde, W.A., Jr. 1963. Studies on growth of Eaton agent in tissue cultures. Proc. Soc. Exp. Biol. Med. 112:905-909.

A method for quantitative studies of Eaton agent with a tissue culture system is described. The agent grew slowly in monkey kidney cell cultures, unlike many PPLO, but resembled other members of the genus Mycoplasma in being resistant to penicillin and sensitive to the tetracyclines. Demonstration of growth-inhibiting effects of both rabbit antiserum and sera of patients convalescent from atypical pneumonia, in confirmation of the work of Eaton, provides another parameter for studying immune response and relating Eaton agent to human disease.

5327

Corstvet, R.E.; Sadler, W.W. 1964. The diagnosis of certain avian diseases with the fluorescent antibody technique. Poultry Sci. 43:1280-1288.

A modified method of conjugate preparation produced conjugates that were satisfactory for differentiating various avian pathogens in artificial media and tissues. This simplified method saved considerable time over previously published methods by eliminating fractionation of the unconjugated serum, dialysis, and concentration. This was desirable since indirect FA did not function with colony imprints. FA can be very useful in evaluating mycoplasma cultures as to purity and greatly simplifies the identification of Mycoplasma spp. or serological types as well as other avian pathogens. The results also showed that tissue imprints of trachea, air sac, and proventriculus could be used in identifying NDV by FA.

5328

Dajani, A.S.; Clyde, W.A., Jr.; Denny, F.W. 1964. Pathogenesis of M. pneumoniae infection in hamsters. Federation Proc. 23:552:192.

An experimental model was designed for the study of the natural history of M. pneumoniae, Eaton PPLO, infection in the Syrian hamster. Following intranasal inoculation, microscopic lung lesions similar to the ones observed in human disease were regularly noted in 10 to 14 days; macroscopic lesions were minimal and inconsistent. Peribronchial infiltrates of predominantly mononuclear cells were the usual findings. Using special staining techniques, including immunofluorescence, organisms were localized primarily on the epithelial surface of large bronchi. Quantitative recovery of the agent from various sites of the respiratory tract indicated that multiplication commenced in 3 days and reached a maximum in 7 to 14 days, and that organisms persisted for at least 10 weeks. The infective dose for 50 per cent was approximately 10 organisms. Study of variables demonstrated that the anesthetic agent used was an important factor and that strain derivation and attenuation on artificial media did not seem to influence infectivity. Infection was not communicable. This experimental model can serve for further investigations of this agent, with particular reference to the effect of antibiotic and prophylactic agents. Complete article.

5329

Dajani, A.S.; Clyde, W.A., Jr.; Denny, F.W. 1965. Experimental infection with Mycoplasma pneumoniae, Eaton's agent. J. Exp. Med. 121:1071-1086.

The pathogenesis of Mycoplasma pneumoniae infection was studied in the Syrian hamster with qualitative and quantitative culture methods and special histopathologic techniques. The animals were readily infected with the mycoplasma, which multiplied throughout the respiratory tract. Sensitivity of this experimental host to infection was indicated by the 50 per cent infective dose, which was 10 colony-forming units of the organism. Inoculation consistently resulted in the production of peribronchial pneumonitis induced by the mycoplasma. The organisms were visualized in a superficial location in the mucosa of involved bronchi by indirect fluorescent antibody staining and by a modification of the Brown and Brenn technique. The data indicate applicability of the hamster to the study of problems concerned with M. pneumoniae disease that are impractical or impossible to resolve in the human host.

5330

Dowdle, W.R.; Robinson, R.Q. 1964. An indirect hemagglutination test for diagnosis of Mycoplasma pneumoniae infections. Proc. Soc. Exp. Biol. Med. 116:947-950.

An indirect HA test for detection of M. pneumoniae antibodies is described. The test appears to be immunologically specific, at least as sensitive as the CF test, and far more sensitive than immunofluorescence with colony impression smears.

5331

Eaton, M.D. 1965. Pleuropneumonia-like organisms and related forms. Annu. Rev. Microbiol. 19:379-406.

As a portion of this general discussion, the use of FA to identify and localize antigen and to titrate PPLO antibodies is reviewed.

5332

Goodburn, G.M.; Marmion, B.P.; Kendall, E.J.C. 1963. Infection with Eaton's primary atypical pneumonia agent in England. Brit. Med. J. 1963:1266-1270.

Sixteen sporadic cases in the general population and six cases in a small school outbreak of infections due to Eaton's primary atypical pneumonia agent are described with two characteristic clinical histories. Diagnosis was made by serological methods; serum antibody was detected principally by immunofluorescent methods, using the indirect staining technique. The development of a complement fixation test for the laboratory diagnosis of infections with Eaton's agent is described, the results of which appear to be in reasonable agreement with those obtained by immunofluorescent methods. The antigenic similarity of agents causing similar illnesses in the United States, Holland, and England is shown, and evidence is presented that infection has been present in this country for at least 12 years.

5333

Hers, J.F.Ph. 1963. Fluorescent antibody technique in respiratory viral diseases. Amer. Rev. Resp. Dis. 88:316-338.

Fluorescent antibody diagnosis of respiratory viral disease is rapid and sensitive. It also provides a tool for pathogenesis study. Among the diseases discussed are influenza, Eaton's agent, and psittacosis. The discussion is particularly informative.

5334

Hers, J.F.Ph.; van der Kuip, L.; Masurel, N.; Mulder, J. 1963. The diagnosis of primary atypical pneumonia by fluorescent-labeled antibody technique. Ned. Tijdschr. Geneesk. 107:74-78. In Dutch.

Diagnosis of primary atypical pneumonia by the fluorescent antibody technique and the complement fixation reaction is described. Diagnosis by this method can be made at a very early stage of the disease by applying this method to smears of sputum or nasal exudate. A study of 14 patients is described.

5335

Jensen, K.E.; Neal, E.J.; May, P.A. 1963. Suppression of growth of Eaton Mycoplasma by immune sera. Federation Proc. 22:982:325.

With the etiologic agent of primary atypical pneumonia established as a Mycoplasma, efforts have been intensified to define the epidemiology. Serology was at first limited to fluorescent antibody staining and, more recently, complement fixation, CF, methods. We have found that when suspensions of the organism are mixed with animal or human immune sera the number of colonies that develop on agar medium is significantly reduced. Since heated serum also produced the effect, complement does not appear to be essential. Studies have included the variation of several factors to find optimal conditions for greater reproducibility of results. Comparisons of this activity with that measured by FA and CF procedures indicate that viability reduction is a sensitive method for demonstrating antibody against the agent. Complete article.

5336

Kim, K.S. 1965. Serological and morphological studies of mycoplasma. Diss. Abstr. 26:1289.

Growth studies on PPLO were performed by viability determinations on serial tenfold dilutions of the cultures. For optimum growth the medium composition and environmental conditions differed from strain to strain. Electron microscopic pictures of different strains showed variation in cell sizes and morphology, which may be of some help in classifying the PPLO. Cell structure is described. An antiserum absorption method was used to detect differences between anti-tissue culture grown PPLO and anti-broth grown PPLO. Agar diffusion tests revealed that there is more than one antigen system present in some PPLO. Eaton agent adhered to a glass surface during growth, a property not observed with other strains. Colonies growing in broth media that stick to the glass surface are visible in 24 hours under low magnification. FA staining shows that they are Eaton agent. Ordinarily it takes 4 to 5 days for colonies to be visible on agar. This method may be useful as a means of rapid recognition of Eaton agent in clinical infections.

When examined under the oil immersion objective the colonies contained short rods, coccoid forms and star shaped forms. Transformation has been demonstrated between Eaton agent and M-PPLO.

5337

Lemcke, R.M.; Shaw, E.J.; Marmion, B.P. 1965. Related antigens in Mycoplasma pneumoniae and Mycoplasma mycoides var. mycoides. Australian J. Exp. Biol. Med. Sci. 43:761-770.

Serological cross-reactions have been observed between Mycoplasma pneumoniae and M. mycoides in tests with convalescent-phase sera from human beings with atypical pneumonia, with sera from rabbits hyper-immunized with one or other of the two strains, and with postinfection sera from cattle experimentally inoculated with M. mycoides. The cross-reactions were most pronounced in CF tests, less by FA and least by growth inhibition. An ethanol extract (Dafaalla B fraction) and a semi-purified polysaccharide (galactan) from M. mycoides reacted in gel-diffusion and hemagglutination with antisera to M. pneumoniae. It is possible, therefore that more than one antigenic component may be shared between the two mycoplasmas.

5338

Malizia, W.F.; Barile, M.F.; Riggs, D.B. 1961. Immunofluorescence of pleuropneumonia-like organisms isolated from tissue cell cultures. Nature 191:190-191.

Fluorescent antibody was used to detect PPLO contamination of continuous tissue cell lines. Similar antigenicity of PPLO strains obtained from various cell lines was demonstrated. The staining pattern morphologically resembled the intracytoplasmic granulation of contaminated tissue culture cells. Fluorescence was never observed in the nucleus.

5339

Marmion, B.P.; Perceval, A.; Ennis, G.C. 1965. Respiratory illness and Mycoplasma pneumoniae (Eaton agent). Med. J. Australia 2:233-235.

A family outbreak of infection with M. pneumoniae (Eaton agent) is described. This involved three adults - the mother, father, and mother-in-law - and three children in a family. The two women were severely ill with, respectively, bilateral basal and right middle-lobe pneumonia. The father and the children had a milder illness with fever and cough. All three adult patients had high or rising antibody titers to M. pneumoniae, which were demonstrated by the complement-fixation, immunofluorescence, or growth-inhibition technique. This small outbreak is described in the hope that it may stimulate a wider search for, and assessment of the part played by, Eaton agent in producing respiratory illness in Australia.

5340

Mufson, M.A.; Ludwig, W.M.; Purcell, R.H.; Cate, T.R.; Taylor-Robinson, D.; Chanock, R.M. 1965. Exudative pharyngitis following experimental Mycoplasma hominis Type 1 infection. J. Amer. Med. Ass. 192:1146-1152.

In two separate studies, a total of 50 male volunteers were inoculated by the nasopharyngeal route with the DC 63 strain oral isolate of Mycoplasma hominis Type 1 in an early passage in broth. Forty-five of the 50 volunteers were infected; the organism was recovered from 42 of the men and, in addition, 38 volunteers had a fourfold or greater rise in indirect hemagglutination antibody level. An afebrile exudative pharyngitis developed in 21 of the volunteers, and an afebrile non-exudative pharyngitis developed in four men. Approximately one-half of the volunteers with pharyngeal involvement also had cervical adenopathy; one-fourth of the men complained of sore throat. Exudative pharyngitis occurred significantly more often in volunteers free of preinoculation indirect hemagglutination antibody than in men with antibody. The findings suggest that M. hominis Type 1, the DC 63 strain, can cause exudative pharyngitis under experimental conditions. The relationship of this organisms to naturally occurring exudative pharyngitis remains to be determined. Indirect FA was used for antibody titration.

5341

Noel, J.K., III. 1964. Tube agglutination and fluorescent antibody techniques for identification of avian pleuropneumonia-like organisms. Diss. Abstr. 24:4922.

Fourteen strains of avian pleuropneumonia-like organisms (PPLO) were used to produce immune sera in rabbits. Each antiserum was tested against all strains used in this study by tube agglutination in order to determine similar antigenic components within the different strains. Agglutination adsorption tests gave further clarification as to the relationships among the different strains that cross-reacted. The relationships are discussed. A serological survey of various flocks of chickens and turkeys distributed throughout Maryland was conducted to determine the most prevalent strain or serological group. Of 22 flocks tested by tube agglutination, only one reacted to the pathogenic strain, MD-3. FITC was used to label pooled immune sera for the direct method and to label anti-rabbit globulin antiserum for the indirect method. Both methods proved satisfactory. Of 25 field cases suspicious of air sac infection, 19 were positive for the presence of PPLO. Bright fluorescing yellowish-green coccoid cells of approximately 0.1 to 0.3 u in diameter were observed in tissue smears, turkey sinus exudate, and filtrates from homogenized tissues.

5342

Noel, J.K.; DeVolt, H.M.; Faber, J.E. 1964. Identification of Mycoplasma gallisepticum in lesion tissue by immunofluorescence. Poultry Sci. 43:145-149.

Because dissemination of air sac infection may occur rapidly within a poultry flock, a rapid and specific diagnostic technique was investigated. It was demonstrated that the FA technique could be successfully applied as a rapid and specific diagnostic method for detecting avian PPLO in air sac infection. In 19 field cases suspicious of air sac infection, bright fluorescing, yellowish-green coccoid cells of approximately 0.1 to 0.3 micron in diameter were observed. PPLO cells were also observed in turkey sinal exudate and filtrates from homogenized tissues.

5343

Sobeslavsky, O.; Syrucek, L.; Bruckova, M.; Abrahamovic, M. 1965. The etiological role of Mycoplasma pneumoniae in otitis media in children. Pediatrics 35:652-657.

This paper reports on the isolation and identification of Mycoplasma pneumoniae as possible etiological agent of otitis media in three naturally infected children. The disease was a complication of a mild respiratory tract illness. All patients recovered in the course of one week. M. pneumoniae isolation was successful both in enriched liquid and on solid PPLO media from throat swabs of two patients and from the ear swab of one after paracentesis of the eardrum. The strains isolated grew on solid media forming typical colonies and producing hemolysin. Their final identification was accomplished by means of FA as well as by the CF test. In the paired sera of two of these patients a significant rise of CF antibody against both the M. pneumoniae standard strain and the strains isolated was established. There were no antibody rises against influenza viruses, adenoviruses, parainfluenza virus Type 1, and RS virus, nor a significant rise of antistreptolysin O.

5344

Thivolet, J.; Sohier, R.; Picard, M.; Blanc, G. 1963. Application of the immunofluorescence technique to the immunological diagnosis of the pneumopathy induced by Eaton's mycoplasma. Ann. Inst. Pasteur Paris 105:749-756. In French.

After a survey of work on the serological diagnosis of this pneumopathy and a description of the technique used, the authors report the results they have obtained in the study of 136 sera from 102 patients. In cases of primary atypical pneumonia with a cold-agglutinin positive reaction and negative serological reactions with the main respiratory viruses, 34.8 per cent of the sera yielded a positive immunofluorescence reaction. The reasons for this low percentage are discussed.

5345

Tully, J.G. 1963. Erythrocyte-modifying capacity and serological reactivity of cell components of human mycoplasma, PPLO, strains. Proc. Soc. Exp. Biol. Med. 114:704-709.

Liquid cultures of several mycoplasma strains were harvested and packed cells were fractionated. Recovery of fractions from alkaline hydrolysis yielded small quantities of material capable of modifying erythrocytes for specific agglutination in homologous PPLO antiserum. Fractions capable of sensitizing erythrocytes were also active in adsorbing specific PPLO antibody reacting in an immunofluorescent test. Hemagglutinin titers of PPLO antisera were compared with agglutinin and indirect fluorescent antibody titers.

5346

Tully, J.G. 1965. Biochemical, morphological, and serologic characterization of mycoplasma of murine origin. J. Infect. Dis. 115:171-185.

