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# THE INTERNATIONAL SYMPOSIUM ON AERO-BIOLOGY (4TH) HELD AT ENSCHEDE, NETHERLANDS 3-7 SEPTEMBER 1972

Arthur W. Frisch

Office of Naval Research
London, England

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By ARTHUR W. FRISCH

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13 ABSTRACT

Almost all aspects of the field of aerobiology were covered in the four day meeting. Relatively few papers contained new material. Many were summary type reviews. Practically all of the aerobiologists in Europe and the United States attended the Symposium. Summaries of the important papers are presented in this report. The complete papers will appear in the forthcoming Symposium Proceedings.

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| AIR SAMPLERS | | | | | | |
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| MACROPHAGES | | | | | | |
| AEROSOL IMMUNIZATION | | | | | | |

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II

TABLE OF CONTENTS

THE IVth INTERNATIONAL SYMPOSIUM ON AEROBIOLOGY
HELD AT ENSCHEDE, THE NETHERLANDS, 3-7 SEPTEMBER 1972

## A. INTRODUCTION

The Twente region of southeastern Holland contains some 19 municipalities of which five, Almelo, Borne, Enschede, Hengelo and Oldenzaal have joined together in a Stedenband project for the purpose of furthering their joint socio-economic development. They anticipate a 30-40% population increase to 420,000 by 1985, and are actively soliciting development through an agency known as the CIVI Office of Stedenband Twente, P.O. Box 217, Enschede (pronounced Enshkadeh).

The site of the Symposium was the Technische Hogeschool Twente (Twente University of Technology) which is only seven years old. Many of the professors and staff serve as advisors or board members in the municipal governments; others serve as consultants to local industry. It is essentially an engineering school with research activities in automatic compiling design, light degradeable plastics, self lubricating artificial joints, air cushion bearings, high energy laser technology, pulse code modulating systems, and computerized determination of plastic properties, to name just a few.

The University is situated between Enschede and Hengelo. The campus is modern and placed in a park-like setting with trees, lawns and water. The 2000 students (coeducational) live on campus as do the staff and faculty. Major buildings include lecture hall-library, physics-electronics, creative arts studio, high pressure laboratory, central workshops, chemical technology and mechanical engineering.

The IVth International Symposium was organized under the direction of Prof. K.C. Winkler from the Laboratory of Microbiology, Utrecht, The Netherlands. His committee was given financial support by the Health and Defense Organizations of the Netherlands by the Ministry of Public Health and Environment and by several commercial firms.

The scientific program for the Symposium was most ambitious and attracted over 200 participants, mostly from Europe and the United States. The mornings were largely devoted to general sessions, the afternoons were taken up by sectional meetings (see appendix). The general topics included airborne viral infections, room ventilation, resistance to infection, the secretory immunoglobulins, transmission of respiratory infections and the epidemiology of airborne animal diseases. The sectional meetings consisted of brief presentations or panels on such topics as methods in aerobiology, cilia and infection, microbial survival in aerosols, isolation systems in human disease, factors influencing pulmonary defense, disease transmission in hospitals, transmission in communities, respiratory immunization, respiratory allergy, ventilation in operating theaters and miscellaneous subjects.

B. ABSTRACTS OF PAPERS

The Symposium was opened by Prof. K.C. Winkler (already mentioned) who defined Aerobiology as a study of the "Airborne Transmission Process where ATP stands for the biological angle." He cited four varieties of aerobiology, i.e., (1) Botanical (pollens, fungal spores, dust, etc.), (2) Medical (disease and inside environment), (3) Experimental (biological warfare, aerosols, etc.), and (4) Industrial (food, air pollution, silicosis, irradiation, etc.). He stressed the complexity of the field and particularly the multifactoral causality of apparently unrelated events as illustrated by the comment that "the true task of aerobiology is to find out why, where and how often the accident happens."

The second paper was given by Dr. R.E. Strange (Microbiology Research Establishment, Porton nr Salisbury, Wiltshire, England) on the "Rapid Detection of Airborne Microbes." He generalized on methodology and stressed principles, pointing out that automated devices have been produced which are claimed to be able to monitor the atmosphere and/or the water for their microbial content. "The requirements for high sensitivity and fast readout have been met, but a major problem is eliminating interference by other components present in samples from natural environment." In general, bacteria are easier to monitor than viruses which require time for replication in tissue culture in order to reach minimally detectable concentrations.

The methods involve use of all of the known chemical, physical and physiological characteristics of microbes, plus the technology of collection concentration and fractionation of samples. Of particular value are large volume air samplers, liquid impinger devices which separate particles into various sizes, and two-phase polymer systems for concentrating and purifying as opposed to membrane filtration.

The major procedures are:

1. Measurement of particle size as determined by counters of the Coulter and Rayco types provided other particles do not interfere.

2. Analyses for protein, lipid, carbohydrate, RNA, and DNA in the absence of other biologic contaminants. Fluorescent gas chromatography, mass spectroscopy and radiochemical techniques have also been utilized.

3. Cultural methods using microcolonies and sensitive devices to determine pH changes, turbidity, gaseous products, labeling with $C^{14}$ glucose, or $P^{32}$.

4. Bacterial enzymes such as phosphatases or esterases; the former releasing p-nitrophenol which is measured in the spectrophotometer; the latter with a fluorescent organic phosphate which is quite a sensitive procedure.

5. Immuno-fluorescence techniques are easy, sensitive, specific. They are automated (Coffey, E.M. et al, 1971, Appl. Microbiol. 21:280) and can be used for the detection of viruses (Hakon, N. and Hankins, W.A., 1970, Appl. Microbiol. 19, 224).

6. Immuno-adherence techniques using red cells as carriers of specific antibody and hemagglutination as the end point are useful for detecting viruses (Mitz, M.A., 1969, Ann. N.Y. Acad. Sci., 158:651).

7. $I^{125}$ labeled antibody has also been employed and counts performed with washed membrane filters. The method is said to detect "500 to 1000 homologous bacteria."

8. Other procedures giving results in a few minutes and sensitive to $10^3$-$10^5$ organisms are (a) the "partichrome" technique in which air sample particles are deposited on a tape and stained. Biological particles take up the protein dye and are detected by phototubes sensitive to different wavelenths of light (see Mitz, above), (b) Luminol chemiluminescence (Oleniacz, W.S. et al in Automation in Analytical Chemistry, Technion Corporation, Ardsley, New York). The method depends on the fact that luminol, 5-amino-2, 3-di-hydro-1, 4 phtalazindione, reacts in the presence of iron containing organic and biologic compounds which function as catalysts, (c) ATP determination (firefly luminescence - see Mitz above), (d) Radioactively-labeled antibody technique (see Strange, R.E. et al, J. gen Microbiol. 67, 349 (1971)).

Some of the more rapid detection methods have been summarized by Strange in the following chart:

Some Rapid Microbial Detection Methods

| Objective | Technique |
|---|---|
| Detection of viable microbes | (1) Slide culture → micro-colonies |
| | (2) Measure pH/turbidity changes in culture (NASA "Wolf Trap") |
| | (3) Measure $^{14}CO_2$ production in cultures (NASA "Gulliver") |
| | (4) Measure uptake of $^{32}PO_4$ |
| Broad spectrum detection | (1) Luminol chemiluminescence (haem compounds) |
| | (2) Firefly luminescence (ATP) |
| | (3) Staining ("Partichrome," "Biosensor," FITC) |

Specific identification

(1) Immunofluorescence
(2) Radio antibody assay
(3) Immunoadherence (viruses)
(4) G.l.c. of bacterial growth products
(5) G.l.c. and Mass spectroscopy of pyrolysis products

A most stimulating study was presented by Drs. R.P. Clark and R.N. Cox (National Institute for Medical Research, London) in a paper titled "The Generation of Aerosols from the Human Body." With a Schlieren optical system they were able to photograph the convection currents generated from the heated surface of the body in contact with the cooler adjacent air. These currents acted as carriers for desquamating skin scales (7 million shed per minute and $10^{10}$ every day) and other particles caught in the aerosol. The convective boundary layer was diverted by solid objects in its path (i.e. the chin) and augmented by the bellows action of loose clothing. The authors constructed a heated model which breathed (reciprocal pump) and then demonstrated that magnesium carbonate particles less than 70 microns in size and fungal spores were carried about by the convection current and could penetrate cotton fabrics. They concluded the paper with the following comments: "Recognition of the natural convective boundary layer flow as a transport mechanism for particles that are shed by the body is of importance in the study of airborne infection. It also has implications in, for instance, the design of operating theaters and infant incubator ventilation systems and in the design of protective clothing."

Dr. T.G. Akers (Naval Biological Research Laboratory, Oakland, California) presented his data on "Some Aspects of the Airborne Inactivation of Viruses." He stressed the importance of the suspending medium, the relative humidity, the temperature, the presence of vitrous oxide and sulfur dioxide gases and solar radiation as factors which can be decisive in systems where viral suspensions are being aerosolized.

An excellent paper was given by Dr. L. Reid (Institute of Diseases of the Chest, London). She covered the broad topic of newer knowledge concerning the anatomy, histology and physiology of the respiratory tree, particularly the bronchi and alveoli. She stressed the fact that the respiratory tract contains 25 generations of bronchi and bronchioles and five generations of alveoli, that all the cell membranes are irregular thus presenting large surface area to infecting agents, and that much of the mucosa is bathed in IgA and covered with a carpet of grass-like cilia which slowly propel a viscus mucous cover toward the oral pharynx. This is the landing site where the virus particles or bacteria must find a favorable environment in order to replicate. Dr. Reid emphasized the fact that nerve

fibers can now be demonstrated in the alveolar wall of the rat and more often in the mouse. She believes that these nerves stimulate the secretory activity of the Type II cell causing surfactin release.

The afternoon portion of the meeting was devoted to the role of cilia in infection and was chaired by Dr. D.A.J. Tyrrell (Clinical Research Centre, Harrow, England). He began his paper by pointing out that we inspire 10 liters of air and many particles every minute, reason enough for most infections to begin in ciliated cells. Tyrrell believes that some viruses and bacteria (Bordetella pertussis, Mycoplasma pneumoniae, parainfluenza and influenza viruses) do attach to the "outer membrane of the cilia of animal tracheal cells," as a means of infecting the respiratory tract. The mycoplasmas replicate at cell surfaces; parainfluenza virus can be shown to attach to cilia and the viron is said to fuse with the membrane. (This phenomenon has not yet been demonstrated with influenza virus.) Ciliostatic and ciliopathic substances of viruses and bacteria were mentioned (more later) and the importance of organ culture techniques for the purpose of studying the function of cilia was stressed.

A paper by Dr. B. Hoorn (Umeå University, Sweden) dealt largely with the organ culture technique and the destruction of ciliated tracheal cells which followed. Thus ciliated human embryo cells inoculated with Rhino HF virus showed vacuolization in eight hours and were lost from the surface within 24 hours; the underlying cells were unaffected. With influenza virus intermediate cells were also damaged. Additional studies with phase microscopy showed cytopathic effects of viruses on ciliated cells. Hoorn pointed out the interesting fact that olfactory cells are not susceptible to the cytopathic action of viruses. In the discussion Dr. V. Knight reported that repeated biopsies of human ciliated mucosa can be obtained with a small curette; this type of material, he said, is suitable for tissue culture.

Dr. F.W. Denny (University of North Carolina School of Medicine at Chapel Hill) reported his studies on a substance derived from encapsulated and non-encapsulated strains of type B H. influenzae which paralyzes cilia in two to six days (organ culture technique and the rat trachea). With human cells the effect is noticed in eight to ten days. The pathology involves injury to the cilia containing cells with resultant rounding and sloughing in 48-96 hours. The bacterial culture supernate is often active in a dilution of 1:80. The ciliopathic substance is formed as a secretory product; it is non-dialyzable, stable at 56° C for 30 minutes, and partially ether sensitive. Denny believes that the material may be endotoxin, but he is not yet certain that this is so.

Dr. R. Dourmashkin (Clinical Research Centre, Harrow, England) presented his electron micrographs showing influenza virus entering chicken tracheal cells where it is most often found in a vacuole surrounded by a double-layered membrane. Some virus particles are seen in the cell cytoplasm and may possibly have been uncoated, although there was no evidence to

prove this speculative claim. With human epithelium he could not demonstrate cilial penetration.

Drs. Sylvia E. Reed and P.S. Nolan (Harvard Hospital, Salisbury, England) demonstrated early infections of bovine trachea with Parainfluenza 3 virus using immunofluorescence. They attempted to free infected cultures of virus by adding antiserum and/or interferon but without success. Virus levels in the supernate remained low until the antibody was removed; thereafter the titer rose precipitously.

Dr. D. Taylor-Robinson (also of the Clinical Research Centre) studied various mycoplasmas growing in tracheal cultures and observed injury to cilia with some but not all strains. M. capri was very active; M. galinarium was inactive; M. pneumoniae required 14-16 days to produce damage. With some mycoplasmas (M. capri) the toxic effect is delayed if catalase is added. He believes that excessive amounts of peroxide are produced with resultant cell injury. In other experiments the addition of nuraminidase was partially preventive. With M. pneumoniae the ciliary toxicity is attributed to a mycoplasmal structure called "terminal bulb," recently described in Sweden. In the discussion Denny claimed that mycoplasmas do produce a toxin if one uses tracheal organ cultures. Dr. Tyrrell pointed out that different parts of the respiratory tract may show different viral susceptibility patterns. Despite the lively comments, the question of whether or not a ciliotoxic material is produced by mycoplasmas is, as yet, an open one.

In the general session dealing with the resistance of the lungs to infectious agents the chairman was Sir C.H. Stuart-Harris of Sheffield. The first paper was a summary one titled "Natural Resistance and Factors Influencing Defense," and was presented by Prof. R. Rylander (Karolinska Institute for Medical Health, Stockholm, Sweden). The anatomic and physiologic factors usually associated with resistance were discussed, i.e., cilia, mucous, blood supply, cough, bacterial flora, macrophage function and the activity of lymphocytes.

The role of secretory antibody was discussed by Prof. J.F. Heremans (University of Louvain, Brussels, Belgium). His material was well organized and contained considerable experimental data from his own laboratory. He talked about the biochemistry of the secretory globulins as well as their biological functions. Of particular interest to me were the following comments: In support of his contention that half of the secretory IgA of animals is derived from the circulation, he cited some experiments in which dogs were transfused with labeled IgA. Half remained in the blood and less than half appeared in the milk, bile and jejunum. In man, thus far, all of the secretory IgA is most probably produced locally. Heremans believes that IgA may act in lytic systems by activating $C'_3$ directly, particularly in the presence of lysozyme. IgA may also act to inhibit bacterial growth because of its ability to bind to apolactoferrin which is

bacteriostatic. Of particular significance is the fact that surface immmunization with potent antigens produces a primary IgA response which is maintained only as long as the antigen is present. Subsequent stimulation produces another primary (not secondary) type response, and under the right conditions, immunologic paralysis may be induced. Of special interest was the comment that the secretory component of IgA protects the antibody from being digested by proteases present in the secretions. In fact, proteolytic enzymes may actually attach to IgA without affecting its antibody activity.

The final paper of the session was given by Dr. A.C. Allison (Clinical Research Centre, Harrow, England) on "Immunologic Factors in Defence and Pathogenesis of Disease." He emphasized some specific aspects of these very large areas of research as follows:

1. Genetic aspects of resistance with particular reference to strains of animals where susceptibility and/or resistance are under the control of one set of genes as in the case of leukosis virus. In these situations the genetic endowmment operates at "the level of all cells in the body."

2. Macrophage aspects - with much emphasis on the thesis that spread of infection to systemic levels is enhanced in situations where intracellular multiplication within macrophages occurs. Localization is the rule when viruses are unable to replicate within macrophages. This generalization holds even for persistent viruses. Examples included are ectromelia infections compared with vaccinia and persistent CM infections in mice.

3. The role of antibody and complement in the recognition and uptake of viruses was stressed. Also noted was the fact that antibody attachment to viruses did not preclude subsequent intracellular replication as exemplified by LCM infection.