A comparative study of murine mycoplasma strains has revealed at least five distinct serotypes. The majority of mycoplasma isolated from the respiratory tract of mice and rats produced a granular type of colony on solid media and are serologically indistinguishable from the prototype strain of Mycoplasma pulmonis. Both neurotoxic and atoxic strains of M. neurolyticum were serologically distinct from other rodent strains. Two M. arthritidis strains were also unrelated to other murine mycoplasma but were immunologically identical to M. hominis Type 2 human genital mycoplasma. The Type C strain of Sabin possessed the biochemical and morphological characteristics of mycoplasma and was serologically distinguishable from other murine strains. Serologic relationships were studied by serum titration by the indirect FA test.

5347

van der Veen, J.; van Nunen, M.C.J. 1963. Role of Mycoplasma pneumoniae in acute respiratory disease in military population. Amer. J. Hyg. 78:293-301.

During an 18-month study at a military training center in The Netherlands, paired sera from 348 patients with acute respiratory disease not associated with influenza or adenovirus infection were tested against Mycoplasma pneumoniae by the fluorescent antibody technique and the complement fixation test. Evidence of M. pneumoniae infection was obtained in 8.9 per cent of the patients. The CF test was about 84 per cent as sensitive as the fluorescent antibody technique in detecting infection. M. pneumoniae was recovered on artificial agar medium from 8 of 25 serologically positive patients. About 7 per cent of the recruits were infected with M. pneumoniae during the first 6 weeks of basic training as measured by the CF test.

5348

van Nunen, M.C.J.; van der Veen, J. 1963. Examination for Mycoplasma pneumoniae (Eaton's agent). Ned. Tidjschr. Geneesk. 107:2141-2145. In Dutch.

Paired sera from 897 patients with acute respiratory disease were tested for complement-fixing antibody to M. pneumoniae. Paired sera from 348 patients were also tested for fluorescent-stainable antibody to M. pneumoniae. Antibody rises were found in 45 patients. The fluorescent antibody technique was slightly more sensitive than the complement fixation test. The test for cold agglutinins was relatively insensitive for the detection of respiratory disease caused by M. pneumoniae. The complement-fixing antigen used in these tests was highly specific. The fluorescent antibody technique showed that a high titer of fluorescent-stainable antibody may persist for at least 4 months after infection. The complement fixation test for M. pneumoniae is recommended for the diagnosis of acute disorders of the respiratory tract and lungs.

X. NEISSERIACEAE

5349

Biegeleisen, J.Z., Jr.; Mitchell, M.S.; Marcus, B.B.; Rhoden, D.L.; Blumberg, R.W. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: I. Clinical evaluation of conjugates on smears prepared directly from cerebrospinal fluid sediments. J. Lab. Clin. Med. 65:976-989.

Techniques were described for the cultivation and immunofluorescent identification of Hemophilus influenzae, Diplococcus pneumoniae, Neisseria meningitidis, and eight less common pathogens in specimens of cerebrospinal fluid from 100 patients with bacterial meningitis. A comparison of the results obtained by conventional methods and by immunofluorescent staining indicated that the latter method was fully as sensitive as the former and was more accurate in treated cases. Some of the dangers involved in the use of the Gram stain of the sediment as a tool for presumptive diagnosis were discussed, as were shortcomings of fluorescent antibody staining, particularly in infections caused by uncommon gram-negative organisms. The immunofluorescent staining technique was recommended for the rapid screening of spinal fluid specimens, as well as of cultural isolates.

5350

Danielsson, D. 1963. The demonstration of N. gonorrhoea with the aid of fluorescent antibodies: I. Immunological studies of antigonococcal sera and their fluorescein-labeled globulins, with particular regard to specificity. Acta Dermatovenerol. 43:451-464.

Antigonococcal sera were prepared in rabbits and their immunological activity was measured by agglutination, complement fixation, and fluorescent antibody techniques. The fluorescein isothiocyanate - conjugated globulins gave strong reactions with meningococci, with certain strains of S. aureus, and with Group G streptococci. Weak reactions were noted with certain Neisseria (flava, flavescens, sicca, and catarrhalis), certain Group A and Group C streptococci, S. aureus, S. albus, and a few types of pneumococci. Cross-reactions with S. aureus were eliminated by absorbing the conjugate with such S. aureus as gave strong reactions. The cross-reactions noted with other cocci, exempting meningococci, were at the same time reduced to a minimum. The immunological significance and the practical consequence of these observations are discussed.

5351

Danielsson, D. 1963. The demonstration of N. gonorrhoeae with the aid of fluorescent antibodies: II. A comparison between the fluorescent antibody technique and conventional methods of detecting N. gonorrhoeae in men and women. Acta Dermatovenerol. 43:511-521.

Specimens collected from male and female patients were tested by FA and the results were compared with those obtained by direct microscopy after staining with methylene blue and Gram stains and by culture. The specimens from males were examined by direct and delayed FA test, those from females were subjected only to a delayed FA test. Seventy-five males were tested 114 times. Gonococci were detected by direct microscopy in males with suspected gonorrheal urethritis in 59.6 per cent of the cases, in 63.5 per cent and 76.9 per cent by direct and delayed FA tests respectively, and in 71.2 per cent by culture tests. Gonococci were detected by direct microscopy in men treated for gonorrhea in 8.3 per cent of the cases, in 11.1 per cent and 30.6 per cent by direct and delayed FA tests respectively, and in 19.4 per cent by culture tests. One hundred and fifteen females were tested 239 times. Gonococci were detected in females with suspected gonorrheal infection or reported as sources of infection in 18.5 per cent of the cases by direct microscopy, in 55.6 per cent by culturing and in 68.5 per cent by delayed FA tests. In females treated for gonorrhea, 1.3 per cent were detected by culture and 16.3 per cent by delayed FA.

5352

Danielsson, D. 1964. Serological studies of N. gonorrhoeae and other Neisseria species by means of the double diffusion in gel technique and the fluorescent antibody method. Acta Pathol. Microbiol. Scand. 60:300.

The precipitin relationship between N. gonorrhoeae strains and other Neisseria species was studied by the double diffusion in gel technique. The Neisseria strains tested were sonic-treated. A reference system was used containing 15 separate precipitating systems. The serum was obtained from a rabbit immunized with formalin-killed gonococci from the gonococcus reference strain. One or two of the precipitinogenic factors demonstrated in the reference strain were missing in some strains of N. gonorrhoeae. Meningococci belonging to groups A, B, C, and D had 11 or 12 antigenic factors, and strains of N. sicca, N. catarrhalis, N. flava, and N. flavescens had 3 to 9 antigenic factors in common with the 15 factors demonstrated in the reference strain. Subculturing of the reference strain did not alter its precipitin pattern. The fluorescein isothiocyanate - conjugated globulin of the reference serum gave a 3 to plus 4 reaction with the reference strain at a maximal dilution of 1:256. A corresponding reaction was obtained with other gonococcal strains at dilutions of 1:64 to 1:128 and with meningococci at dilutions of 1:16 to 1:32. Some strains of the so-called apathogenic Neisseria species gave a weak 1 to plus 2 reaction

at a maximal dilution of 1:4 to 1:8. Preliminary results indicated that the majority of the antigenic factors demonstrated by the diffusion in gel technique is of intracellular origin, especially those in common for the different species within the Neisseria group.

5353

Danielsson, D. 1965. The demonstration of N. gonorrhoeae with the aid of fluorescent antibodies: 3. Studies by immunofluorescence and double-diffusion-in-gel technique on the antigenic relationship between strains of N. gonorrhoeae. Acta Pathol Microbiol. Scand. 64:243-266.

The fluorescent antibody method and the gel diffusion technique were used to study the antigenic relationship between different strains of N. gonorrhoeae and the antigens taking part in FA staining and gel precipitation reactions. Both heat-labile and heat-stable antigenic factors were shown to take part in the two reactions. A close antigenic relationship among different gonococcal strains was demonstrated by both techniques. The fluorescein isothiocyanate - labeled globulin of one species' antiserum seems to allow the diagnosis of all gonococcal strains. Absorption experiments indicated, however, the occurrence of strain-specific antigenic factor. A group of antigenic factors demonstrated by the gel diffusion technique were found to be of no importance for the FA staining. They were of intracellular origin.

5354

Danielsson, D. 1965. The demonstration of N. gonorrhoeae with the aid of fluorescent antibodies: 4. Studies by immunofluorescence and double-diffusion-in-gel technique on the antigenic relationship between N. gonorrhoeae and other Neisseria strains. Acta Pathol. Microbiol. Scand. 64:267-276.

The antigenic relationship between N. gonorrhoeae and other Neisseria species was studied by comparative fluorescent antibody tests and gel diffusion tests with the use of a gonococcus reference system. Strong reactions were obtained between fluorescein isothiocyanate - labeled antigonococcus globulin and meningococci, but only weak reactions at low dilutions of the conjugate with so-called apathogenic Neisseria. A conjugate, specific for gonococci only, can be obtained by absorption. From a practical point of view this does not seem to be necessary for the diagnosis of genitourinary gonorrhea. Gel diffusion tests showed a relatively close antigenic relationship between gonococci and meningococci and certain apathogenic Neisseria strains. Most of these antigens were of intracellular origin. The gel diffusion tests also demonstrated the occurrence of species-specific antigens in gonococci and some of these were of importance for the FA staining.

5355

Danielsson, D. 1965. The demonstration of Neisseria gonorrhoeae with the aid of fluorescent antibodies: 5. A comparison of different techniques--absorption, one-step inhibition, and counterstaining--for elimination of cross reactions. Acta Dermato-Venereol. 45:61-73.

FITC conjugated rabbit antigonococcus globulins and rabbit normal globulin were tested for reactions with Neisseria strains, staphylococci, and streptococci. Strong reactions were observed between antigonococcus conjugates and meningococci. Both the antigonococcus and normal globulin conjugates gave strong reactions with some of the staphylococcus and Group G streptococcus strains. These could be eliminated by absorbing with strongly reacting strains of Staphylococcus aureus. At the same time FA titers of the antigonococcus conjugates with gonococci and meningococci were lowered. The staining of staphylococci was somewhat inhibited by adding human serum or normal rabbit serum to antigonococcus conjugates. Adding antistaphylococcal rabbit serum to FITC-labeled antigonococcus globulins had a more pronounced inhibiting effect. RB 200-labeled globulin of the antistaphylococcal rabbit serum had the same effect. The application of delayed FA tests on clinical specimens showed that it was necessary to take precautions against nonspecific reactions. In males, delayed FA tests and culture were of equal value for diagnosis of gonococci. In females, delayed FA tests gave a higher yield than culture.

5356

Danielsson, D. 1965. The demonstration of Neisseria gonorrhoeae with the aid of fluorescent antibodies: 6. A comparison of conventional methods and fluorescent antibody (FA) techniques with counterstaining for the demonstration of N. gonorrhoeae. Acta Dermato-Venereol. 45:74-80.

The FA technique is a rapid and sensitive means for the identification of N. gonorrhoeae. During a period of a more extensive use of cultural methods a close agreement was obtained between the two method, which seems to indicate that the FA technique has a high rate of specificity, thus giving evidence that satisfactory precautions can be carried through against cross-reactions.

5357

Frank, C.W. 1965. Gonococcal arthritis in children. Arth. Rheum. 8:442-443.

Gonococcal arthritis, usually not thought of as a disease of children, has been observed in five girls and one boy. The ages ranged from 2 to 13 years. The arthritis was migratory in five patients. Symptoms

eventually localized predominantly in one large joint. Gonococci were cultured from the joint or vagina in five of the six patients. In the sixth, the arthritis began shortly after that of a culture-positive sister, and showed a classic response to penicillin. The sera of all patients contained gonococcal antibody as demonstrated by the fluorescent antibody technique. Gonococcal arthritis should be considered in the differential diagnosis of polyarthritis in the child.

5358

Fry, C.S.; Wilkinson, A.E. 1964. Immunofluorescence techniques as an aid to the diagnosis of gonorrhoea. Brit. J. Vener. Dis. 40:125-128.

Immunofluorescence tests using an antigonococcal serum conjugated with fluorescein isothiocyanate were carried out on 100 male and 94 female patients. The results are compared with those of Gram-stained smears and cultures. A higher proportion of positive results was obtained by the fluorescence methods than by either smears or cultures. The advantages of the method and the importance of the use of a specific antiserum that does not cross-react with other organisms are discussed.

5359

Hess, E.V.; Hunter, D.K.; Ziff, M. 1965. Gonococcal antibodies in acute arthritis. J. Amer. Med. Ass. 191:531-534.

Diagnosis of acute gonococcal arthritis has been hampered by the lack of a reliable and specific procedure for the detection of gonococcal antibodies. The indirect immunofluorescent method, using formalin-fixed smears of Neisseria gonorrhoeae organisms, has provided a sensitive and specific method for measurement of this antibody. Gonococcal antibody was present in 86 per cent of patients with definite and 49 per cent of patients with presumptive gonococcal arthritis. Only seven of 42 patients, or 17 per cent, with active Reiter's syndrome gave positive reactions. Patients with acute infectious arthritis caused by other organisms gave negative results. Cross-reactivity of gonococcal arthritis sera with Mimea polymorpha was not observed. The results indicate that the method described provides a means for specific recognition of gonococcal antibodies, and permits epidemiologic study of gonococcal infection and its complications.

5360

Hirschberg, N. 1964. Hemagglutination tests in the detection of antibodies to Neisseria gonorrhoeae. Bacteriol. Proc. M192:80.

An extract of the gonococcus in saline, which attaches easily to tanned sheep cells to provide hemagglutination antigen to test for gonococcal antibodies in serum and in vaginal secretions, has been prepared from

recently isolated strains of the organism and from old laboratory cultures. The antigen is primarily protein and extremely heat-labile, but may be maintained up to 2 months at 4 C and for a year or more in the frozen state. Of 30 sera tested from patients with chronic and acute joint diseases, nine case histories showed positive hemagglutination tests with titers from 1:16 to 1:512; one showed a weak complement-fixation reaction. In acute gonorrhea the tests on sera were negative. In chronic cases and old cured cases, however, a number of sera showed positive hemagglutination tests; 14 specimens from vaginal washings of a series of 100 female patients were positive; seven showed positive smears or cultures. The positive results correlated well with the clinical histories. Fluorescent antibody studies on the sera from this series of patients showed some agreement. Fluorescent antibody appears to be different from the hemagglutinating antibody. With a few exceptions, both tests were negative in a large series of tests on sera of young normal males.

5361

Holman, M.S.; Koornhof, H.J.; Hayden-Smith, S. 1964. The laboratory diagnosis of gonorrhea in the female. S. Afr. J. Lab. Clin. Med. 10:95-98.

Compared with Gram-stained smears, the extra expense and work involved in the direct FA test seem to be well warranted. It is specific and a definite laboratory diagnosis can be given. The delayed FA technique gave considerably better results than culture on blood agar. Subinoculation from Stuart's medium onto Difco gonococcal medium, followed by the delayed FA techniques, gave superior results to Stuart's medium followed by ordinary cultures. The results of Stuart's medium used as a transport medium for the delayed FA techniques, compared with the FA technique after immediate incubation, show that Stuart's transport medium is a very useful adjunct to the conventional culture methods.