4. Susceptibility and resistance as a function of age was explained by (a) differences in capacity to produce antibody and (b) by the fact that macrophages from young mice are unable to prevent viral replication. Whereas those from older animals are virucidal. Of particular interest were some passive transfer experiments of macrophages into young animals to make them resistant and the administration of anti-macrophage serum to old mice to make them susceptible.

5. The role of antibody as an adjunct to the phagocytosis of viruses by macrophages was stressed and experiments cited in which humoral immunity was suppressed by cyclophosphamide with cellular immunity being retained. Under these conditions, viremia persists.

6. The important role of antibody in protecting animals in experimental infections with coxsackie B3 virus was emphasized using cyclophosphamide as a suppressant of humoral immunity, the human counterpart being agamma-

globulinemics who are unable to handle poliomyelitis infections and to some extent infectious hepatitis. The loss of cell mediated immunity as induced by anti-macrophage serum is of particular significance in pox virus, herpes virus and lymphocytic choriomeningitis infections, the important human counterparts being herpes, varicella, cytomegalo, vaccinia and measles virus infections.

Of course cell mediated immunity is of major importance in oncolytic viral infections as is readily demonstrated experimentally. The human counterpart in this case being patients with kidney grafts on immunosuppressive therapy who develop malignancies.

7. Dr. Allison concluded his remarks with a brief discussion of "killer" or effector cells which attack other cells carrying an antibody marker. In his opinion these cells are neither macrophages nor ordinary lymphocytes but a special type of cell about which much needs to be learned.

8. In the discussion, the role of interferon was downgraded in favor of the macrophage role, recognizing that interferon was also being produced in situations where cell mediated immunity was functioning. Allison would like to relegate interferon to a secondary position in the defense heirarchy.

"Immunoglobulins and Resistance to Viruses in the Gastro-intestinal Tract" was the title of the paper presented by P.L. Ogra, M.D. (Associate Professor, Dept. of Pediatrics, School of Medicine, State University of New York, Buffalo). Infection of the bowel with echo viruses and poliovirus vaccines is followed by secretory IgA response often not associated with IgA in the serum. Of particular interest are some studies of Australian antigen (hepatitis associated antigen) which may be found in the feces for as long as three or four months. When this antigen disappears, IgA antibody appears and may persist in the feces for as long as seven to eight months. Small amounts of Australia antibody appeared in the serum, IgG and a little IgM, but IgA is not found. The role of a secretory IgA in providing protection again various infections was discussed.

Dr. G.C. Schild (WHO, World Influenza Centre, National Institutes for Medical Research, Mill Hill, London NW7 1AA, England) has developed a "single-radial-diffusion test which is a sensitive rapid and convenient method for the estimation of antibodies to the hemagglutinin and neuraminidase of the influenza virus." Virus is incorporated into an agarose gel and the test antiserums are added to wells. Zones of opalescence around the wells became evident in three to four hours and reach maximum diameter in seven to eight hours. Readings are carried out at the end of 16-18 hours by measurement with an eye-piece micrometer or from photographic enlargements. The following advantages are claimed: (1) the test is simple to do, (2) it is unaffected by non-specific inhibitors, (3) finger blood can be used as a source of serum, (4) gels containing virus can be stored at 4° C for up to six months.

"The Role of Local Antibody in Respiratory Syncital (R.S.) Virus Infection" by P.S. Gardner, M. McQuillin and R. Scott (University of Newcastle upon Tyne, Newcastle upon Tyne, England). The authors obtained second samples of secretions from 54 patients known to be infected with R.S. virus as previously determined by isolation and by immunofluorescence techniques. This time, after an average of only seven days, virus was isolated from 31%, even though 76% showed the virus to be present in stained preparations. Thus "virus antigen persisted and could still be detected by immunofluorescence in later stages of the illness when virus could no longer be isolated." Whether the results were due to antigen-decay with loss of infectivity or to neutralization by antibody followed by loss of infectivity, is unknown; the second hypothesis is favored.

"Antibody Response to Influenza Virus Vaccine in the Sputum and Serum of Persons with Obstructive Bronchitis," S. Shore, C.W. Potter, and C. Stuart-Harris (The Royal Hospital, Sheffield, England). The authors immunized three groups of patients with chronic lung disease (10 per group) using a bivalent, formolized, saline vaccine containing influenza strains A2/HK/68 and B/ENG/66. The intranasal route was ineffective (two doses). A single dose of subcutaneous vaccine yielded a good serum response as well as a rise in sputum antibody in 70%. The combination of one dose intranasally and one subcutaneously gave results in between the above. It was concluded that in patients with chronic lung disease, two doses by the subcutaneous route was "the most efficient method of administering inactivated vaccine."

A considerable portion of the program on the second day was devoted to ventilation and isolation requirements for hospitalized patients. Among the various presentations was a two-hour panel on ventilation systems in which nine speakers participated. The titles of their papers are listed in the appendix. Two papers were of particular interest to me. The first was by A. Abel (Royal Institute of Technology, Stockholm), who discussed the measurement of performance, maintenance, and methods for indicating that the air supply and extract systems were functioning properly. He recommends that minimal surveillance requires (1) a knowledge of the ventilating airflow as measured by using $N_2O$ as a tracer gas and determining the outflow by infrared absorption, (2) the number of airborne particles in multiple random samples to be counted by some reliable method, (3) the simple measurement of temperature and humidity, and (4) complete checks of the performance of recently installed filters.

Dr. E.J.L. Lowbury (Birmingham Accident Hospital, Birmingham, England) talked about the "Air Isolation Requirements for Patients in the Context of the Risk of Contact and Autogenous Infection." The basic premise behind the study is that infections acquired through multiple channels cannot be prevented by the "blocking of one or even two of these channels." But if one route is "more important than the others, blocking that route alone should protect the patient." Thus, airborne and contact contamination of burned patients by hospital strains of S. aureus can be influenced by isolation

suites, but infections of some patients by endogenous fecal staphylococci and all patients by proteus and coliform organisms cannot be prevented by such methods.

In an attempt to assess the significance of airborne versus direct contact infection "air curtains" were compared with open-tipped plastic isolations. Control and "air curtain" patients acquired P. aeruginosa infections half the time, but ten patients who were managed in the plastic isolators were not infected at all. S. aureus colonization was somewhat reduced but occurred with equal frequency in both groups.

Exposure of patients to infection in the operating room theater is short when compared with the exposure of the burned skin or a granulating wound during treatment. As Lowbury points out, "the longer the patient is in the ward, the harder it is to keep him free from cross infection."

The general session on the third day was concerned with transmission of infections in closed environments.

Dr. M.T. Parker (Central Public Health Laboratory, London) gave a general paper titled "Transmission in Hospitals." He began by saying that the more expensive the device the more we should be certain that it will do for the patient what is expected of it. He noted that there are times when the transmission is so complex that it may be necessary to run the risk of including preventive procedures which are almost certain to be worthless. Furthermore, a consortive approach is almost always necessary; therefore, everyone involved has to agree, in advance, to carry out their accepted assignments. Since clinical trials detect only large differences, it is necessary to support the conclusions with bacteriological data. In Parker's opinion attempts to prevent infection should have the following priorities, i.e.,

1. to reduce the incidence of clinical disease
2. to decrease the colonization rate
3. to reduce the accessibility of susceptible patients
4. to lower the number of organisms in the immediate environment
5. to reduce the number of organisms in the environment.

The comments of Dr. P.S. Brachman (Center for Disease Contol, Atlanta, Ga.) concerning the Parker paper were instructive. In a study involving 70 hospitals it was found that 5% of the patients were infected; 50% of all infections involved the urinary tract; 15% affected the respiratory tract and 30% of these were due to aerosol equipment. Many species of microorganisms were involved; only 5-10% of the respiratory infections were caused by staphylococci. Brachman also pointed out that the incidence of staphylococcal infections in hospital nurseries has increased sixfold since the withdrawl of hexachlorophene powder from the market.