5362

Hunter, D.K.; Ziff, M.; Hess, E.V. 1963. Gonococcal antibody response in gonococcal arthritis and Reiter's syndrome by an immunofluorescent method. Texas Rep. Biol. Med. 21:466-467.

Diagnosis of acute gonococcal arthritis and etiological studies in Reiter's syndrome have been hampered by the lack of a reliable and specific test procedure for the detection of gonococcal antibodies. This report details results obtained utilizing indirect FA. N. gonorrhoeae were cultured on standard media, and formalin-fixed smears were prepared from cultures harvested at 18 hours. Test sera were reacted with the fixed organisms for one hour. Strong fluorescence of N. gonorrhoeae was noted with 1:100 or greater dilutions of the acute sera of all of 15 patients with definite gonococcal arthritis. Titers up to 1:1600 were observed. Sera of 14 of 21 patients with a clinical diagnosis of gonococcal arthritis were positive at a dilution of 1:100. Only

5 of 24 patients with Reiter's syndrome gave positive reactions. Eleven patients with other diseases were negative. Positive results were obtained in 5 of 27 acute gonorrhea cases. FA provides a sensitive means of titrating gonococcal antibody and permits epidemiologic study of Reiter's syndrome, non-gonococcal urethritis, and related conditions. Complete article.

5363

Grossman, M.; Sussman, S.; Gottfried, D.; Quock, C.; Ticknor, W. 1964. Immunofluorescent techniques in bacterial meningitis: Identification of Neisseria meningitidis and Hemophilus influenzae. Amer. J. Dis. Children 107:356-362.

This study was designed to investigate the application of the fluorescent antibody technique to the rapid diagnosis of meningococcal and H. influenzae meningitis by staining cerebrospinal fluid smears. Conjugates of polyvalent meningococcal and H. influenzae antisera were of excellent potency and specificity. The application of these conjugates to the diagnosis of meningitis from initial cerebrospinal fluid smears was disappointing. In the case of meningococci, the fluorescent antibody technique was comparable to Gram stain and in the case of H. influenzae it was not quite as good as Gram stain. The application of the fluorescent antibody technique to cerebrospinal fluid smears shares the limitation of other direct fluorescent antibody examination of clinical material in bacterial disease. Further trial of this method seems warranted, perhaps with the modification of incubating the cerebrospinal fluid for a few hours before examination.

5364

Kellogg, D.S., Jr.; Deacon, W.E. 1964. A new rapid immunofluorescent staining technique for identification of Treponema pallidum and Neisseria gonorrhoeae. Proc. Soc. Exp. Biol. Med. 115:963-965.

An immunofluorescent staining technique has been developed that specifically stains T. pallidum or N. gonorrhoeae in less than one minute. Application of the technique to direct smears of either organism has demonstrated its practicability as a diagnostic tool.

5365

Kellogg, D.S., Jr.; Peacock, W.L., Jr.; Deacon, W.E.; Brown, L.; Pirkle, C.I. 1963. Neisseria gonorrhoeae: I. Virulence genetically linked to clonal variation. J. Bacteriol. 85:1274-1279.

One type, obtained from the purulent exudate of acute gonorrhea, was maintained by 69 selective in vitro passages, at which point the organisms produced infections in human volunteers. A predominence

of clonal types found in laboratory strains and a lack of ability to infect human volunteers resulted from 69 nonselective in vitro passages. Physiological and serological characteristics of the clonal types are compared. We are now in a position to study Neisseria gonorrhoeae organisms in their virulent form. Organisms were identified in exudates by direct and indirect FA.

5366

Migulina, V.M. 1961. Employment of the method of fluorescent antibodies for the diagnosis of gonococcus. Vestn. Dermatol. Venerol. 35:54-58. In Russian.

FA was used for differential diagnosis of the gonococcus. Rabbit anti-gonococcus and anti-staphylococcal sera were used, conjugated with FIC. Direct FA was used to stain cultures of each bacterium. Cross-staining was observed. Staining brilliance varied with culture age and other factors.

5367

Mitchell, M.S.; Biegeleisen, J.Z., Jr. 1965. The effect of penicillin on immunofluorescent staining of Diplococcus pneumoniae, Neisseria meningitidis, and Hemophilus influenzae in cerebrospinal fluid in vitro. J. Lab. Clin. Med. 66:53-63.

Several strains of Diplococcus pneumoniae, Neisseria meningitidis, and Hemophilus influenzae were exposed to a range of concentrations of penicillin G in pooled human cerebrospinal fluid at 37 C in vitro. Pneumococci were killed and lysed at bactericidal concentrations of penicillin, but a large percentage of these cells seemed to be unchanged in appearance from the untreated state on immunofluorescent staining. At subinhibitory concentrations, organisms of this species may have been slightly enlarged, but capsular staining was again totally unaffected. Neisseria meningitidis, Groups A and C, became enlarged and bloated, and lost most of their capsular staining at bactericidal concentrations of penicillin, but the only difference observed at subinhibitory concentrations was the enlargement of some cells. Neisseria meningitidis, Group B, seemed unchanged in size and staining characteristics at all concentrations of the antibiotic. Hemophilus influenzae, Type b, organisms lost all capsular staining, but were not disrupted at bactericidal concentrations of penicillin. At subinhibitory concentrations, generally little or no change in morphology and integrity of capsular antigen was observed, although 1 to 2 per cent of the cells were long forms with somewhat shaggy capsules.

5368

Mitchell, M.S.; Marcus, B.B.; Biegeleisen, J.Z., Jr. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: II. Growth, viability, and immunofluorescent staining of Hemophilus influenzae, Neisseria meningitidis, and Diplococcus pneumoniae in cerebrospinal fluid. J. Lab. Clin. Med. 65:990-1003.

H. influenzae, N. meningitidis, and D. pneumoniae were studied. H. influenzae, Type b, could be isolated in 7 to 9 days at 4 or 25 C, and intense FA staining of capsules was observed for as long as 2 weeks. At 37 C the organism could not be cultured beyond 2 to 3 days. The capsules appeared ragged, as judged by the FA staining reaction, and by 18 to 24 hours, fluorescence was considerably diminished. Ability to be stained was lost in most cases after 48 hours of incubation at 37 C. N. meningitidis, Group B, could be cultured from specimens of CSF for 7 to 8 days when stored at 4 or 25 C and an average of 15 days at 37 C. FA staining seemed equally bright indefinitely at all storage temperatures, although after 5 to 7 days the staining may well have been somatic rather than capsular. Pneumococci were cultured from CSF specimens for an average of 17 days at 4 or 37 C and an average of 21 days at 25 C. FA staining remained. In normal cerebrospinal fluid seeded with cultures of the above three species of organisms in vitro, immunofluorescent staining characteristics were very similar to those in clinical specimens, but viability was, in almost all instances, considerably shorter. All cultures tested multiplied consistently with the exception of meningococci of Groups A and C, which only rarely grew in CSF.

5369

Mitchell, M.S.; Rhoden, D.L.; King, E.O. 1965. Lactose-fermenting organisms resembling Neisseria meningitidis. J. Bacteriol. 90:560.

Organisms resembling meningococci morphologically, culturally, and serologically were isolated during a study of asymptomatic carriers. They differed in that they fermented lactose repeatedly. Slide agglutination and FA staining grouped these organisms serologically.

5370

Moore, M.B., Jr.; VanderStoep, E.M.; Wende, R.D.; Knox, J.M. 1963. Fluorescent gonococcal antibody technique in gonorrhea in the male. Public Health Rep. 78:90-92.

The delayed fluorescent antibody test for gonorrhea was compared with a routine culture technique in 477 men. Men were selected because diagnosis, both clinically and culturally, is more accurate in men than in women. The results of the two tests were in agreement in 92.4 per

cent of the cases. In 5.7 per cent the FA test was positive and the culture was negative; in 1.9 per cent the culture was positive and the FA test negative. This study indicates that the delayed FA test for gonorrhea will be available additon to diagnostic tests available for the study of urethritis in men.

5371

Mukobayashi, H. 1964. Immunohistochemical studies of the site of human chorionic gonadotropin production. Wakayama Med. Rep. 8:63-73.

This survey deals with the bacteriological diagnosis of 263 Bantu females suspected of gonorrhea on account of lower abdominal pain, burning on micturition, and a vaginal discharge. It is shown that the recently described fluorescent antibody techniques have very definite advantages over the conventional methods. A summary and an evaluation of the various methods, together with recommendations of procedures, are presented in detail.

5372

Ocinnikov, N.M. 1963. An appraisal of the fluorescent antibody method in gonorrhoea. Bull. WHO 29:781-788.

Fluorescent antibody procedures have in a short time become valued techniques for the detection of many pathogenic microorganisms, and are used in syphilis, for instance. A fluorescent antibody test has also been proposed for use in gonorrhoea, but the author suggests that there are still many questions to be answered before that test can be recommended for widespread practical application. In particular, large-scale further research is necessary on the antigenic structure of Neisseria gonorrhoeae, and of other organisms of the Neisseria group, before reliance can be placed on the strict specificity of this test. The author also describes the fluorescent antibody technique for gonorrhoea used in the USSR, discussing its advantages and disadvantages.

5373

Ovchinnikov, N.M. 1964. Immunofluorescent method in the diagnosis of gonorrhea. Vestn. Dermatol. Vener. 38:53-59. In Russian.

On the strength of the study of antigenic properties of Vi, O, and H cultures and the protective properties of pure Vi, O, and H antisera of Salmonella, the study of immunogenic capacity of gonococcal vaccines prepared by different techniques, and ultra-thin sections of gonococci examined in the electron microscope, the author denies the identity of the structure of gonococcal K-antigen and Salmonella. There is only an external similarity; the essence of the immunity is different: typhoid produces immunity in the convalescent, but no immunity remains

after gonorrhea. The author believes that the current methods of immunofluorescence do not permit differentiation between species of the genus Neisseria in the original material, that not all the altered gonococci have similar fluorescence. The author questions the statement that gonorrhea may be diagnosed on the basis of a pair of fluorescent gonococci. This method should be investigated, but so far cannot be recommended as a definitive method for practical purposes.

5374

Pariser, H.; Farmer, A.D.; Marino, A.F. 1964. Asymptomatic gonorrhea in the male. Southern Med. J. 57:688-690.

Evidence is presented by testing for gonorrhea with the fluorescent antibody method and culture technique that the male can harbor the organism without signs or symptoms of infection; he may acquire an asymptomatic infection or may harbor organisms after treatment, despite the disappearance of signs or symptoms. One can only postulate if in this asymptomatic state sufficient viable organisms are present to transmit the disease. Clinically and epidemiologically, transmission by an asymptomatic male seems probable. Cure cannot be determined solely on the basis of the disappearance of signs and symptoms. Post-treatment examinations of urine and prostatic secretions by fluorescent antibody tests and cultures provide the most accurate gauge of cure.

5375

Peacock, W.L., Jr.; Martin, J.E., Jr.; Thayer, J.D.; Schroeter, A.L. 1964. Immunofluorescent detection of serum antibodies to the gonococcus among meningococcal carriers. Antimicrobial Agents and Chemotherapy. 1964, 649-651.

Conventional medium and Thayer-Martin selective medium were used to detect the presence of Neisseria in the nasopharynx of 26 confined male subjects. Eleven of these were found to be asymptomatic nasopharyngeal carriers of meningococci. Serum from each subject was tested by the indirect fluorescent antibody method with a formalin-treated suspension of the Neisseria isolate from the subject and with virulent Type 1 gonococci. Sera from confined subjects free from any Neisseria, from females who had had contact with males with gonorrhea, and from presumably normal young boys were also tested by indirect FA against virulent Type 1 gonococci. Meningococcal carriers are shown to have antibody titers similar to those found in patients with gonorrhea. The indirect FA method, therefore, was ineffective in differentiating meningococcal and gonococcal antibodies.

5376

Peacock, W.L.; Thayer, J.D. 1964. Direct FA technique using Flazo orange counterstain in identification of Neisseria gonorrhoeae. Public Health Rep. 79:1119-1122.

Laboratory diagnosis of gram-stained cervical smears from women suspected of having gonorrhea was so unproductive in the past that when the culture method was used the stained smear procedure was not. Even after immunofluorescent methods had greatly improved diagnosis of direct smears, results were still about 50 per cent inferior to those obtained with the delayed fluorescent antibody procedure or with the culture method. By the use of Flazo orange as a counterstain, nonspecific background fluorescence was quenched without obscuring points of reference, such as pus cells. Eye strain was notably reduced. One hundred fifty-six female contacts of male gonorrheal patients were examined. In the more rapid FA procedure the method of smear preparation and counterstain resulted in specific diagnoses of gonorrhea to within 2 per cent of results by the culture method.

5377

Prince, L.N.; Randall, E.L.; Lentz, J.W.; Shapiro, L.H. 1964. The evaluation of oxytetracycline in the treatment of gonorrhea in the prenatal patient: A preliminary report. Amer. J. Obstet. Gynecol. 90:202-204.

During a study to determine the relative efficiency of the culture method and the fluorescent antibody technique in the detection of gonococcus in the pregnant woman, an incidence rate in 1,470 prenatal patients studied over a period of a year was found to be 2.99 per cent. Twenty-nine of the 32 patients included in the drug evaluation group met the criteria of cure, 90.6 per cent success.

5378

Reyn, A. 1963. Current problems in the diagnosis and therapy of gonorrhea from the laboratory point of view. Acta Dermatovenerol. 43:380-393.

The advantages and disadvantages of the two diagnostic methods, microscopy and culturing, are discussed. The balance between the microscopy and culture results depends on a number of factors, of which the method of transporting the material is most significant. The introduction of Stuart's transportation medium all over Scandinavia is an important step forward. For practical reasons one must adhere to 24 hours as permissible transport time and negative results obtained after more than 36 hours of transportation are less reliable. The classical bacteriological technique may be supplemented with or supplanted by the fluorescent antibody technique; as a consequence of antibiotic therapy it is shown in gonococcal strains isolated in Denmark that the sensitivity to antibiotics has changed.

5379

Shapiro, L.H. 1963. Gonorrhea in females. GP 28:78-82.

The diagnosis of gonorrhea in females is difficult. Although most infected females are asymptomatic, they are no less infectious. A high index of suspicion and a follow-up of contacts yield a high percentage of positive diagnoses. Examination of gram-stained smears in the female is of little or no value. Culture techniques are extremely useful but they must be thorough. Endocervical curettage greatly increases the possibility of a positive culture. The fluorescent antibody technique also helps detect the gonococcus.

5380

Shapiro, L.H.; Lentz, J.W. 1963. Fluorescent antibody technique in diagnosis of gonorrhea in females. Obstet. Gynecol. 21:435-437.

The delayed fluorescent antibody technique is of great value in the diagnosis and epidemiology of gonorrhea because it greatly reduces the time factor in making a laboratory diagnosis in females and because, in a study of 148 women named as contacts, it enabled us to make the diagnosis in 47.3 per cent. Curettement of the endocervix continues to be a useful means of obtaining material diagnostic of gonorrhea.

5381

Vickers, F.N.; Maloney, P.J. 1964. Gonococcal perihepatitis: Report of three cases with comments on diagnosis and treatment. Arch. Intern. Med. 114:120-123.

Gonococcal perihepatitis is a disease of young women, usually reporting right upper quadrant pain as a major symptom. Previous history of pelvic inflammatory disease can usually, though not always, be obtained. Differential diagnostic considerations include pyelonephritis, cholecystitis, hepatitis, and pleurisy. Vaginal smear and Gram stain will usually not be conclusive. Culture for the gonococcus or the new fluorescent antibody methods are necessary for definitive diagnosis. The mechanism of jaundice in the occasional patient with this syndrome is not as yet clear. The treatment for this condition is penicillin. Three illustrative cases from our experience have been presented.

5387

White, L.A.; Kellogg, D.S., Jr. 1965. Neisseria gonorrhoeae identification in direct smears by a fluorescent antibody counterstain method. Appl. Microbiol. 13:171-174.

Direct smears from female patients have been considered unreliable for the detection of Neisseria gonorrhoeae by FA methods because of the inadequate background contrast of the fluorescein-stained smears and a scarcity of organisms on the smear. Evans blue dye employed as a counterstain eliminated the nonspecific background staining and increased the reliability of the direct FA procedure. Direct smears demonstrating positive fluorescence were obtained from 86 per cent of a group of culturally positive females. The FA-counterstain technique is as sensitive as the presently recommended cultural procedures.

XI. PSEUDOMONADALES

A. GENERAL

5383

Biegeleisen, J.Z., Jr.; Mitchell, M.S.; Marcus, B.B.; Rhoden, D.L.; Blumberg, R.W. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: I. Clinical evaluation of conjugates on smears prepared directly from cerebrospinal fluid sediments. J. Lab. Clin. Med. 65:976-989.

Techniques were described for the cultivation and immunofluorescent identification of Hemophilus influenzae, Diplococcus pneumoniae, Neisseria meningitidis, and eight less common pathogens in specimens of cerebrospinal fluid from 100 patients with bacterial meningitis. A comparison of the results obtained by conventional methods and by immunofluorescent staining indicated that the latter method was fully as sensitive as the former and was more accurate in treated cases. Some of the dangers involved in the use of the Gram stain of the sediment as a tool for presumptive diagnosis were discussed, as were shortcomings of fluorescent antibody staining, particularly in infections caused by uncommon gram-negative organisms. The immunofluorescent staining technique was recommended for the rapid screening of spinal fluid specimens, as well as of cultural isolates.

5384

Biegeleisen, J.Z., Jr.; Mosquera, R.; Cherry, W. 1964. A case of human melioidosis: Clinical, epidemiological, and laboratory findings. Amer. J. Trop. Med. Hyg. 13:89-99.

The clinical, epidemiological, and laboratory aspects of the first confirmed case of human melioidosis reported in Ecuador are presented. The results of conventional bacteriological and serological testing and of animal inoculation confirmed the presumptive diagnosis of infection with Pseudomonas pseudomallei made by the fluorescent antibody test. Specifically stained fluorescent organisms were demonstrated in tissue impression smears prepared from the organs of guinea pigs experimentally infected with the specimen cultures. The value of immunofluorescence for screening specimens was emphasized by the rapidity with which Pasteurella pestis and P. tularensis were excluded from consideration as probable etiologic agents of this infection and the short period of time required to make a presumptive identification of P. pseudomallei. The role of fluorescent antibody procedures in bacteriological and histopathological laboratories is discussed. A procedure for minimizing erroneous diagnoses of melioidosis is proposed.

5385

Tanaka, C.; Teraoka, A. 1964. Site of action of bacterial pyrogen immunofluorescent studies in mice. Jap. J. Pharmacol. 14:232-233.

FA demonstrated pyrogen in cytoplasm of polymorphs. In frozen sections it was seen in Kupffer cells of the liver, reticulum cells of the spleen, glomeruli, tubular epithelium, lung interstitium, vessel walls, intravascular spaces, leukocytes, and macrophages.

B. VIBRIO

5386

Belden, E.L.; Robertstad, G.W. 1965. Application of fluorescent antibody technique for serotyping Vibrio fetus. Amer. J. Vet. Res. 26:1437-1441.

Fluorescein conjugated antisera to Montana serotypes I, II, III, and V were used to serotype 33 Vibrio fetus isolates of human, bovine, and ovine origin. Isolates were placed in serogroups I, II, V, or II:V, depending on their reactions with fluorescent antisera. The FA reaction was more sensitive than the tube agglutination test to detect cross-reactions. Cross-reactions did not occur with eight saprophytic Vibrio-like organisms. In addition, two V. fetus isolates could not be placed into the typing scheme. A pooled conjugated antiserum was used to positively stain all but two of the V. fetus isolates. A pooled antiserum is suggested for diagnostic test purposes.

5387

Felsenfeld, O. 1964. Present status of the El Tor vibrio problem. Bacteriol. Rev. 28:72-86.

The taxonomic position of the El Tor vibrios has not been clarified as yet. Since hemolysis, a textbook characteristic of El Tor strains, may be slow or absent, phage susceptibility is recommended as a criterion in separating El Tor from true cholera vibrios. Additional tests are listed that may be of value in the differential diagnosis of these organisms. The culture of the El Tor organisms for diagnostic purposes in the laboratory does not present greater problems than that of true cholera vibrios. Vaccine field trial under well-controlled conditions should be carried out. FA is mentioned as a research tool and for identification of vibrios in water samples.

5388

Mellick, P.W.; Winter, A.J.; McEntee, K. 1965. Diagnosis of vibriosis in the bull by use of the fluorescent antibody technic. Cornell Vet. 55:280-294.

An FA conjugate was prepared by labeling the gamma globulin fraction from a pool of rabbit antisera for one strain of Vibrio fetus venerealis with FITC. Nonspecific fluorescence was minimized by using a fraction of the conjugate separated by ion-exchange chromatography. Cross-reactions in the FA reaction were observed with intestinal and venereal strains of V. fetus, but not with V. bubulus or 17 other species of bacteria tested. The conjugate was used to stain smears of preputial fluid from a group of 24 bulls. This group included known carriers and bulls of various ages from which V. fetus had not been isolated. Samples from each bull were examined weekly for 6 consecutive weeks. Complete agreement was obtained between the results of FA on preputial fluid and the results of cultural examination of semen samples from these bulls. FA provides a highly accurate and sensitive method for the detection of V. fetus carrier bulls.

5389

O'Berry, P.A. 1964. A comparison of three methods of serum fractionation in the preparation of Vibrio fetus fluorescent antibody conjugates. Amer. J. Vet. Res. 25:1669-1672.

Lapine and bovine hyperimmune sera were prepared by parenteral injection of two venereal strains of Vibrio fetus. These sera and those from rabbits and cows negative for V. fetus agglutinins were fractionated by procedures utilizing ethanol, ammonium sulfate, and ethodin. Globulin fractions were conjugated to fluorescein isothiocyanate, studied electrophoretically, and found to be composed of gamma globulins with a trace of beta globulins. Homologous and heterologous V. fetus cell suspensions and smears were stained with each fluorescent antibody conjugate. Conjugates prepared from serum fractionated with ammonium sulfate were superior. Bright staining was observed more frequently with bovine serum conjugates than with rabbit serum conjugates. Although the staining of cell suspensions was rapid and simple, better results were obtained by staining smears.

5390

Pan, I.-H. 1965. Rapid detection of V. cholerae by fluorescent antibody technique. J. Formosan Med. Ass. 64:313-317.

The fluorescent antibody technique was applied for the rapid diagnosis of V. cholerae. The results showed not type-specific but species-specific fluorescence. Inhibition tests using a ten-times diluted serum demonstrated a positive result. The serum without heat treatment was proved to give a good fluorescence at pH 7.2.

5391

Sack, R.B.; Barua, D. 1964. The fluorescent antibody technique in the diagnosis of cholera. Indian J. Med. Res. 52:848-854.

Direct stool smears stained by the fluorescent antibody technique have been evaluated in the diagnosis of cholera. In a series of 80 patients there was approximately 95 per cent correlation between the fluorescent smear and the bacteriological findings. The specificity and sensitivity of the technique have been studied.

5392

Schimmelpfennig, H.; Mitscherlich, E. 1964. The use of fluorescence serology in bacteriological diagnosis: I. Differentiation of Vibrio fetus from Vibrio El Tor by fluorescent antibody. Zentralbl. Veterinarmed. Reihe B 11:5:393-406. In German.

A simple and rapid method for differentiating V. fetus and V. El Tor strains by means of FA is described. It is necessary to have high-titer specific sera and complete vibrio antigens. Specificity of the V. fetus sera and conjugates is obtained by absorption with an antigen mixture of several vibrio types. A marked and an unmarked El Tor serum behaved specifically after absorption with a mixture of antigens from two vibrios isolated from water. With the help of specific sera, 71 of 74 V. fetus and V. bubulus strains were differentiated. In addition three strains of vibrio El Tor and three other strains belonging to Group 1 cholera-O were differentiated from 11 non-pathogenic Vibrio strains isolated from water.

XII. SPIROCHAETALES

A. LEPTOSPIROSES

5393

Chernukha, Y.C.; Korn, M.Y. 1965. Results of use of the fluorescence technique in study of leptospirae. J. Hyg. Epidemiol. Microbiol. Immunol. 9:240-246.

Experiments were carried out with anti - L. grippotyphosa and anti - L. icterohaemorrhagiae sera labeled with fluorescein isocyanate. Before staining, the Leptospira preparations were fixed with 96 per cent ethanol. In additon to homologous strains, the test sera stained 51 heterologous strains of leptospirae belonging to 36 serotypes. Supplementary control experiments with absorbed labeled sera, however, showed that the fluorescence of leptospiral cells treated with labeled heterologous anti-leptospiral serum was specific.

5394

Hartmann, L.; Filitti-Wurmser, S.; Jacquot-Armond, Y.; Mailloux, M.; Hurez, D.; Fauvert, R. 1964. Macromolecular nature of an antibody of Leptospira australis. Biochim. Biophys. Acta 82:249-259.

Infection by Leptospira australis or L. icterohaemorrhagiae induce the formation of antibodies that are macroglobulins and have a common antigenic determinant, beta-2M. It is highly probable that an unknown structural difference gives rise to an agglutination lysis specific to each serotype of the leptospire.

5395

Imamura, S.; Ashizawa, Y. 1965. Studies on the serological reactions of Leptospira, especially on the sensitized-erythrocyte lysis test and the fluorescent antibody test. Nippon Eiseigaku Zasshi 19:365-368. In Japanese.

Efforts were made to find a serodiagnostic test for leptospirosis with high sensitivity and specificity that is easily performed without the use of living leptospirae. The leptospiral sensitized-erythrocyte lysis test developed by Chang and others was studied using freeze-dried antigen obtained from sonically disrupted cells and the results were compared with those of the agglutination-lysis test. The test was a valuable diagnostic procedure. The indirect FA test was also studied, and the results were compared with those of the agglutination-lysis test. Test results with the two procedures showed good correlation, but the FA test was less sensitive than the agglutination-lysis test.

5396

Maestrone, G. 1963. Demonstration of leptospiral and viral antigens in formalin-fixed tissues. Nature 197:409-410.

Modifications of tissue preparation procedures for FA staining are described. These modifications warrant further investigation because of the following practical implications: Elimination of the hazard of handling infectious material; simplification of collection, shipment, and storage of suspected specimens; investigation by FA of specimens submitted for routine histopathological examination; and possibility of a more extensive use of this relatively new tool.

B. TREPONEMATOSES

5397

Anonymous. 1965. Blood tests for syphilis. Brit. Med. J. 1:76-77.

This is a discussion of the historical development of serologic tests for syphilis. Reference is made to the relative testing ease and interpretation of results. Problems in testing are reviewed.

5398

Anonymous. 1965. Persistence of treponemes after treatment of syphilis. Lancet 1:693-694.

The virulence of persisting treponemes may be attenuated: they may have come to terms with their human host and perhaps be kept in check by an immune response to their continued presence. Such treponemes are presumably not actively dividing, and they may be walled off in fibrous tissue, both of which circumstances would lessen the efficacy of penicillin against them. The American workers rightly point out that their results are not evidence against the efficacy of penicillin in syphilis, and the possibility of reinfection in their patients cannot be excluded. Plans for the eradication of syphilis must still be based on a determined search for cases and their prompt and effective treatment, and penicillin remains the best treatment available. The FTA test was used.

5399

Argonza, W.S.; Jue, R.F.; Puffer, J.; Bodily, H.L.; Wood, R.M. 1965. A useful adaptation of the fluorescent treponemal antibody test for syphilis: The simultaneous use of the FTA-5 and the FTA-200 (FTA-5-200). Health Lab. Sci. 2:246-249.

A fluorescent treponemal antibody procedure, the FTA-5-200, has been shown to give results equivalent in sensitivity and specifity to those obtained by the Treponema pallidum immobilization test in two-thirds of the specimens submitted for TPI tests. Its use as a screening procedure for specimens on which TPI tests are requested offers advantages.

5400

Bellone, A.G.; Leigher, G. 1964. Observations on the FTA test with particular regard to untreated primary syphilis. G. Ital. Dermatol. 105:175-184. In Italian.

The FTA test at serum dilutions of 1:5, 1:50, and 1:200 was tested, together with two complement fixation quantitative tests, the VDRL and the qualitative TPI test, on 281 luetic patients in different stages of the infection, either not yet treated or in treatment, and on many control subjects. It was possible to show that in untreated primary syphilis the FTA test was highly specific and provided almost the same sensitivity as the complement fixation test with Reiter's treponemic antigen and was slightly more sensitive than the VDRL. In other stages of syphilis, and in the controls, the FTA test had a high sensitivity and specificity, with parallel results to the TPI test. Following treatment the FTA test persists reactive for a longer time than the other serologic tests.

5401

Beurey, J.; Morel, J.; Gury, C. 1964. Our experience with immunofluorescence: Relating to 250 sera studied at the Nancy Dermatological Clinic. Bull. Soc. Franc. Dermatol. Syph. 71:676-678. In French.

Using the indirect immunofluorescence method, quantitative determinations of antibodies of 250 sera in the diagnosis of syphilis were made and the method compared with the classic serological test and the treponemal immobilization test. Reproducibility, sensitivity and specificity are adequate. The method is useful in late diagnosis of the disease, but the technique is still not standardized.

5402

Bradford, L.L.; Bodily, H.L.; Ketterer, W.A.; Puffer, J.; Thomas, J.E.; Tuffanelli, D.L. 1965. FTA-200, FTA-ABS, and TPI tests in serodiagnosis of syphilis. Public Health Rep. 80:797-804.