A rather stimulating and controversial paper titled "Studies of the Aerobiology of Dentistry" was presented by Dr. R.L. Miller (Pleasant Hill, Calif., USA). He believes that dentists are especially vulnerable to aerosols created by the nature of their contact with patients and by their instrumentation. Dental students and young practitioners contract all sorts of airborne infections, but in later years they acquire resistance and remain in good health until they succumb to cardiac disease. Miller demonstrated that aerosols, generated by most dental procedures, resulted in the projection of numerous organisms into the atmosphere. These were particularly evident when water sprays were used or when drilling procedures were being carried out. High-velocity suction devices reduced the dissemination by 99%. The dentist can protect himself or, if he has a cold, his patients, by wearing a special fiber-glass mask. Another problem is the failure of the dentist to recognize that the water reservoir is often contaminated and may contain a million or more organisms (pseudomonads) per milliter. The splatter problem is a difficult one to prevent, and data were presented to indicate that during operative procedures, saliva is thrown several feet in all directions. Partial bacteriological control can be obtained by employing a rubber dam, by having patients use an effective mouth wash (quaternary type), by replacing the air-water spray with a hand-operated stream containing a small amount of chlorine, by continuous suction during operative procedures and by wearing protective glasses. Miller pointed out that he knew of several dentists who had suffered the loss of an eye because of a dendritic ulcer (herpes simplex) contracted from a patient carrier; a few dentists have contracted catastrophic infections with M. tuberculosis.

Miller was asked why he recommends these procedures since dentists are generally healthy despite the hazards. The obvious reply was that both the patient and the dentist need to be protected from each other and that it is only proper hygenic practice to avoid the exchange of aerosols. Stoxil ointment was recommended by Miller for the treatment of early herpetic lesions.

Dr. H.A. Behagel (The Netherlands) gave a substitute paper on "Aerosol Transmission in Industry." He presented data on an attempt to determine the cause of contamination in a biologic bottling procedure by inserting blanks into the operation. The contamination was not airborne but was due to technician contact. Dr. Rylander (Sweden) commented on a situation in a sewage plant in Sweden where gastrointestinal disturbances in the workers were due to aerosols generated in the plant.

Dr. B. Buch (Research Institute of Swedish National Defence, Sundbyberg) discussed "Bacterial Numbers in Air" using a "semi-continuous slit-sampler, working according to correct aerodynamic principles" even in sub-zero weather. He observed that the bacterial, air content is greatest during the summer and autumn depending on the wind direction and its velocity. As might be expected, the counts were highest in city air where values of 1,000 organisms/$m^3$ were obtained. He amplified the previous comments of Rylander by giving specific details. Thus, for sewage plants the aerosols contain

700,000 microbes/$m^3$. Where chemical devices are used for destroying garbage, values of 13,000 are reached. "In both types of plants, the numbers of airborne coliforms (35° C) may exceed 'normal' total indoor concentrations." Transport distances as great as 1800 km (just over 1,100 miles) may be reached.

The "Transfer of Micro-Organisms by Marine Aerosols" was discussed by Mme J. and Dr. M. Aubert (C.E.R.B.O.M.-I.N.S.E.R.E.M., Nice, France). Aerosoles originating on the ocean surfaces from "bursting bubbles" of dissolved gases, are picked up and carried for long distances by the prevailing winds. (Blancard, D.C. and Woodcock, A.H., 1957, Tellus 9: 145-158). As a result, it is estimated that NaCl fallout on the continents is of the order of $10^9$ tons per year (Dryssen, D., 1972, Ambio 1: 22-25). The microdroplets (20-100 microns) may also contain bacteria and plankton, trace elements or exogenous substances (hydrocarbons and detergents)." (Buat Menard, P., 1970, These 3e cycle, Paris, 1-95).

The Auberts examined transmission of particles from polluted areas using a large volume filter sampler mounted on an oceanographic boat. Land data were gathered by exposing large plates for 10 minutes at appropriate intervals; cultures were examined after incubation at 20° C and 37° C in order to detect terrestrial and marine bacteria.

Both coliform and marine organisms were found in aerosol samples taken 160 m from the discharge outlet. Maximal counts were 300-500/10 $m^3$ of air. In altitude studies marine bacteria were always recovered from samples taken up to 70 m above sea level. An experimental water basin was constructed with controlled surface evaporation and markers (Rhodamine B and S. marcescens) for collecting more precise data under reproducible conditions.

During the last hundred years the Shetland Islands have been receiving birch and pine pollens from Scandinavia and Britain based on data derived from meteorological charts by J.E. Tyldesley (Lerwick Observatory). Shetland is situated at a latitude of 60° N and a longitude of 1° W; the nearest land is some 155 miles away. "It is found that about 90% of the tree pollen arrives on days of five types only." Weather records going back to 1873 are available. By relating the pollen counts to the weather types it was estimated that the number of days per year (ten-year mean) for effective tree pollen transport was 15 $\pm$ 5.

R.G. Thomson, L.E. Lillie and F. Gilka (Dept. of Pathology, Guelph, Canada) reported on lung clearance studies in calves using the pathogen Pasteurella hemolytica and the Parainfluenza-3 virus. The naturally occurring infection in cattle is associated with stress (transport) plus viral plus P. hemolyticus infection followed by a severe fibrinous pneumonia which is more damaging than the viral or bacterial infection alone. They devised an instrument for administering aerosols and compared P.

hemolyticus clearances with S. aureus in mice and in cattle. The curves for the two are similar with 10.52% of organisms being retained at four hours in calves given P. hemolyticus. Clearance values are significantly increased by the administration of Bacterin, Cortisol and Aerosol Immunization; they are decreased following endotoxin. These studies were carried out with the objective of establishing base values for subsequent work on the effects of viruses and stress on bacterial infections. The instrumentation and the design of the experiments were well conceived.

P. Camner, C. Jarstrand and K. Philipson (Karolinska Institute, Stockholm) reported on "Tracheobronchial Clearance in Patients Infected by Mycoplasma Pneumoniae." In previous publications (Camner, P. et al., 1971, Int. J. Appl. Radiat. Isotopes 22: 731 and 1971, Arch. Environm. Hlth. 22: 444) reported on their method of studying tracheobronchial clearance of inhaled 6-μm teflon particles tagged with $^{99m}T_c$, a gamma emitter with a half-life of six hours. In the present study they scanned chests for radioactivity at one week, at one and at three months after the onset of mycoplasmal pneumonia as demonstrated by rises in CF and cold agglutinin titers. Average retention of radioactivity, 120 minutes after particle inhalation, is shown in the six pneumonia patients as compared with the 17 controls without pneumonia.

Percent Retention after 120 Minutes

| | 1 wk | 1 month | 3 months | 17 controls |
|---|---|---|---|---|
| 6 M. pneumoniae patients | 72.7* | 59.5* | 57.9* | 40.9* |

* One pneumonia patient and one control had practically no retention and should have been discarded.

Impairment of clearance is most marked early in the disease returning toward normal with time ($p=0.05$). The data are variable, i.e., ranges of 1.0% to 73.0% in the controls and 3.4% to 94.8% in the pneumonias are noted. No comments were made regarding the fate and safety of the teflon particles.

Dr. A.I. Gromyko (Ivanovsky Institute of Virology, Academy of Medical Sciences of USSR, Moscow) read a paper entitled "Experimental Mixed Infection Induced by Aerosols of Ornithosis and Influenza Agents." The presentation was difficult to follow, but the essential findings were that a synergistic effect is obtained as evidenced by the augmentation of the lesions and increased concentrations of both agents in the lungs, blood-stream, and distant organs. His data support the observations of others who have attempted synergistic experiments of a similar type (see references in the paper).

Dr. Chien Liu (Dept. of Medicine and Pediatrics, University of Kansas School of Medicine, Kansas City, Kansas) presented a paper titled "Potentiation of Pneumococcal Septicema Development by Mycoplasma pneumoniae Infection." The multiplication of pneumococcus type I following intranasal inoculation of the hamster demonstrated pneumonic pathology and consistent bacteremia (1-10 million organisms/mliter). Infection of animals with pneumocci followed by M. pneumoniae inoculation resulted in higher degrees of bacteremia in shorter periods of time. This potentiating effect was absent if infection with M. pneumoniae was induced first. The model did not show clear cut augmentation because of the unusual virulence of the pneumococci (1-2 organisms produce eventual bacteremia).