Previous reports have suggested the possibility of substituting fluorescent treponemal antibody (FTA) methods for the more expensive and technically complex Treponema pallidum immobilization (TPI) test for the serodiagnosis of syphilis. This study reports on the reproducibility of the FTA-200 test and compares the sensitivity and specificity of TPI, FTA-200, and FTA absorption (FTA-ABS) tests. The FTA-200 test had a high level of reproducibility and specificity and was significantly more sensitive than the TPI test in primary syphilis, of about equal sensitivity in secondary and early latent syphilis, and less sensitive in late syphilis. The FTA-ABS procedure had a comparable level of specificity but was more sensitive than either the TPI or FTA-200 test. The FTA-200 test is a useful tool in the serodiagnosis of syphilis, although it cannot entirely replace the TPI test. Preliminary results with the FTA-ABS procedure are promising.

5403

Brophy, E.M.; Ashworth, C.T.; Arias, M.; Reynolds, J. 1964. Acute syphilitic nephrosis in pregnancy. Obstet. Gynecol. 24:930-937.

A case of acute syphilitic nephrosis appearing in the 32nd week of pregnancy is described. Electron micrographs of a renal biopsy taken after delivery are presented. Syphilitic osteomyelitis of the skull was a complication in this case. The patient developed a convulsive disorder following this pregnancy and died of fulminant viral hepatitis 5 months after delivery.

5404

Brown, W.J.; Meyer, P.E.; Nevin, T.A. 1965. Some properties of a treponemal group antigen. Bacteriol. Proc. M83:53.

A treponemal group antigen originally described by Deacon et al. is the subject of continuing study. Current investigations reveal that it can be extracted from Reiter treponemes with dilute mineral acids or trichloroacetic acid. It is insoluble in ether, acetone, or ethanol solutions stronger than 80 per cent. The antigen remained stable when boiled in 0.5 N hydrochloric acid for 10 to 20 minutes. Spirochetal morphology was retained after extraction. Repeated precipitation of the trichloroacetic acid - extracted material from 95 per cent ethanol yielded a pure product as indicated by a single spot on paper chromatograms and a single band on gel diffusion plates. Antigenic activity as demonstrated by immunofluorescence was not reduced. Sonic fractions

of extracted organisms developed three distinct bands in gel diffusion plates, but no group antigen was demonstrable by immunofluorescence. Sonic fractions of unextracted organisms, however, produced three and sometimes four bands with no loss of group antigenicity. It is probable, therefore, that the group antigen may requently be one of the other three bands.

5405

Brown, W.J.; Simpson, W.G.; Moore, M.B.; Price, E.V.; Weinstein, S. 1963. Oral propionyl erythromycin in treating early syphilis. Public Health Rep. 78:911-917.

Oral propionyl erythromycin was used in the treatment of 554 patients with darkfield-positive early syphilis. No serious reactions were reported. Ten grams of erythromycin administered in a period of 8 to 10 days proved inadequate for the treatment of early syphilis. No significant difference was observed between 15 grams and 20 grams given in a 10-day period. The combined results of the two higher dosage schedules showed a re-treatment rate in secondary syphilis of 12.5 per cent compared with a 48 per cent rate for the 10-gram schedule. Erythromycin was more effective in the treatment of females than males, probably because of greater adherence to the prescribed schedule. Results of VDRL slide, KRP, FTA, and TPCF-50 test, performed routinely on pre-treatment and post-treatment sera, are compared.

5406

Buck, A.A.; Spruyt, D.J. 1964. Seroreactivity in the Venereal Disease Research Laboratory slide test and the fluorescent treponemal antibody test: A study of patterns in selected disease and control groups in Ethiopia. Amer. J. Hyg. 80:91-102.

Comparative studies were made between FTA and the VDRL slide test to determine the diagnostic competence of the latter. The 587 persons in the study represented the following: malaria, early syphilis, leprosy, a random sample of a town, and healthy controls. In children under the age of 15 years, the reactions in the VDRL and FTA tests were distributed as if they were independent of each other. After sexual maturity, results of the tests were associated. In children under the age of 2, FTA positive prevalence was highest when seroreactivity in the VDRL tests was lowest. The high prevalence of FTA reactions in infancy followed by a rapid drop suggests that FTA in infants may be residual maternal antibodies. Excess reactivity in the VDRL tests unrelated to treponemal infections was observed in patients with lepromatous leprosy, but not with tuberculoid leprosy. The difference in seroreactivity was found only in the VDRL. The percentage of VDRL reactions unconfirmed by the FTA test was highest in the population of an area with hyperendemic malaria. For three different diagnostic criteria, i.e., splenomegaly, presence of FA to Plasmodium falciparum,

and recovery of plasmodia in thick blood films, the percentage of 'biologic false positive reactions' was higher in the individuals with a positive diagnosis.

5407

Bugiardini, G.; Ferrucci, M.; Docimo, C. 1964. Fluorescence microscopy in serologic diagnosis of syphilis. Arcisped. S. Anna Ferrara 17:857-878. In Italian.

The authors have carried out FTA and complement deviation tests, Meincke II, Kahn, and VDRL tests with 539 sera, of which 179 belonged to syphilitic subjects and 460 to non-syphilitic subjects. FTA was the most sensitive of the tests. FTA gave some false biological positives. These were fewer than in the other tests. The authors evaluate FTA as a test of high sensitivity and good specificity in the diagnosis of syphilis.

5408

Carpenter, C.M.; Boak, R.A.; Miller, J.N.; Le Clair, R.A. 1965. Serologic diagnosis of syphilis. Calif. Med. 102:14-16.

Comparative data from four different laboratories on the TPI and FTA tests on VDRL-reactive sera from patients with no symptoms of syphilis showed ranging agreement of results between the FTA test and the TPI test. The FTA test is complex and requires additional research before its use as a routine public health laboratory procedure can be recommended. The TPI test appears to be unequivocally the test of choice for the diagnosis of late and latent syphilis.

5409

Catalano, P.M.; Schragger, A.H. 1965. Early and latent syphilis. Arch. Dermatol. 92:433-435.

As a portion of this clinical discussion, the authors express the hope that the FTA-ABS test will supplant the TPI.

5410

Colombani, J.; Ripault, J. 1964. The immunofluorescence test applied to the diagnosis of syphilis, comparison with the Nelson test and classical serology: I. Study of 3,156 sera. Pathol. Biol. 12:56-64. In French.

The immunofluorescence test, studied in 3,156 sera in a parallel series with the TPI and SCl tests, has shown identical reactions with TPI in 87.26 per cent of the cases and with classical serology in 83.39 per cent of the cases. The IF test is positive alone principally in the course of primary syphilis at an early stage. It can persist during the course of primary syphilis for 1 to 2 years after the start of

treatment. In rare cases, the IF test can be negative when the TPI is positive, in tertiary and in all forms of syphilis after treatment. It is a highly specific test, where the positive result is due to an antibody that is surely distinct from any other. The possibility of carrying out the IF test quantitatively makes it a useful method for control of the serological evolution in the infected subject.

5411

Daguet, G.L. 1964. Immunofluorescence applications for dermato-venereology. Bull. Soc. Franc. Dermatol. Syph. 71:359-363. In French.

In dermatovenereology, as in many other fields, immunofluorescence is a valuable research method. Its practical applications can only be conducted in the framework of studies comprising indisputable reference standards based on clinical data and conventional methods of demonstrating antigens and antibodies.

5412

Eng, J.; Nielsen, H.A.; Wereide, K. 1963. A comparative study of fluorescent treponemal antibody (FTA) and Treponema pallidum immobilization (TPI) testing in 50 untreated syphilitic patients. Bull. WHO 28:533-535.

This investigation is believed to represent new knowledge of the sensitivity of FTA-200. What appears to be disagreement between the TPI-18 results in Oslo and Copenhagen is believed to be due to a small difference in the TPI sensitivity level in the two laboratories at the time of the study. In the present material, which includes so many sera with only a small amount of immobilizing antibody, even a minor sensitivity difference could have important numerical implications for the over-all results.

5413

Evans, A.J.; Summerly, R. 1964. Pseudo-chancre redux with negative serology: A case report. Brit. J. Vener. Dis. 40:222-224.

This clinical case history included a negative FTA test. TPI and RPCF tests were also negative.

5414

Fegeler, F.; Schoessler, H. 1965. The fluorescence treponema antibody test (FTA) in the framework of specific luesserology. Arch. Klin. Exp. Dermatol. 222:106-114. In German.

Of 238 sera, sent in for examination by TPI test because the diagnosis could not be made either clinically or through classic serology, the results of the FTA tests and TPI tests were compared. A far-reaching

agreement between the two specific serological test methods was the result. Thus, the FTA test represents an important supplement to the TPI test. However, it can be substituted only at certain indications.

5415

Fiumara, N.J. 1963. Biologic false-positive reaction for syphilis. New Engl. J. Med. 268:402-405.

Reagin tests are still valuable in the diagnosis of syphilis. Even when diagnostic problems arise, almost 80 per cent of patients with a persistently positive blood reagin test have or have had syphilis. For this reason, such patients must be considered to be syphilitic until proved otherwise. Today, a diagnosis of biologic false positive should not be made without the benefit of the Reiter-protein CF test as a screening device. If this test is negative, the TPI test should be performed. Only when both treponemal tests are negative can the diagnosis of biologic false-positive reaction be entertained in a patient with a persistently positive reagin blood test. FTA may someday replace the TPI test.

5416

Fiumara, N.J. 1965. Diagnosis of neurosyphilis. J. Amer. Med. Ass. 192:1111.

Even a couple of drops of blood in the spinal fluid may result in a positive FTA test, particularly if the more sensitive FTA absorption test is used. For the FTA or TPI test to be useful in spinal fluid examinations, the spinal fluid must not have any red blood cells in it. These tests are no more useful than the cheaper and faster reagin tests for spinal fluid.

5417

Fribourg-Blanc, A.; Niel, G.; Mollaret, H.H. 1963. Notes on some immunological aspects of the African cynomolgus: I. Antigenic relationship of its gamma globulin with human gamma globulin; II. Guinean endemic focus of treponematosis. Bull. Soc. Pathol. Exot. 56:474-485.

The authors have used the immunofluorescence technique to detect serum antibodies and antigens, and to identify microbial strains. This technique allows demonstration of an antigenic relationship between human and cynomolgus gamma globulins. Guinean cynomolgus tested with Nichol's strain of Treponema pallidum do not provide differentiation of T. pallidum from T. pertenue, the agent of yaws, and from T. carateum. In 50 per cent of the cases they probably react to yaws antigen, and this fact suggests that eradication of endemic human yaws should also involve an action against monkeys.

5418

Fry, C.S.; Wilkinson, A.E. 1963. A note on the use of Evans blue as a background stain in the fluorescent treponemal antibody test. Brit. J. Vener. Dis. 39:190-191.

The FTA test was improved by using Evans blue as a counterstain that quenched background fluorescence.

5419

Garbin, S.; Piacentini, I. 1964. The importance of immunofluorescence in the serodiagnosis of syphilis. Boll. Ist. Sieroterap. Milan 43:240-248. In Italian.

The immunofluorescence test was compared with the common serological reactions for syphilitic serodiagnosis in 400 subjects. The FTA always turned out to be negative in nonsyphilitic sera, even though these latter showed positivity in other serological reactions. On the contrary, it turned out to be positive in the sera of formerly syphilitic patients, even though the other serological reactions were negative in these patients, with the exception of five cases, old syphilitics under treatment, who should be considered completely recovered. Only one case of positivity of the FTA without illness was shown in a fetus, son of a syphilitic mother. The antibodies, passively transmitted through the placentar route, were detected only with FTA and disappeared after some weeks.

5420

Garbin, S.; Piacentini, I. 1965. Contribution to the study of passage of antitreponemal antibodies from the mother to the fetus. Boll. Ist. Sieroterap. Milan 44:102-103. In Italian.

A healthy child, born of a syphilitic mother, showed at his birth antibodies detectable only by means of the FTA, with absence of complement-deviating and flocculating antibodies. These fluorescent antibodies disappeared from the child's serum within 4 months. As far as we know, the demonstration of the passage of these antibodies through the placentar filter was never found in healthy subjects.

5421

Gross, W.M.; Ball, M.R. 1964. Use of fluorescein-labeled antibody to study Borrelia anserina infection (avian spirochetosis) in the chicken. Amer. J. Vet. Res. 25:1734-1739.

The direct fluorescent antibody technique was used to detect Borrelia anserina in the tissues and blood of experimentally infected chickens. During the acute and crisis stages of spirochetosis, organisms were found in the liver, spleen, lungs, kidneys, and brain but were con-

fined to the vascular system. Spirochetal antigen could not be demonstrated in the tissues of recovered and challenged immune birds. The efficacy of the FA test for detecting Borrelia in blood fi.. was compared with the Wright staining technique. Results obtained from both procedures were in complete agreement, but FA allowed a more rapid examination of specimens.

5422

Guarguaglini, M. 1964. Evaluation of the treponema agglutination (TPA) test as compared with the Nelson-Mayer (TPI) test and immunofluorescence (FTA) test in serodiagnosis of syphilis. Boll. Ist. Sieroterap. Milan 43:221-233. In Italian.

The author, after outlining the improvements already brought to the TPA technique, illustrates the results obtained in the study of experimental infection in the rabbit and after treatment with penicillin and in the study of 350 human sera and fluids from subjects in different phases of syphilis, from healthy people, and from people of unknown clinical history. The comparative study of the TPA with other serological reactions, TPI, FTA, and standard serology, clearly pointed out that this test is deeply sensitive. This test is a valuable tool of diagnostic serology of syphilis within the reach of any laboratory.

5423

Hadida, M.E.; Allinne, M.; Orfila, J. 1963. New serological reactions in syphilis. Bull. Soc. Franc. Dermatol. Syph. 70:660-662. In French.

The Nelson test was compared to the reactions to the lipidic antigens, the complement fixation reactions to the treponemic antigens, both pathogenic and nonpathogeni and the immunofluorescence reaction. An attempt was made to evaluate the sensitivity and specificity of these various reactions. Results indicated that the Nelson test continues to be necessary in judging the disputed cases.

5424

Hamashima, Y. 1963. Rapid diagnosis by fluorescent antibody. Jap. J. Clin. Pathol. 11:467-475. In Japanese.

Streptococcus, Staphylococcus, Treponema pallidum and Toxoplasma gondii can be specifically detected by the fluorescent antibody technique. This technique is applicable for clinical use. This technique, if some related problems are solved, can also be applied to the detection of other pathogenic bacteria.

5425

Hepler, J.K.; Connally, J.; Fiumara, N.J. 1965. Syphilis and post-treatment serologic response. J. Amer. Med. Ass. 191:961.

This letter and answer by Dr. Fiumara concern a case of confusing serology following apparently successful treatment of syphilis. The longer time before reversion to negative serology exhibited by the FTA test as compared with other tests is discussed briefly.

5426

Huan-Ying, L. 1964. The use of fluorescent antibody in the serodiagnosis of syphilis. Chin. Med. J. 83:11-16.

A self-made fluorescence microsope has been used in the examination of 793 serum samples from syphilitic and 1,069 serum samples from dermatologic cases by the indirect fluorescent antibody test. It is concluded that the sensitivity and reproducibility of the fluorescent treponemal antibody test are both superior to those of the routine serologic tests, and that the specificity is comparable with that of the Treponema pallidum immobilization test.

5427

Hu[illegible]er, E.F.; Deacon, W.E.; Meyer, P.E. 1964. An improved FTA test for sy[illegible]ilis, the absorption procedure FTA-ABS. Public Health Rep. 79:410-412.

An absorption technique designed to remove nonspecific treponemal antibodies from human sera has resulted in an improved FTA test modification designated the FTA-ABS test procedure. The sorbing agent is a sonicate of the Reiter treponeme containing common or group nonspecific treponemal antigen. Experimental test results indicate that the new procedure is more than twice as sensitive as the FTA-200 test, and specificity equals that obtainable by the TPI test procedure.

5428

Kellogg, D.S., Jr.; Deacon, W.E. 1964. A new rapid immunofluorescent staining technique for identification of Treponema pallidum and Neisseria gonorrhoeae. Proc. Soc. Exp. Biol. Med. 115:963-965.

An immunofluorescent staining technique has been developed that specifically stains T. pallidum or N. gonorrhoeae in less than one minute. Application of the technique to direct smears of either organism has demonstrated its practicability as a diagnostic tool.

5429

Kellogg, D.S., Jr.; Deacon, W.E. 1965. Fluorescent antibody dark-field detection of Treponema pallidum. Bacteriol. Proc. M128:60.

This paper presents the results of applying a rapid, direct fluorescent antibody technique, employing fluorescein-conjugated T. pallidum antisera, to the detection of T. pallidum in lesion material. Comparisons of data developed from triplicate specimens, one examined by conventional dark-field microscopy as a freshly prepared specimen and two fixed and examined by fluorescent antibody dark-field techniques, both locally and after mail to distant laboratories, show close agreement between the two procedures. The fluorescent antibody dark-field technique specifically detects T. pallidum in specimens from the oral cavity that contained treponemes morphologically indistinguishable from T. pallidum. The fluorescent antibody dark-field technique offers answers to several problems inherent to the conventional dark-field technique.

5430

Kent, J.F.; DeWeerdt, J.B.; Lawson, W.B.; Kinch, W.H. 1964. Course of the serologic reactions in rabbits inoculated with different numbers of Treponema pallidum. Amer. J. Clin. Pathol. 42:33-36.

Pairs of rabbits inoculated respectively with 10^8, 10^5, and 10^2 Treponema pallidum were tested serologically during a period of 128 days for anticardiolipin, anti-Reiter protein, fluorescent trepomenal, and treponemal immobilizing antibodies. The rapidity as well as order of appearance of the antibodies was a function of size of the inoculum. Chemotherapy resulted in decreases in the titers of anticardiolipin and anti-Reiter protein more rapidly than those of fluorescent or treponemal immobilizing antibodies.

5431

Kiraly, K.; Jobbagy, A.; Mecher, T. 1965. Fluorescent treponemal antibody testing. Bull. WHO 33:687-703.

Contradictory reports have been published on the value of FTA as a serological test for syphilis. This is due mainly to differences in technique and especially to variations in the quality of the conjugate used. They describe the preparation of an FITC immune serum conjugate, that is characterized by its antihuman-globulin titer, FITC/protein ratio, and staining effect. The FTA-50 test performed with this conjugate gave better agreement with the TPI test than any of four other serological tests with which it was compared. Its sensitivity was good, being equal to that of the T. pallidum complement-fixation (TPCF) test. Nevertheless, the FTA-50 test was not absolutely specific and, as a screening procedure before TPI testing, a combination of the TPCF test with the cardiolipin complement-fixation test appeared to be simpler, more sensitive, and cheaper. Further study was needed with a view

to the elimination of nonspecific staining and standardization of the conjugate and antigen before the FTA-50 test could replace the TPI test.

5432

Leibovitz, A.; Oberhofer, T.R.; Meacham, J.T; Diestelhorst, T.N. 1963. Enhancement of specificity of the fluorescent treponemal antibody test as compared with the TPI test. Amer. J. Clin. Pathol. 40:480-486.

The production of Treponema pallidum antigen that readily adheres to glass slides is further resolved by suspension of organisms treated with sodium hypochlorite in normal saline solution that contains 5 per cent inactivated normal rabbit serum This solution also permits the storage of treponemes in the deep freeze without diminution of fluorescence. The fluorescent treponemal antibody (FTA) 100 test was as specific and as sensitive as the Treponema pallidum immobilization test to rule out biologic false positive reactors. Paired serum specimens enhanced the reliability of both tests in rendering a definite diagnosis.

5433

Manikowska-Lesinska, W. 1963. Investigations into a simplified modification of the immunofluorescence test for treponemes (IFTT). Przegl. Dermatol. 50:195-196. In Polish.

The results of study of 175 individuals presented here, although in need of confirmation on a larger scale, indicated that the simplified modification of the test has a great sensitiveness and seem to be applicable to mass examination. The technique is mainly concerned with blood serum collection by finger stick.

5434

Manikowska-Lesinska, W. 1965. Studies on a simplified modification of treponema immunofluorescence reaction Przegl. Dermatol. 52:353-358. In Polish.

Treponema FA test is sensitive, considerably specific, and comparatively easy to accomplish, which renders it suitable to serologic mass examinations. An analysis of 2,800 examinations proves that sensitivity and specificity of the simplified modification equals that of the Vaisman method.

5435

Longhi, A. 1964 The FTA immunofluorescence test for syphilis. Boll. Sci. Med. 136:246-250. In Italian.

After a review of various diagnostic methods, results of the indirect FTA test during various stages of clinical development of treponemic

infection are reported and compared with the other methods. In primary pre-serological syphilis, the usual blood tests are negative, whereas FTA gives positive results very early. In syphilis with chancre dating back some weeks or already receding but untreated, FTA and blood tests are positive, but the TPI test is usually negative. In secondary syphilis, all of the tests are positive, but treatment can lead to negative reactions. Tertiary syphilis with central nervous system involvement shows a positive FTA for blood or spinal fluid in 94 per cent of cases. Conventional blood tests are 70 per cent positive. In late syphilis with no apparent symptoms, FTA is positive in a considerable number of cases, even after treatment. Course and duration of treatment at various stages, as well as in congenital syphilis, must be evaluated in light of the test results. The FTA method is recommended for its broad margin of biological safety and practical possibilities.

5436

Longhi, A.; Caleffi, M.L.; Toniutti, M. 1964. Observations on the value and significance of the FA test in the serological study of syphilis. Arch. Ital. Dermatol. Venereal. 32:256-300. In Italian.

A serologic study was made on sera obtained from syphilitics in all stages of syphilis and also normals. The antibody response was studied by the FA test, which was compared with the classical reactions (CF and flocculation test) and the TPI test. FA is a sensitive and highly specific test. It reacts very early in infection, when all of the other reactions are negative. It behaves in a manner analogous to the TPI test in secondary, tertiary, old, latent, and congenital syphilis. In recent, cured syphilis it becomes negative more slowly than in the Nelson test during the first 3 years, and becomes completely negative during the 4th year.

5437

Luger, A.; Ebner, H. 1963. The classical syphilis serology following Salvarsan and penicillin treatment. Wiener Klin. Wochensch. 75:629-634. In German.

The course of reagin titer following treatment of syphilis in various stages is reported. FA is briefly mentioned.

5438

Metzger, M.; Ruczkowska, J. 1964. Influence of lysozyme upon the reactivity of Treponema pallidum in the fluorescent antibody reaction. Arch. Immunol. Therap. Exp. 12:702-708.

Serum and tissue lysozyme plays an essential role in the development of reactivity in previously nonreactive treponemes. The ability of freshly extracted treponemes to combine with fluorochrome-labeled antibody

developed at a much faster rate in the presence of tissue-extracted lysozyme; removal of this enzyme by repeated washing of treponeme suspensions caused distinct prolongation of the time required for the organisms to become fully reactive. Egg white lysozyme was also shown to have an accelerating effect on the rate at which the reactivity of these organisms developed. Antibody-combining capacity of treponemes occurred faster when they were incubated at 4 C than at 37 C.

5439

Meyer-Rohn, J. 1964. First trials and results of the FTA test. Hautarzt. Z. Dermatol. Venerol. 15:673-676. In German.

First experiences and results with the FTA test in 350 tests on non-selected material are described. The results in world literature are confirmed: the FTA test, which shows true syphilis antibodies, distinguishes itself by high specificity. It demands little expenditure but much experience and should always be carried out by the same technician. It can be a routine diagnosis.

5440

Miller, J.N.; Whang, S.J.; Boak, R.A.; Carpenter, C.M. 1964. Complexities of the fluorescent treponemal antibody test, and its preliminary evaluation in the serologic diagnosis of syphilis. Tech. Bull. Regist. Med. Technol. 34:37-39. Also, Amer. J. Clin. Pathol. 41:337-339.

The use of Treponema pallidum antigen suspended in Nelson's medium in the fluorescent treponemal antibody (FTA) test resulted in a reduction of 90 to 95 per cent in the numbers of organisms present after the test was performed, as well as a fragmentation of many organisms. When the treponemes were suspended in sterile 0.85 per cent saline solution and preserved with 1:10,000 final dilution of merthiolate, neither loss of antigen nor fragmentation was observed. The contents of each individual vial of labeled antihuman globulin should be titered and stored at -20 C. Subjectiveness involved in determining the intensity of the fluorescent reaction necessitated close comparison of the results of the test with positive control sera. Of 35 diagnostic problem sera that were reactive in the TPI test, 29 (82.9 per cent) were FTA-reactive. In the same category 94.2 per cent were FTA-nonreactive. Thus, the FTA test failed as a means of detecting 17.1 per cent of the patients with latent syphilis. Greater experience and extensive research are essential before the FTA test can be used as a dependable procedure for the diagnosis of syphilis.

5441

Naumann, G. 1964. Fluorescence serological investigations for the demonstration of Treponema-specific antibodies. Z. Ges. Hyg. 10:518-523. In German.

The method of fluorescence-serological detection of Treponema-specific antibodies (the FTA test) was tried out in 200 sera. The results were compared with those of the Treponema pallidum immobilization test (TPI test). The results of both reactions were in conformity in at least 82.5 per cent of the sera tested; in at least 6 per cent of the sera the results showed discrepancies between the two tests. Of our findings, 11.5 per cent did not permit a clear interpretation.

5442

Naumann, G.; Wildfuhr, G. 1965. The significance of immunofluorescence for serodiagnosis of infectious diseases. Munchen Med. Wochensch. 107:1384-1386. In German.

The author discusses the results obtained by the immunofluorescence method in serodiagnosis of various infectious diseases. Comparative studies with standard methods show that this method is of greater significance in serological diagnosis of syphilis, toxoplasmosis, pseudotuberculosis, cryptococcosis, and trichinellosis.

5443

Neblett, T.R.; Merriam, L.R.; Burnham, T.K.; Fine, G. 1964. A source of false-positive fluorescent treponemal antibody reactions. J. Invest. Dermatol. 43:439-440.

Non-syphilitic sera that demonstrated antinuclear factor with tumor imprints also produced a reactive fluorescent treponemal antibody (FTA) test whose intensity seemed to parallel that of nuclear immunofluorescence. Such reactive FTA results were rendered negative by absorption with human tumor homogenates; they were diminished partially by normal tissue homogenate absorption, but remained unaffected by animal tissue powder absorptions. Patients furnishing such sera were considered non-syphilitic. Known syphilitic sera absorbed with tumor or normal tissue homogenates could not be rendered negative to the FTA, and these did not produce nuclear immunofluorescence. False-positive FTA test results may indicate the presence of an autoimmune disorder.

5444

Niel, G ; Fribourg-Blanc, A 1963. Immunofluorescence and syphilis serology Bull. WHO 29:429-442. In French.

Comparative studies on 12,000 sera with the FTA test, cardiolipin reactions, and the Treponema pallidum immobilization (TPI) test are reported Provided that the FTA test is carried out with scrupulous care, it has proved to be highly sensitive, easily reproducible, and sufficiently specific Its simplicity allows it to be used as a routine test for case-finding and evaluation of treatment. It also allows the quantitative expression of results. The quantitative titers obtained in the FTA test, ranging from 0 to 100,000, are an accurate reflection of the antibody elicited and hence of the degree of infection. Although it is not quite as highly specific as the TPI test, the FTA technique has the important advantage of revealing syphilitic infection earlier than other tests. The authors do not think the various criticisms that have in the past been levelled at the FTA test are fully justified if the test is meticulously performed, but they emphasize that strict standardization of the test procedure, of the reagents used, and of the manner of recording results is essential. Once this is achieved, they consider that the optimum diagnostic procedure might well be the routine performance of the FTA test together with a Kline or VDRL flocculation test on all sera.

5445

Niel, G.; Fribourg-Blanc, A. 1965. Quantitative immunofluorescence and syphilis serology: A recent statistical study. Bull. WHO 33:89-105. In French

This deals with studies on 5,169 sera and describes modifications in the authors' technique for the performance of the FTA test. The authors have perfected optical instrumentation and developed strictly standardized reagents that permit excellent reproducibility for quantitative testing. Stress is laid on the importance of the quantitative expression of results For this purpose it is essential that the technique be followed to the letter in every instance. In confirmation of earlier studies, the results detailed in this paper show the FTA test to be more sensitive at all stages of syphilis than the VDRL, Kline, or Kolmer cardiolipin tests or the TPI test with lysozyme. In primary syphilis, the FTA test is usually positive earlier than the cardiolipin tests, and in long-standing treated syphilis it is exceptional for sera to be both FTA-negative and TPI-positive. Although some sera do give nonspecific FTA reactions, these are always at low titer. More than 1,080 normal sera were subjected to parallel VDRL and FTA testing, and those reacting positively were further checked with the Kolmer and TPI tests. The FTA test detected twice as many syphilitic sera (confirmed by the TPI test) as did the cardiolipin reactions. The efficiency of the FTA test as a case-finding technique has been amply demonstrated.

5446

Nielsen, H.A.; Idsoe, O. 1963. Evaluation of the fluorescent treponemal antibody test. Acta Pathol. Microbiol. Scand. 57:331-347.

The results obtained in the FTA test are reviewed, as are the techniques used by the different laboratories. An investigation of 1,194 sera and 76 cerebrospinal fluid samples from 1,237 persons is reported. The methods are described and the results tabulated. The sera used originate from persons with syphilis and with other diseases. The sensitivity, specificity, and reproducibility of the FTA test are discussed, and its technique is examined in detail. The FTA method is of considerable interest for research work, and may possibly be of use for diagnostic studies in laboratories where for various reasons the TPI test is difficult to carry out.

5447

Ninu, E. 1964. Value of the immunofluorescence test (FTA) on the identification of biologically false positive reactions in the serological diagnosis of syphilis. Ann. Sclavo 6:797-808. In Italian.

The results of serologic tests for syphilis carried out during 4 years on 34,019 individuals are reported. There were 212 positive serum samples belonging to 156 individuals checked by the FTA test. This test proved to be highly specific at the two serum dilutions employed (1/50 and 1/200) and allowed the recognition of all the biologically false positives (37 of 134) already ascertained by anamnesis. Thus the incidence of serologically difficult cases was reduced from 50.7 to 4.4 per cent.