D.H. Barry and L.E. Mawdesley-Thomas (Huntingdon Research Centre, Huntingdon, England) gave an interesting paper on the "Laboratory Evaluation of Respiratory Irritation." They operated on the principle that irritation results in an increase in the number and activity of goblet cells. Their animals were put through an experimental procedure after which sections were obtained from the trachea and stained for fat. The number of goblet cells was determined in serial sections (8,000 cells counter) with the aid of an image analyzer which reacts to contrast. Alveolar damage was assessed by machine counting of the number of alveolar septa which intercept a line on the slide. Test rats (cigarette smokers) show fewer intercepts than the controls. Other studies showed that alveolar macrophages are not increased as a result of irritants, but they do contain more acid phosphatase. As a result the cells are stained darker, and this effect is measurable, with a special type of densitometer, by means of light passing through the macrophage. As the irritation increases the enzyme content of the cells rises proportionately. The assorted devices seemed to provide good quantitative data on the irritating potential of chemicals and gases when applied to the respiratory mucosa.

"Bacterial Dispersion from the Body Surface" by K.R. May and N.P. Pomeroy (Microbiological Research Establishment, Porton, Wiltshire, England). In this paper the authors used human subjects placed in a box through which air is drawn, thus generating a surface aerosol containing viable organisms in skin scales, clothes, dust, etc. (consult paper for details of the procedure). The subjects (male and female) were studied clothed and unclothed. In general significantly fewer organisms were obtained from females than males. Unclothed males shed four times more organisms than clothed ones; female counts were lower and unaffected by clothing. The site of shedding in the male was found to be the inguinal area. This was prevented if the subject wore tightly woven undergarments. The results again point up the fact that ordinary hospital clothing is highly permeable to body aerosols which are generated by movements of all sorts. New methods for containing these organisms while still keeping the surgeon or nurse comfortable are urgently needed.

Confirmatory data using a similar apparatus were reported by R. Blowers, J. Hill and A. Howell (Clinical Research Centre, Harrow, England). Of 200 males examined 15 (7.5%) were dispersers (caused S. aureus counts to rise to

360 per $m^3$). On the other hand, none among 313 young women were classed as dispersers. Again the perineal area of the male was the principle source of the organisms.

A related study titled "Dispersal and Skin Carriage of Staphylococci in Healthy Male and Female Subjects and Patients with Skin Diseases" was carried out by G.A.J. Ayliffe, J.R. Babb and B.J. Collins (Hospital Infection Research Laboratory and the Industrial Injuries and Burns Research Unit, Birmingham, England). In this study "11/32 (32%) of males, 1/30 (3.3%) of females, 4/28 (14%) females with pyogenic lesions and 9/12 (75%) of patients with skin diseases were dispersers." Of a total of 104 persons with skin lesions who acted as S. aureus carriers, 55% showed 0-5 colonies in 50 $ft^3$ of air, 22% showed 5-20, 8% showed 21-50 and 17% shed more than 50 colonies in the collected sample. The observation that nasal carriers and persons with skin diseases shed S. aureus from the skin has also been made by several other investigators (see references in the paper).

Dr. J. Dijkman (Nijmengen, The Netherlands) discussed the role of organic pollutants in "allergic" diseases of the lung. He was particularly interested in occupational diseases peculiar to workers in food industries, and talked in some detail about mushroom grower's respiratory illnesses with particular emphasis on the possible etiologic role of the actinomycetes in the compost. Other unusual conditions mentioned include farmer's lung, paprika splitter's lung, lung diseases in malt, wheat and coffee workers, tea taster's and cheese washer's disease, pigeon breeders, hen breeders and bird fancier's lung, weaver's cough, sisal worker's lung, maple bark stripper's disease and others.

The introductory paper in the general session on "Respiratory Immunization" was given by Dr. H.C. Bartlema (Medical Biological Laboratory TNO, Rijswijk, Z.H., The Netherlands). His was a summary type paper emphasizing local versus humoral immunity, the role of IgA, IgG and lymphoid cells. The author reacted favorably toward efforts to immunize against the respiratory viruses using aerosolized vaccines, but questions their use in diseases which are not primarily respiratory in nature. He, himself, has been studying the primary response in mice given tetanus toxoid aerosols with adjuvants and secondary boosters for a previous primary subcutaneous immunization. The results indicate than an interstitial pneumonitis is produced which may be due to delayed hypersensitivity. Support for this concept is obtained from the observation that mice immunized to tetanus toxoid by the intranasal route are protected through "activated" macrophages against 25 LD/50 of L. monocytogenes; subcutaneously immunized mice remained susceptible. A colleague, Dr. Gerbrandy, has observed that greater cellular reaction is produced when the toxoid is introduced directly into the trachea. Intranasal immunization results in high IgA and low IgG levels in the bronchial washings.

"The Bacteriology of Operating Theatres with and without Laminar Flow" is the title of the paper presented by J.C. Gould, F.J. Bine and J.H.S. Scott (Central Microbiological Laboratories in Edinburgh, Scotland). A horizontal laminar flow module was installed in one operating theater with the second room used as a control. Infection rates were determined for all operations and separately for the arthroplastys only. The infections were classed as serious if they were deep and interfered with recovery and as superficial if they did not significantly delay recovery. Much to the surprise of the hospital surgeons, the infection rates for the year prior to installation of the laminar flow device turned out to be only 2.6% for deep infections and 3.3% for arthroplastics. Laminar air flow figures were 2.5% over all and 1.6% for hip arthroplastics (not a significant difference). Studies of bacteria-containing particles in air samples from both theaters were done. The corresponding figures at the operating sites were 20 versus 48/$m^3$ of air and 64 versus 52 at the sides and rear of the theater (not significant). The predominant organisms were Gram-positive cocci and the distribution remained the same after the introduction of the laminar flow chamber. Gould did not indicate that any special precautions were taken in surgery and on the wards which might be responsible for the very low rates of infection, particularly in the orthopedic section.

Whyte and R.H. Saw (Building Services Research Unit, University of Glasgow) reported on their experiences with a laminar-flow operating room compared with a conventional one. Samples were obtained with a high volume split sampler close to the operation site. In the conventional theater they found an average of 282 bacteria/$m^3$ as compared with 21 in the laminar flow system. They attributed the drop to fewer persons being present and the increased air turnover. "The 15 times reduction would be about what was expected on the basis of the extra dilution and isolation." Despite the 90-95% decrease in bacteria, they do not believe that a good case for prevention of infection has, as yet, been presented by those favoring the laminar airflow system. They noted that in the US "there are about 250 such units .... and others are being installed at a rate of 20 per month." Advantages and disadvantages of various aspects of the system were discussed. A particular point which they consider important is that the operating team in the laminar flow unit is isolated from the rest of the theater. This fact seemed more significant to them than the system itself.

Lena Ewetz and S.J. Ludin (Research Institute of National Defense, Dept 1, S-172 04, Sundbyberg 4, Sweden) reported on "Luminol Chemiluminescense. Technique for Detecting Microorganisms." The luminol method was adapted to the autotechnicon thus obtaining sensitivity and accuracy within the range of $10^4$ to $10^7$ E. coli per ml with a standard error of the mean of $\pm$ 10%. Contact the authors for detailed information.

C.S. Cox (Microbiological Research Establishment, Porton Down, nr Salisbury, Wilts., England) talked on aerosol survival of Escherichia coli:

"Effects of Shaking during Growth of Culture and the Influence of Rehydration before Sampling." The author demonstrated that aeration of cultures during growth produces good survival of E. coli on subsequent aerosolization. The effect is presumably due to production of a "deeper" resting phase. Furthermore, when aerosols are humidified before collection, the survival is also increased.

H.A. Druett and A.M. Hood (Microbiological Research Establishment, Porton Down nr Salisbury, Wilts., England) discussed "A Safety Cabinet for Exposing Pathogens to the Open Air." The authors presented a new apparatus for exposing pathogens on micro-threads to the open air. The instrument is smaller than the previous ones and contains improved safety features. For specific details the reader is referred to the authors or to the Symposium proceedings to be published.

R.E. Davids and D.C. O'Connel (Defence Research Establishment Suffield, Ralston Alta, Canada) reported on "A Novel Particulate Aerosol Sampler." An aerosol sampler is attached to a face mask in such a way that the expired air impinges on a filter-paper disc. The device has been designed for the purpose of obtaining "a representative aerosol sample" from man. For additional details contact the authors or see the Symposium proceedings.