5448

Orhel, I. 1964. Treponema antibody fluorescence in the diagnosis of syphilis. Rad. Med. Fak. Zagrebu 12:37-49. In Serbian.

Sera from 144 cases of primary and secondary syphilis were studied. Comparisons were made with the lipin and TPI tests.

5449

Ottolenghi, F. 1965. The complement deviation test for syphilis with a lipoprotein antigen. Dermatologica 130:88-100.

In modern syphilitic serology, a lipoprotein antigen consisting of cardiolipin and protein from Reiter's treponeme in optimal concentration of their serological activity, may find a useful employment. This antigen, which does not cause zone phenomena, possesses sensitivity higher than those of antigens constituted by simple cardiolipin and by Reiter's treponema protein, although they are strongly specific.

The percentage of agreement with the FTA that, not reported here, has reached a higher level with the lipo-proteic antigen than with other antigens, including VDRL. The mixed antigen seems more specific even if less sensitive than VDRL. Favorable comparisons were made with other syphilis serologic tests.

5450

Ovchinnikov, N.M.; Lur'e, S.S.; Bednova, V.N. 1961. Immunofluorescence method in the serological diagnosis of syphilis. Vestn. Dermatol. Venerol. 35:27-32. In Russian.

The fluorescent treponemal antibody test was used for serodiagnosis on 171 syphilis patient sera and 197 control sera for comparison with the Treponema pallidum immobilization, Wassermann, and precipitin tests. Agreement of all tests was 72.4 per cent. The final two tests gave 85.4 per cent agreement.

5451

Ovchinnikov, N.M.; Lur'e, S.S.; Sazonova, L.V.; Bednova, V.N. 1964. Fluorescent method in the diagnosis of syphilis. Cesk. Dermatol. 39:297-303. In Czech.

The authors tested the diagnosis of syphilis by fluorescent antibodies. They examined 2,266 sera, including 1,581 from patients with different forms of syphilis, treated and not treated, and 685 control sera. The results were compared with those of the BWR, cardiolipin, nonspecific antigen, Kahn's reaction, and the cytocholic test. In addition 1,461 sera were investigated also by TPI. In 1,152 sera (78.8 per cent) the results of the fluorescent reaction, TPI, BWR, and cardiolipin tests agreed. The bet agreement of results of the fluorescent reaction was obtained with TPI (87.4 per cent). Disagreement of the fluorescent reaction with other reactions was 21.1 per cent. These sera, mostly from patients undergoing treatment, were FTA-positive. The remaining 17.4 per cent were negative in the fluorescence reaction and positive in the BWR, TPI, or both. The authors assume that false-positive BWR reactions were involved. In sera of the control groups positive results of the fluorescent reaction were obtained in two of 635 sera. The BWR and IPI were also positive. The fluorescent reaction is very valuable in the event of false-positive results of the BWR.

5452

Ovchinnikov, N.M.; Lur'e, S.S.; Sazonova, L.V.; Bednova, V.N. 1964. Fluorescence serological method in the diagnosis of syphilis. Lab. Delo 10.302-306. In Russian.

The fluorescent antibody method is specific, sensitive, and rapid. On the basis of its sensitivity, the reaction of immunofluorescence

in the serum diagnosis of syphilis surpasses not only the Wassermann reaction with cardiolipin antigen, but also the Treponema pallidum immobilization reaction. This method has great importance for investigating sera that give a false-positive result in the Wassermann and precipitation reactions.

5453

Pagnes, P. 1963. The influence of complement in the FTA. Minerva Dermatol. 38:337-339. In Italian.

Alexin is not involved in this reaction, and it probably does not contribute to false-positive results.

5454

Petzoldt, V.D.; Tupath-Barniske, R. 1965. On specific syphilis serodiagnosis: FTA test and TPI test. Deut. Med. Wochensch. 90:950-954. In German.

Three hundred and sixty-one sera, of which the precipitation of classic sero-reactions were known, were tested with FTA and TPI tests. The results of both test procedures coincide well. Only in primary syphilis may the FTA be positive more often and the TPI negative, since the TPI test shows immobilizing antibodies in the serum only at the end of the primary period. Aside from this, results of both tests showed agreement of 97.2 per cent. The cases in which the results of the two test procedures did not agree are discussed individually. This points out that it is sometimes difficult to decide which of the two tests gave a false result.

5455

Puccinelli, V. 1961. The value and significance of the fluorescent antibody test in the serology of syphilis. Arcisped. S. Anna Ferrera 14:781-788. In Italian.

Practicality, performance simplicity, excellent specificity, and clinical conformity made the fluorescent antibody test desirable for diagnosis of syphilis. Diagnostically, it was at least the equal of the treponemal immobilization test.

5456

Ripault, J.; Colombani, J. 1964. The immunofluorescence test applied to the diagnosis of syphilis, comparison with the Nelson test and classical serology: II. Study of all cerebrospinal fluids. Pathol. Biol. 12:276-285. In French.

The IF test, using Treponema pallidum as antigen, has been carried out on cerebrospinal fluids (CSF). The study of the CSF from 109 non-syphilitic patients has shown that pure CSF can be used without fear of false-positive reactions. The CSF from 302 syphilitic subjects has been simultaneously studied with the IF test, the TPI, and the complement fixation reactions using Reiter's treponema as antigen as well as cardiolipidic antigen. The IF test has shown results comparable with those of TPI. It is the most sensitive of all the tests used, its concentration of positivity varying from 1:1 to 1:800, with an average concentration of 1:57. It is less strongly positive when it is used singly than when it is combined with one or several other positive tests. It is practically always positive in neurosyphilis. It is positive only in 50 per cent of the cases of recent syphilis, where the clinical signs of neuromeningeal involvement are absent or discrete. A negative IF test thus excludes the possibility of a neuromeningeal lesion of syphilitic origin. IF testing of both serum and CSF is recommended.

5457

Ruczkowska, J. 1965. The fluorescent treponemal antibody inhibition test as a new method for the diagnosis of syphilis. Arch. Immunol. Therap. Exp. 13:602-613.

A new method for the detection of treponemal antibodies in sera and cerebrospinal fluids has been developed based on the fact that fluorescence of an antigen induced by FA can be inhibited or markedly diminished by first exposing the antigen to specific unlabeled antibody. After investigating various factors that affect the phenomenon, a qualitative and quantitative technique of the fluorescent treponemal antibody inhibition test has been developed. With this test, 1,500 sera and 100 cerebrospinal fluids were examined, and the results were compared with those obtained with the Nelson-Mayer test and FTA-indirect test. The elaborated FTA-inhibition test was found to be as sensitive and specific as the FTA-indirect method and Nelson-Mayer test. Its advantages are that it is less expensive, easier to perform, and allows examination of a large number of sera in shorter time.

5458

Sasahira, T. 1963. Serodiagnosis of syphilis by fluorescent treponemal antibody test: I. On fluorescent treponemal antibody test using the Reiter treponeme, RFTA test. Jap. J. Bacteriol. 18:335-343. In Japanese.

In 228 problem sera, the results obtained by fluorescent treponemal antibody test using the Reiter and Nichol's strain of Treponema pallidum were compared and a high degree of correlation existed between the results obtained by both methods; RFTA test was as specific for syphilis as the FTA test. The complement method of fluorescent treponemal antibody test for syphilitic sera with anticomplementary action gave negative results, this method should not be used as a routine technique of FTA or RFTA. The author devised a quantitative technique for the RFTA test by using twofold dilutions of syphilitic sera. The RFTA titers of the same specimens were always higher than the RPCF or reagin titers. Furthermore, the RFTA titers did not correspond to the FTA titers. Possible explanations for this result are discussed.

5459

Sasahira, T. 1965. Serological diagnosis of syphilis by fluorescent antibody technique: II. Changes in 7S antibody in different stages of syphilis and specificity of fluorescein treponemal antibody test. Jap. J. Bacteriol. 20:183-194. In Japanese.

From 200 ml of normal human serum 800 mg of pure 7S gamma globulin was obtained by first treating with ammonium sulfate and then filtering through a DEAE cellulose column with 0.015 M NaCl and 0.01 M phosphate buffer, pH 7.5. With this gamma globulin as antigen, antihuman 7S gamma globulin rabbit serum was obtained. Rabbit gamma globulin was then labeled with FITC. With this FITC-labeled anti-7S gamma globulin, and the FITC-labeled anti-gamma globulin described in part I of this series, 7S antibody and 19S antibody were detected, and the changes in them at various stages of syphilis were observed. 7S antibody appeared in primary, secondary, late and congenital syphilis. 19S antibody was detected in primary syphilis. The higher specificity of the FA technique compared with that of RPEF, WR, and VDRL was confirmed by correlating the serological results and clinical findings obtained in 137 cases of syphilis

5460

Schroeter, A.L. 1965 Transmission of syphilis. J. Amer. Med. Ass. 191:165

The patient described probably had latent syphilis. Comfirmation serology by FTA-200 or FTA-ABS is suggested.

5461

Sedati, P.; Mancini, L.; Rossi, M. 1962. The use of immunofluorescence technique in the serological diagnosis of syphilis. Med. Clin. Sperim. 12:257-274. In Italian.

A review of the recent advances in syphilis serology is given. Original work in which the immunofluorescence test was compared with the conventional complement-fixation and flocculation tests is reported. The FTA test proved to be very useful in the diagnosis of syphilis in various evolution stages. A particular advantage of the test was shown by its aptitude for detecting false biologic reactions. Simultaneous use of the FTA and the conventional tests, in order to improve the possibility and the security in the serologic diagnosis of syphilis, is suggested

5462

Smith, J L.; Singer, J.A.; Moore, M.B., Jr.; Yobs, A.R. 1965. Seronegative ocular and neurosyphilis. Amer. J. Ophthalmol. 59:753-762.

Syphilis is rapidly increasing in incidence in the United States. There is no paucity of clinical signs of late syphilis, despite the infrequency of reactive serologic tests in such patients. The magnitude of the problem is indicated by the fact that over 100 cases of ocular and neurosyphilis have been found in one city hospital within the past year. The importance of the TPI and the FTA absorption tests in the diagnosis of seronegative syphilis is emphasized. The fact that a negative blood test does not rule out syphilis cannot be too highly stressed.

5463

Spangler, A.S.; Jackson, J.H.; Fiumara, N.J.; Warthin, T.A. 1964. Syphilis with a negative blood test reaction. J. Amer. Med. Ass. 189:87-90.

The reappearance of syphilis as an important infectious disease in this country prompted a review of the methodology of diagnosis as viewed by the practitioner. Blind reliance on standard serologic tests for syphilis may be misleading. In our study, the disease, in various stages, was present in 24 patients whose serologic tests were negative. In 16, the negative test was the result of a positive prozone reaction, and the reagin became positive upon dilution of the serum. The newer treponemal tests (RPCF and TPI) dark-field and spinal fluid examinations were also valuable in establishing the diagnosis. The clinician must be aware of the possibility of active syphilis with a false negative serology.

5464

Stout, G.W.; Falcone, V.H.; Moore, M B., Jr. 1964. Protocols for testing reagents employed in serologic tests for syphilis. Health Lab. Sci. 1:119-128.

As a portion of this, procedures for FA reagents are given.

5465

Taglieri, G.; Tresca, G. 1964. Report of sero-epidemiology of a sample of the population of Asmara, Eritrea. II. Study of antibodies against the Nichols treponeme with the immunofluores nt method. Arch. Ital. Sci. Med Trop. Parassitol 45:163-180. In Italian.

The authors conducted a sero-epidemiological screening with fluorescent antibodies to investigate the frequency of syphilis in a sample of the population of Asmara, Eritrea. The individuals were selected at random among the socio-economic classes. They did not present clinical manifestation of the disease. There were a total of 21 positive cases among 160 examined, or 13.1 per cent. Among these positive cases there were 16 among 124 subjects between 6 months and 9 years of age, or 12.9 per cent. There is a high incidence of syphilis in that region, and there is a high frequency of the disease in infancy.

5466

Thivolet, J.; Sepetdjian, M. 1963. Endo-urethral ulcer; problems of diagnosis. Bull. Soc. Franc. Dermatol. Syph. 70:953-954. In French.

One case is reported in which the FTA test results made possible a positive diagnosis of syphilitic endo-urethral ulcer when other diagnostic procedures gave inconclusive results.

5467

Tomizawa, T.; Kasamatsu, S.; Yamaya, S ; Murata, R. 964. Studies on the fluorescent antibody technique applied to the serodiagnosis of syphilis. Jap. J. Med. Sci. Biol. 17:280.

FA is specific as TPI and TPCF in which virulent Treponema pallidum is employed as antigen. This method may be more practical than the other tests since the method is less laborious in antigen preparation and performing the test. However, it has been noticed that false positive reactions appeared at low dilutions of both test and conjugated sera. It has not been explained whether this false reaction is a specific antigen-antibody reaction or a component in the test serum merely adherent to the organisms. The titer of FITC-labeled anti-HGG and antigenicity of T. pallidum drop during storage. Studies will be needed to prevent the reduction in the potencies.

5468

Vaisman, A.; Hamelin, A.; Guthe, T. 1963. Use of the fluorescent antibody technique for dried blood eluents: Comparison of the FTA, TPI, and the improved lipoid antigen application to serum. Bull. WHO 29:1-6.

Past attempts to use dried finger-puncture blood for lipoidal antigen testing of the eluent were successful when blotting paper was used as the adsorption medium. With the introduction of the immunofluorescent technique in syphilis and other treponematoses, it was decided to undertake a fluorescent treponemal antibody (FTA) testing study of dried blood eluents using blotting paper as the absorption medium, since there is need for a simple procedure in areas where information on specific serotesting for treponematoses is required and where venipuncture is impracticable. The authors describe the preliminary results of their FTA tests. The serological reactivity to FTA, TPI, and lipoidal antigen was also examined in venipuncture sera from the same individuals. The variations found in sensitivity, specificity, and reproducibility of the blotting paper disc FTA-100 procedure were not significant, and the results were practically the same as those obtained independently by FTA-100 examination of venipuncture sera from the same individuals. The advantages of finger-puncture blood sampling are outlined.

5469

Vaisman, A.; Paris-Hamelin, A.; Guthe, T. 1963. Treponema immunofluorescence test applied to dry blood. Presse Med. 71:2653-2654. In French.

The treponema immunofluorescence test for the serological diagnosis of syphilis can be performed on whole blood; the sample is then adsorbed on paper and dried. This blood sampl can be mailed to a laboratory. The inconvenience of preservation and mailing of fresh blood or serum is avoided. The excellent preservation of the immunological properties of the blood in this condition, plus the sensitivity and specificity advantages of the immunofluorescence reaction complement each other.

5470

Wilkinson, A.E. 1963. The fluorescent treponemal antibody test in the serological diagnosis of syphilis. Proc Roy. Soc. Med. 56:478-481.

The FTA-200 test was more specific but less sensitive than the TPI test.

5471

Yobs, A.R., Brown, L.; Hunter, E.F. 1964. Fluorescent antibody technique in early syphilis: As applied to the demonstration of T. pallidum in lesions in the rabbit and in the human. Arch. Pathol. 77:220-225.