M.L. Rotter, W. Koller, H. Flamm, W. Resch and J. Schedling (University of Vienna, Institute of Hygiene, Vienna, Austria) reported on "Sampling of Airborne Bacteria by Gelatine Filters in an Automatic Sampler." The authors devised an interesting air sampler consisting of 12 gelatin foam filters which operate automatically collecting particles from measured volumes of air. The filters are then either melted or dissolved in solvent and bacterial cultures are made from measured aliquots. From the data shown, the initial results were variable and a standard procedure for culturing from the filters is yet to be developed. The automatic sampling device, however, functioned well.

E. Israeli (Israel Institute for Biological Research, Tel-Aviv University Medical School, Ness-Ziona, Israel) discussed "Effects of Drying by Aerosolization and Lyophilization on Macromolecular Synthesis in E. coli." The author examined E. coli "killed" by spraying into an aerosol chamber or exposed to air after lyophilization. The organisms were tested for their ability to incorporate tritiated thymdine into DNA. In both instances the uptake mechanism in the "dead" cells remained intact but only for one cycle of DNA replication. The permease system, transcription to mRNA and translation to protein were not oxygen sensitive (lyophilization experiments) but were damaged by drying from an aerosol. It was concluded that "death in lyophilized, oxygen-treated bacteria is not due to defects in DNA, RNA or protein synthesis but seems to depend on damage to the DNA initiation point."

P.C. Trexler (Royal Veterinary College, London) discussed "Ventilation of Plastic Isolation Systems." The system is designed as a mechanical barrier; therefore the incoming air is filtered and air flows restricted to levels suitable for patient comfort (8GO liters perminute). Parameters

requiring control are sterilization of filters and chamber (irradiation or ethylene-oxide) temperature (a fan may be needed). Special precautions are necessary to guard against filter damage during transport, maintenance of a slight positive pressure for "exclusion isolation" and a "slight negative pressure for containment." An adequate "alarm system," activated through the air flow system is mandatory.

D. van der Waaij, J.M. Vossen, H.B. Kal and T.M. Speltie (Radiobiological Institute, TNO, Lange Kleiwag 151, Rijswijk (Z.H.) and Dept. of Pediatrics, University Hospital, Leiden, The Netherlands) reported on "Biotyping of Enterobactereaceae - An Important Tool in the Evaluation of Systems of Protective Isolation." The authors used the "biotyping" method of Bettelheim and Taylor (J. Med. Microbiol. 1969, 2, 225) in their study of normal as compared with 10 acute myelogenous leukemia patients kept in protective isolation. Colonization by exogenous enterobacteriaceae was more frequent in leukemics than in normals.

R.F. Sellers, D.F. Barlow, A.T. Donaldson, K.A.T. Herniman and J. Parker discussed airborne foot and mouth disease in a nicely documented summary type presentation including a great deal of work done by their own group at the Animal Institute Research Institute, Pirbright, Woking, Surrey, England. At wind speeds of 5 m/sec the virus may travel 36 km (22.5 miles) in two hours ..... and over 100 km (62.5 miles) in three hours.

SUMMARY

The dominant theme of the conference was on host defenses against infectious agents including immunization via the respiratory tract. As in other areas of microbial pathogenesis, the trend is toward the attempt to define the precise biochemical, cellular and immunological phenomena which lead to the establishment, nourishment and replication of the parasite in the tissues. Equally important is the need to indicate cell and organ reaction in biochemical or physiological terms, the type of immunological response both humoral and cellular, their effects on inflammation, phagocytosis, and parasite survival.

It was clear from the data presented that damage to cilia can be induced by a variety of infectious agents, i.e. bacterial, viral and mycoplasmal. With some viruses the point of first contact with the respiratory epithelium may be ciliary and entry of the virus into the cell may well occur from this site. A portion of the Tuesday morning session was devoted to IgA; its possible role in activating $C\bar{1}_3$ was discussed and the interesting notion was advanced that the IgA response is always a primary one. The concept put forth by Dr. Allison that systemic infections are more likely to occur when bacteria and viruses replicate within macrophages is also interesting. The idea that the "killer" cell is a new cell type will need considerable experimental support before it is generally accepted.

The conference had a new look at naturally occurring aerosols, particularly those which are generated by body movement through convection currents from the skin surface and from the perineal region, particularly in the male. The reported experiments indicate clearly that epidermal cells with their entrapped microorganisms are capable of passing through ordinary clothing and that they are being shed into the environment by these heat activated convection currents. A correlated finding is the demonstration that the office of the dentist is an unusually good place for creating aerosols by means of drilling, brushing and spraying operations.

Much effort is currently going into the development of patient isolation systems with an aim to establish and maintain the "germ free" state. Similarly, air flow systems for operating theaters which incorporate the isolation concept, are gaining in popularity. With the growing evidence that endogenous infections are on the increase, one might question the general use of such devices until their effectiveness has been proven beyond doubt.

I did not feel that great advances are currently being made in the areas of research dealing with the artificial aerosols, i.e., delivery systems, preservation of viability and sampling. The role of air pollutants in the pathogenesis of respiratory diseases is on the descending limb of the popularity arc. The transmission of infection in the hospital environment is still attracting considerable interest and will come into more prominence now that bacterial aerosols from the body surfaces have been identified. The aerobiology group is, as yet, undecided about respiratory vaccines. The Russian scientists view their use as an accepted accomplishment. Others worry about reactions, possible damage to the lung from delayed hypersensitivity reactions, and the relationships between resistance and the type of immunoglobulin produced via this route.

The conference was a rewarding one and extremely well organized; the meeting rooms and the acoustics were excellent; our hosts did everything in their power to make our stay a pleasant and memorable one.

## C. APPENDIX

The titles and authors of the panels and papers in the section meetings are attached for perusal by interested persons.

SECTION MEETINGS - MONDAY, SEPTEMBER 4th, 1972

14.30 hr SECTION A. METHODS IN AEROBIOLOGY

Convener and chairman: K.R. May (Porton)

L. Ewetz and S.J. Lundin (Sundbyberg): Luminol chemiluminiescence technique for detection of micro-organisms.

L.J. Goldberg (Oakland), I. Ford and M.V. Wildensten (Oakland): A quantitative technique, compatible with fluorescent antibody identification, for aerosol sampling onto a thermoplastic rubber: an improvement in film preparation.

C.S. Cox (Porton): Aerosol survival of Escherichia coli effects of shaking during growth of culture and the influence of re-hydration before sampling.

E.W. Larson (Frederick): Comparison of sampling devices.

W.S. Miller (Raleigh): A system for testing integrity of sterile product packaging with aerosols.

H.A. Druett (Porton): Safety cabinet for exposing pathogens to the open air.

D.E. Davids and D.C. O'Connel (Canada): A novel particular aerosol sampler.

M.L. Rotter, W. Koller, H. Flamm, W. Resch, J. Schedling (Vienna): Sampling of airborne bacteria by gelatine filters in an automatic sampler.

14.30 hr SECTION $C_1$. CILIA IN INFECTION.

Convener and chairman: D.A.J. Tyrrell (Harrow)

F.W. Denny (Chapel Hill): The damage of cilia by bacteria.

R. Dourmashkin and D.A.J. Tyrrell (Harrow): The attachement and entry of influenza virus to ciliated cells.

B. Hoorn (Umeå)L Effect of respiratory viruses on ciliated epithelium in organ culture.

S.E. Reed and P.S. Nolan (Salisbury): The cyto-pathology of infection with parainfluenza 3 virus in organ cultures of ciliated epithelium, and the effect of treatment with antiserum.

D. Taylor-Robinson (Harrow): Damage of cilia by mycoplasmas.

TUESDAY, SEPTEMBER 5th, 1972 PANEL ON VENTILATION SYSTEMS

11.00 hr Ventilation systems

Chairman and convener: D.v.d. Waay (Rijswijk)

P.C. Trexler (London): Ventilation systems in patient isolation facilities.

11.30 hr Panel Discussion

Chairman: O.M. Lidwell (London)

Panel Members: N. Foord (London); R.E.O. Williams (London); A. Hambraeus (Uppsala); E. Abel (Stockholm): J.F. Burke (Boston); A.G. Towers (London); H.M. Darlow (Porton); E.J.L. Lowbury (Birmingham); W. Whyte and B.H. Shaw (Glasgow)

Subjects.

Ventilation rates and air pressure differences in isolation rooms: N. Foord (London).

Experience with a simple ventilating system in a multi-room isolation unit. R.E.O. Williams (London).

Air flow through doorways. W. Whyte and B. H. Shaw (Glasgow).

A completely plenum ventilated unit with airlocks. A. Hambraeus (Uppsala).

Measurement of performance, maintenance and indication of correct function of the air supply and extract systems. E. Abel (Stockholm).

Unidirectional airflow for ventilation of patient rooms. O.M. Lidwell (London).

Unidirectional airflow in rooms for several patients. A.G. Towers (London).

Air filters for recirculating systems, minimum efficiency requirements for bacteria and viruses. H.M. Darlow (Porton).

Air isolation requirements for patients in the context of the risk of contact and autogenous infection. E.J.L. Lowbury (Birmingham)

12.30-12.45 hr GENERAL DISCUSSION

SECTION MEETINGS - TUESDAY, SEPTEMBER 5th, 1972

14.00 hr SECTION B.

Conveners: C.S. Cox (Porton) and J.C. de Jong (Utrecht)
Chairman: R. Ehrlich (Chicago)

14.00 - 14.45 hr Item 1. EFFECT OF ENVIRONMENTAL VARIABLES ON MICROBIAL SURVIVAL IN AIR

Introduction: E.W. Larson (Frederick): Fundamental elements of environmental effects in aerobiology.

Discussants: L.J. Goldberg (Oakland): Studies on microbial aerosol survival as a function of temperature humidity, oxygen concentration and additives.

H.A. Druett (Porton): Effect on the viability of micro-organisms in aerosols of the rapid rarefaction of the surrounding air.

M.A. Chatigny, H. Wolochow, W.R. Lief and J. Herbert (Oakland): The toxicity of nitrogen oxides for an airborne microbe: effects of relative humidity, test procedures and containment and composition of spray suspension.

F.A. Dark and D.S. Callow (Porton): The effect of growth conditions on the survival of airborne E. coli.

GENERAL DISCUSSION

14.45 - 15.20 hr Item 2. DAMAGE AND REPAIR OF BACTERIA IN AIR

Introduction: R.L. Dimmick (Oakland): Damage and repair: are they time-dependent?

Discussants: J.E. Benbough and P. Hambleton (Porton): Damage to gramnegative bacterial cell envelopes following aerosolization.

M.T. Hatch (Oakland): Analysis of DNA repair systems and the genetic basis of resistance in airborne Escherichia coli

S.J. Webb (Saskatoon): The membrane and the repair of dehydration damage. (Withdrawn)

GENERAL DISCUSSION

15.20 - 15.50 hr Tea

15.50 - 16.30 hr Item 3. DECAY DURING LYOPHILIZATION AND SPRAYING FROM DRY POWDERS

Introduction: R.J. Heckly (Oakland): Responses of micro-organisms to dehydration and rehydration.

Discussants: C.S. Cox (Porton): A kinetic model for oxygen-induced death in dehydrated bacteria.

E. Israeli (Ness-Ziona): Effect of drying by aerosolization and lyophilization on macromolecular synthesis in E. Coli.

D. Greiff (Milwaukee): Cryobiology of viruses classified according to their dichotomous chemical, structural and physical properties.

D. Grieff (Milwaukee): Factors effecting the stabilities of viruses dried by sublimation of ice in vacuo.

GENERAL DISCUSSION

16.30 - 17.30 hr Item 4. MECHANISMS OF INACTIVATION OF VIRUSES AND MACROMOLECULES IN AIR

Introduction: J.C. de Jong, T. Trouwborst and K.C. Winkler (Utrecht): The mechanism of virus decay in aerosols.

Discussants: T.G. Akers (Oakland): Airborne stability of phage and virus nucleic acids.

J.E. Benbough (Porton): Inactivation of airborne viruses

T. Trouwborst and J.C. de Jong (Utrecht): Surface inactivation, an important mechanism of aerosol inactivation for viruses, inactivated at high relative humidity.

17.00 - 17.30 hr GENERAL DISCUSSION

14.00 hr Section $C_2$. Convener and chairman: A.C. Allison (Harrow)

Immunological factors in defence and in pathogenesis of disease.

P.L. Ogra (Buffalo): Immunoglobulins and resistance to viruses in the gastro-intestinal tract.

G.C. Schild (London): A quantitative, single-radial-diffusion test for immunological studies with influenza virus.

R.V. Blanden (Canberra): The role of cell-mediated immunity in virus infections. (Withdrawn)

P.S. Gardner, J. McQuillin and R. Scott (Newcastle): The role of local antibody in respiratory syncytial virus infections.

M.S. Artenstein and B.L. Brandt (Washington): Local immunity of the respiratory tract following meningococcal immunization.

S. Shore, C.W. Potter and C.H. Stuart-Harris (Sheffield): Antibody response to influenza virus vaccine in the sputum and serum of persons with obstructive chronic bronchitis.

14.00 hr SECTION $D_1$. ISOLATION SYSTEMS IN HUMAN PATIENTS

Convener and chairman: D. v.d. Waay (Rijswijk)

I. Introduction: M.H.E.A. Dietrich (Ulm)

II. VENTILATION.

P.C. Trexler (London): (a) Ventilation of plastic isolation systems.

O.M. Lidwell (London): (b) Unidirectional ('Laminar') air flow for ventilation of patient rooms.

L.G. Herman and L.J. Hart (Bethesda): (c) The contamination of a life island environment by 22 patients.

III. BARRIER FUNCTION AND OTHER ISOLATION PRECAUTIONS.

F. Wendt (Essen): (a) Sterilization and germfree techniques.

J. Klastersky (Brussels): (b) Prophylactic treatment by oral and systemic antibiotics.

IV. INEFFICIENCY OF THE ISOLATION PROCEDURES.

W.C. Noble (London): (a) Evaluating patient isolation efficiency by typing Staphylococcus aureus and Pseudomonas aeruginosa

D. v.d. Waay, H.B. Kal, T.M. Speltie (Rijswijk) and J.M. Vossen (Leiden): (b) Biotyping of Enterobacteriaceae - an important tool in the evaluation of systems for protective isolation

A. Levine (Bethesda): (c) Clinical parameters for the evaluation of isolation procedures. (Withdrawn)

V. COLONIZATION RESISTANCE: PARAMETER OF INCREASED SUSCEPTIBILITY TO INFECTION?

D. v.d. Waay (Rijswijk): (a) The role of colonization resistance in the defence system.

J.M. Vossen (Leiden): (b) Recolonization after decontamination: clinical experiences.

M.H.E.A. Dietrich (Ulm): (c) Conventionalization after germfree state: clinical experiences.

VI. TESTING OF THE DEFENCE MECHANISMS WITH REGARD TO THE INDICATION OR TERMINATION OF ISOLATION.

F.M. Collins (Saranac Lake): (a) Testing host defence mechanisms impaired iatrogenically or by disease.

W.J. Stoop (Utrecht): (b) Clinical tests for inventory and follow up capable to be used as parameters for indication of isolation.

SECTION MEETINGS WEDNESDAY, SEPTEMBER 6th, 1972

14.00 hr JOINT MEETING OF SECTION $A_1$ AND $E_2$.

Conveners: J.T. Bartlett and K.R. May (Porton)
Chairman: J.T. Bartlett (Porton)

H. Morrow Brown (Derby): An automatic volumetric culture plate slit sampler.
G. Tamasi (Budapest): Isolation of mycoplasma from the air.
K.E. Myrbäck (Stockholm): Airborne tularemia.
B. Bucht (Sundbyberg): Bacterial numbers in air.
R. Fontanges (Lyon): A new apparatus with large air intake for the sampling of atmospheric microconstituants.
L.C. Lloyd (Melbourne) (read by proxy): Infective particles generated by cattle with contagious bovine pleuropneumonia.
K.R. May (Porton): High volume sampling.
M. Aubert and J. Aubert (Nice): Marine aerosols.

Panel discussion: Problems of sampling in the open air.