Procedures for fluorescent staining of T. pallidum in tissue smears and sections by direct and indirect methods are presented in detail. Fluorescent antibody staining was easily performed with consistently reproducible results. This simple technique for a specific histochemical stain will be valuable in the diagnosis of lesion syphilis in the field and in studies of pathogenesis of syphilis.

5472

Yobs, A.R.; Olansky, S.; Rockwell, D.H.; Clark, J.W., Jr. 1965. Does Treponema pallidum persist after treatment of late syphilis? Ann. Intern. Med. 62:1088-1089.

Because of the implication that penicillin is ineffective in late syphilis, a similar study was undertaken using TPI-reactive male volunteers whose previous treatment for syphilis could be documented. A surgically excised inguinal lymph node from each of 45 volunteers was studied extensively by darkfield microscopy, FA staining, silver staining, and rabbit inoculation. Nodes from five men were shown to contain treponeme-like forms by at least one test. Two of these contained virulent organisms, as shown by the development of disease in inoculated rabbits. Each of these five men was retreated under our supervision. Four received recommended schedules of benzathine penicillin G, and one, with a history of penicillin allergy, received erythromycin. Another inguinal node removed from each of these five men 3 months after the retreatment was negative by all tests.

5473

Yobs, A.R., Olansky, S., Rockwell, D.H.; Clark, J.W., Jr. 1965. Do treponemes survive adequate treatment of late syphilis? Arch. Dermatol. 91:379-389.

Nodes were studied from 45 men who had been treated for syphilis in varying stages of the disease. Five of these nodes contained treponeme-like forms, which in two cases proved to be virulent. Repeat node study after supervised treatment was completely negative in all five men. Possible reinfection and inadequacy of previous treatment undoubtedly are important factors in the original findings. Penicillin is the drug of choice in the treatment of syphilis. There is no evidence that Treponema pallidum survives in humans after adequate penicillin treatment. FA was used to identify the treponemes in sections and smears.

5474

Yobs, A.R.; Rockwell, D.H.; Clark, J.W., Jr. 1964. Treponemal survival in humans after penicillin therapy: A preliminary report. Brit. J. Vener. Dis. 40:248-253.

Inguinal nodes of 45 men who had been treated for syphilis at various stages of the disease were studied. Five nodes were shown to contain treponemal forms, and in two cases these were shown to be virulent. Findings were completely negative in an additional node removed from each of four men after supervised treatment. The possibility of re-infection must be absolutely ruled out before these findings can be interpreted as demonstrating treponemal survival in humans after adequate penicillin treatment.

XIII. OTHER BACTERIAL STUDIES

5475

Albach, R.A.; Shaffer, J.G.; Watson, R.H. 1965. Morphology, antigenicity, and nucleic acid content of the Bacteroides sp. used in the culture of Entamoeba histolytica. J. Bacteriol. 90:1045-1053.

Certain changes are described in morphology, antigenicity, and nucleic acid content that occur in a culture of Bacteroides sp. in the presence of penicillin G in CLG medium. This variant is one of seven recovered in several laboratories, all of which are descendants of the original Bacteroides isolated by Shaffer and Frye. Penicillin-inhibited cells of this culture are currently being used in the routine propagation of Entamoeba histolytica in CLG medium. Evidence is presented for the loss of ability to react with antibody in these penicillin-inhibited bacteria in CLG medium, when studied by fluorescent antibody techniques. The implications of the antigenic changes observed as they pertain to similar antigenic studies of the amoebas are discussed. A pronounced reduction in the ribonucleic acid (RNA) content of such penicillin-inhibited cells was also observed. The potential importance of the changes that occur in the RNA of these cells with respect to considerations of the growth requirements of the amoebas is also discussed.

5476

Biegeleisen, J.Z., Jr.; Mitchell, M.S.; Marcus, B.B.; Bloden, D.L.; Blumberg, R.W. 1965. Immunofluorescence techniques for demonstrating bacterial pathogens associated with cerebrospinal meningitis: I. Clinical evaluation of conjugates on smears prepared directly from cerebrospinal fluid sediments. J. Lab. Clin. Med. 65:976-989.

Techniques were described for the cultivation and immunofluorescent identification of Hemophilus influenzae, Diplococcus pneumoniae, Neisseria meningitidis, and eight less common pathogens in specimens of cerebrospinal fluid from 100 patients with bacterial meningitis. A comparison of the results obtained by conventional methods and by immunofluorescent staining indicated that the latter method was fully as sensitive as the former and was more accurate in treated cases. Some of the dangers involved in the use of the Gram stain of the sediment as a tool for presumptive diagnosis were discussed, as were shortcomings of fluorescent antibody staining, particularly in infections caused by uncommon gram-negative organisms. The immunofluorescent staining technique was recommended for the rapid screening of spinal fluid specimens, as well as of cultural isolates.

5477

Callao, V.; Olivares, J. 1964. Preparation of fluorescent antibodies against Rhizobium leguminosarum. Microbiol. Esp. 17:181-188. In Spanish.

Anti-Rhizobium leguminosarum antibodies have been labeled with fluorescein isothiocyanate and rhodamine isothiocyanate. These bacteria have been observed in leguminous nodules in different stages of their development. It has been proved that the fluorescent antibody method is very useful in the study of some aspects of plant biology, especially in infectious diseases.

5478

Danielsson, D. 1965. A membrane filter method for the demonstration of bacteria by the fluorescent antibody technique: 1. A methodological study. Acta Pathol. Microbiol. Scand. 63:597-603.

Bacteria suspended in tap water or cultured in broth, and then trapped on non-fluorescent membrane filters, could be identified within one hour by means of the fluorescent antibody method. For this purpose the fluorescence microscope was equipped for incident illumination. The technique described allowed a quantitative determination of the bacteria identified serologically.

5479

Davies, M.E. 1965. Cellulolytic bacteria in some ruminants and herbivores as shown by fluorescent antibody. J. Gen. Microbiol. 39:139-141.

A method is described for the demonstration and enumeration in situ of antigenically related cellulolytic bacteria in the intestinal contents of some herbivores and ruminants, by means of a fluorescent antibody staining technique.

5480

Geason, D.J.; Romano, A.H. 1964. Sheath formation in Sphaerotilus natans, followed by immunofluorescence. Bacteriol. Proc. G132:38.

Labeled homologous antisheath globulin was obtained by injecting rabbits with purified sheath material from S. natans, fractionating the serum with ammonium sulfate, and conjugating with fluorescein isothiocyanate. S. natans was grown on slides immersed in Stokes medium at 28 C for 9 hours and reacted with the homologous labeled globulin. The slide cultures were then washed with buffered saline, pH 7.2, and incubated further in fresh medium. Samples were removed at intervals and examined by fluorescence and phase microscopy. Old portions of the sheath remained

discretely labeled with no diminution in intensity of fluorescence, but nonfluorescent new sheath material was observed at the tips of the filaments. New sheath material is not formed by intercalation or intussusception, but by linear extension of pre-existing sheath as new cells are formed at the ends of filaments. This interpretation was confirmed by an alternative procedure, in which old sheath was reacted with unlabeled homologous globulin and new sheath was identified by the addition of labeled globulin after incubation. Fluorescence was most intense at the growing tips of the filaments.

5481

Hill, E.C.; Lewis, S. 1964. L forms of bacteria isolated from surgical infections. Bacteriol. Proc. M26:48.

Transitional and stable L forms have been isolated from a variety of specimens obtained from patients: blood; spinal, pleural, and synovial fluids; abscesses; lymph nodes; and acne pustules. So called transitional L forms of Clostridium perfringens, C. sporogenes, Peptostreptococcus sp., and Sphaerophorus sp. have been recovered on primary culture media without use of inducing or selective agents. Aerobic and anaerobic cultures were planted on brain heart infusion agar, PPLO agar, Brewer-modified thioglycolate medium, and Castaneda blood culture medium, with and without supplements of yeast extract, human blood, ascitic fluid, or PPLO serum fraction. Use of 2,3,5-triphenyltetrazolium chloride at 0.0025 per cent in the agar media aided in the detection of L colonies. Evidence is presented for the in vivo occurrence of L forms in a patient with thrombophlebitis. L forms were observed in direct mounts of spinal fluid. Organisms of similar morphology were recovered from cultures of the spinal fluid, thrombus, and blood. Immunofluorescence indicated that the L forms recovered from the thrombus were serologically related to S. necrophorus, and transitional L forms of Sphaerophorus sp. were isolated from blood cultures. L forms have persisted in the blood stream of this patient for 5 months postoperatively.

5482

Hsu, K.C.; Rifkind, R.A. 1963. Fluorescent, electron microscopic, and immunoelectrophoretic studies of labeled antibodies. Science 142:1471-1473.

Antibodies, produced in rabbits, to each of three bacterial species have been doubly labeled with fluorescein and ferritin. Irrespective of which label was conjugated to the antibody first, immunologic activity was maintained. Moreover, these preparations gave as high a degree of specificity in fluorescent and electron microscopic studies as did singly labeled antibodies. Immunoelectrophoretic analyses and other immunologic tests further confirmed that the antibodies were conjugated to both labels without loss of specific activity. The technique thus permits the relatively simple method of immunoflurescence

to be used as an aid in selecting optimum ferritin antibody conjugates for localizing antigen at the molecular level by electron microscopy.

5483

Karasek, E. 1965. Studies on the preparation of tissue sections for immuno-histology. Arch. Exp. Veterinaermed. 19:113-121. In German.

The relative advantages of several methods of preparing tissue sections for FA demonstration of bacterial antigens were compared on organ material from experimentally infected mice. Paraffin sections were well suited for anthrax bacilli and erysipelas bacteria. Usable photographs of salmonellae could be obtained only from freeze-dried material. Fresh frozen sections were less suitable. Negative staining with rhodanil albumin reduced the nonspecific fluorescence. BA-46-108471.

5484

Margherita, S.S. 1964. Serological variation of Butyrivibrio in the bovine rumen. Bacteriol. Proc. A42:8.

Strains of the rumen bacterium Butyrivibrio isolated from different areas have been shown to be serologically heterogeneous by agglutination, immunodiffusion, indirect hemolysis, and immunofluorescence. Further studies utilizing the fluorescent antibody technique were conducted to determine the feasibility of in situ identification of these organisms. Fresh rumen contents were filtered to remove coarse particulate matter, washed in buffer, and centrifuged. When the cellular sediments were incubated with fluorescein-conjugated antisera and examined by fluorescence microscopy, morphological types similar to those present in the control preparations containing added homologous cells were not detectable. Stained cells were not detected with antisera prepared either against a strain of the type species, B. fibrisolvens, or against strains isolated from the test animal 2 years previously. Altering the method used to prepare rumen contents, or varying the time of sampling, did not affect the findings. These results suggest in vivo antigenic variation.

5485

Margherita, S. Hungate, R.E.; Storz, H. 1964. Variation in rumen Butyri strains. J. Bacteriol. 87:1304-1308.

Five strains of Butyrivibrio isolated from the rumen of a single animal on an alfalfa hay ration were tested for serological relationships by agglutination and immunofluorescence. The main finding was a serological monospecificity of the strains. A cross-reaction between two strains was detected by agglutination and a second cross-reaction by immunofluorescence, but the cross-reacting pairs were different. Two years after the strains were isolated, fluorescein-conjugated

antisera against three of them were used to test rumen contents of the same animal for homologous cell types. None was found. The findings indicate great variability in the serological characteristics of rumen butyrivibrios

5486

Mikhailov, I.F 1963. Criteria of specific fluorescence of bacteria stained with fluorescent antibodies. Zh. Mikrobiol. Epidemiol. i Immunobiol. 40:7:94-97. In Russian.

As established, the only authentic criterion of specific fluorescent sera interaction with homologous bacteria possessing a membrane was a characteristic marginal fluorescence of a microbial cell. The intensity of this fluorescence is relative. Uniform fluorescence of bacteria possessing cellular membrane may be caused by nonspecific factors such as autofluorescence or secondary fluorescence. Patchy fluorescence of bacteria is connected with specific staining of localized antigens and points to the antigenic commonness with bacteria against which the fluorescent serum was prepared.

5487

Paton, A.M 1964. The adaptation of the immunofluorescence technique for use in bacteriological investigations of plant tissue. J. Appl. Bacteriol. 27:237-243.

The preparation of plant tissue for examination by FA is described. The procedures suggested are intended to overcome some of the major obstacles encountered and the efficacy of the method for plant investigations is upheld by its successful application in a simple test system.

5488

Romano, A.H ; Geason, D.J. 1964. Pattern of sheath synthesis in Sphaerotilus natans. J. Bacteriol. 88:1145-1150.

Formation of the characteristic sheath of Sphaerotilus natans was followed by immunofluorescence. Fluorescent antisheath antibody was obtained by injecting rabbits with purified sheath material from S. natans, fractionating the serum with ammonium sulfate, and conjugating the globulin fraction with fluorescein isothiocyanate. To follow sheath formation, S. natans was grown on slides immersed in Stokes medium at 28 C for 9 hours, and was reacted with labeled antibody. The slide cultures were then washed to remove unbound antibody, and were incubated further in fresh medium. Samples were removed at intervals and examined by fluorescence microscopy. Old portions of the sheath remained discretely labeled with no diminution in intensity of fluorescence, but nonfluorescent new sheath material appeared at the ends of the filaments. These results indicate that sheath synthesis does not take

place by intussusception or diffuse intercalation, but by linear extension of pre-existing sheath. This interpretation was confirmed by a reverse procedure, whereby old sheath was reacted with unlabeled antibody, and new sheath was identified by addition of labeled antibody after further incubation. In this procedure, fluorescence was most intense at the growing tips of the filaments.

5489

Slotnick, I.J.; Mertz, J.A.; Dougherty, M. 1964. Fluorescent antibody detection of human occurrence of an unclassified bacterial group causing endocarditis. J. Infect. Disc. 114:503-505.

Specific fluorescent antibody staining and cultural analysis of the human respiratory tract flora has revealed that Group II D bacteria are normal residents in 68 per cent of the individuals studied.

5490

Zubzhitskii, Yu.N. 1964. Origin of the 'shining halo' phenomenon when fluorescent sera are used. Dokl. Akad. Nauk SSSR 155:4:927-929. In Russian.

To stain microbial cultures, homologous O, OB, and ON fluorescent sera were used, obtained by tagging immune gamma globulins with FITC. A drop of microbial suspension was placed on a slide and mixed with a drop of homologous conjugate. In every position of the freely moving cell (rotation around the long and short axes, turning one of the poles toward the observer) the shining zone around the cell in the form of a halo was retained. The middle part of the cell appeared darker. Only when one and the same layer of FA is observed in various positions, is alternation of dark and shining zones possible. The eye will perceive the light of about 54 molecules of protein. BA-46-39566.

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13. ABSTRACT
This volume is one of a series of six in the second edition of an annotated bibliography on various aspects of immunofluorescence and its use. The first six-volume edition was published in 1965 and included citations for the period 1905 through 1962. The present edition covers the period 1963 through 1965; Volume I contains 490 annotated literature citations, arranged according to major subject areas, and a complete author index.

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