SECTION F. (papers read by title)

J.E.W. Morris (Alverstoke) and R.J. Fallon (Glasgow): Airborne bacteria and the nasopharyngeal flora of submariners.
B.S. Gusmann and I.I. Terskikh (Moscow): Morphology of immunogenesis after aerosol vaccination.
A.K. Fowler, A. Hellman (Bethesda) and R.L. Dimmick (Oakland): Environmental pollutants as activators of C-type RNA tumor virus information.
A.I. Danilov (Moscow): The basis for vaccination against measles by aerosols of live vaccine.

14.00 hr SECTION $C_3$

Convener and chairman: R. Rylander (Stockholm)

EXTERNAL FACTORS INFLUENCING PULMONARY DEFENCE TO AIRBORNE MICROORGANISMS

R.G. Thomson, L.E. Lillie and F. Gilka (Guelph): Factors which influence the removal or retention of bacteria reported in the lung by aerosol.
D.H. Barry and L.E. Mawdesley-Thomas (Huntingdon): Laboratory evaluation of respiratory irritation.
G.M. Green (Burlington): Effect of external factors on pulmonary macrophages.

R. Ehrlich (Chicago): Interaction between air pollutants and respiratory infection.

C.G. Loosli, R.D. Buckley, S.Y. Hwang-Kow, M.S. Hertweck, J.D. Hardy and R. Serebrin (Los Angeles): Effect of air pollutants on resistance of mice to airborne influenza A virus infection.

E. Goldstein, D. Warshauer, P.D. Hoeprich and M.C. Eagle (Davis): Effect of ozone and nitrogen dioxyde on murine resistance to pulmonary bacterial infections.

S.A. Buch, B. Woolsgrave, R. Binns (Huntingdon): The effect of prolonged exposure to dilute cigarette smoke on the particle clearance mechanisms of the rat lung.

P. Camner, C. Jarstrand and K. Philipson (Stockholm): Tracheobronchial clearance in patients infected by mycoplasma pneumoniae.

C. Liu (Kansas City): Potentation of pneumococcal septicemia development by mycoplasma pneumoniae infection.

B. Morein (Stockholm): Mechanisms of Respiratory tract infection with bovine parainfluenza-3 virus.

C.G. Loosli, S.F. Stinson, S.Y. Hwang Kow, D.P. Ryan, J.A. Joyce, J.D. Hardy and R.D. Buckely (Los Angeles): Effect of vitamin A intake on the pathology of airborne influenza A virus infection.

A.I. Gromyko (Moscow): Experimental mixed infection induced by aerosols of ornithosis and influenza agents.

W.D. Won and H. Ross (Oakland): Studies on the influence of low temperature environment on mouse resistance to airborne infection.

M.P.C. Karelse (Rijswijk) and A. Billiau (Louvain): Protection against lung virus infection by intratracheal COAM injections.

14.00 hr SECTION $E_1$

Convener: M.T. Parker (London)
Chairman: G. Laurell (Uppsala)

TRANSMISSION IN HOSPITALS

A. MacDonald, H.G. Smylie (Aberdeen): Ward design in relation to post-operative wound infection.

N. Foord (London): Use of gas and particle tracers in the study of infection transmission.

O.M. Lidwell (London): The dispersal of Staphylococcus aureus in hospitals.

G. Laurell, A. Hambraeus (Uppsala): Measurement of airborne exposure to infection in a burns unit.

P. Chadwick (Kingston): Relative importance of airborne and other routes in the infection of tracheostomised patients with Pseudomonas aeruginosa.

B. Jameson (London): Acquisition of infection by patients in two forms of isolation.

S. Krynski and E. Becla (Gdansk): Resistance index of Micrococcaceae and sensitivity to mercury salt as a method of sanitary evaluation in closed communities.

R.E.O. Williams (London): Air sampling for the measurement of exposure to airborne infection in isolation wards.

K.R. May and N.P. Pomeroy (Porton): Bacterial dispersion from the body surface.

R. Blowers, J. Hill and A. Howell (Harrow): Shedding of Staphylococcus aureus by human carriers.

G.A.J. Ayliffe, J.R. Babb and B.J. Collins (Birmingham): Dispersal and skin carriage of staphylococci in healthy male and female subjects and patients with skin disease.

11.00 hr. SECTION $C_4$

Convener and chairman: H.C. Bartlema (Rijswijk)

RESPIRATORY IMMUNIZATION

A. Bacterial infections

I.I. Terskikh (Moscow): Theoretical bases for vaccination with fine-dispersed aerosols.

J.M. Fournier (Lyon): Kinetics of hemagglutinating antibody formation in Cynocephalus monkeys after aerosol immunization with lyophilized Pasteurella pestis $EV_{40}$.

R. Waldman, E. Fox, A. Mauceri and A. Dorfman (Gainesville): Aerosol immunization with streptococcal M-Protein.

R.B. Hornick, H.T. Eigelsbach, W.R. Griffith (Frederick): Continued studies on aerogenic immunization of man with live tularemia vaccine.

12.00 hr

B. Virus infections

K.S. Liem, E.A. Marcu, J. Jacobs and R. van Strik (Weesp): Intranasal immunization with inactivated influenza virus vaccine.

G. Thomas (Porton): Respiratory immunization with inactivated influenza aerosol vaccine in man, followed by live challenge.

C.J. Bradish (Porton): Respiratory immunization and cross-protection by Semliki Forest virus and VEE virus.

W.J.C. Bogaerts (Rijswijk): Respiratory immunization of mice against encephalomyocarditis (EMC)-virus

11.00 hr SECTION $C_5$

Convener and chairman: N.G.M. Orie (Groningen)

RESPIRATORY ALLERGY

A. v.d. Assem (Utrecht): Daily census of airborne pollen in the Netherlands, especially in relation to hay fever.

R. Davies (London): Airborne spores and seasonal disease.

J. Charpin, C. Boutin and J. Aubert (Marseille): Correlations between atmospheric studies and clinical data in pollinosis. Computer study.

K. de Vries (Groningen): Prevalence of skin tests with and precipitins against some airborne fungi in a randomly selected group of a rural population.

H. Morrow-Brown (Derby): Geographical and circadian distribution of fungi spores and relation to clinical phenomena.

W.S. Benninghoff (Ann Arbor): Criteria for minimal aerobiological monitoring in regional and global systems.

A.W. Frankland (London): Insects as allergens.

11.00 hr SECTION $D_2$

Convener: D. v.d. Waay (Rijswijk)

VENTILATION OF OPERATING THEATRES

The ventilation of surgical operation rooms. The last 50 years. O.M. Lidwell (London)

PANEL DISCUSSION

Chairman: R. Cook (Porton)

J.C.N. Westwood, E. Criddle, S.A. Sattar, E.J. Synek and P. Neals (Ottawa): Sterile air-bath unit in surgery.

J.G. Gould, F.J. Bone and J.H.S. Scott (Edinburgh): The bacteriology of operating theatres with and without laminar flow

W. Whyte and B.H. Shaw (Glasgow): The design and comparative advantages of laminar flow systems.

H. Laufman (Bronx, N.Y.): Confusion in application of clean air systems to operating rooms.

D. v.d. Waay and J.F. v.d. Wal: "Laminar" airflow ventilation: crossflow or downflow?

E. Abel (Stockholm): Allender ceiling for operating rooms.

P.A. Bossers, R.D. Crommelin and E. van Gunst (Delft): Flow patterns and distribution of particles.

H.U. Wanner (Zürich): Airborne bacteria in operating theatres with different ventilation systems.

GENERAL DISCUSSION

THURSDAY, SEPTEMBER 7, 1972

14.00 - 14.45 hr

THE OPEN AIR FACTOR

Chairman: I.H. Silver (Porton)

Introduction: H.A. Druett (Porton). The open air factor

A.M.G. Hood (Porton): Open air factor in enclosed systems.

G.J. Harper (Porton): The influence of urban and rural air on the survival of micro-organisms on microthreads.

G. de Mik (Rijswijk): The survival of Escherichia coli in the open air in different parts of the Netherlands

T. Nash (Porton): Detection of presumed OAF using a nucleus counter.

GENERAL DISCUSSION

14.45 hr R.F. Sellers, D.F. Barlow, A.I. Donaldson, K.A.J. Herniman and J. Parker (Pirbright): Foot and mouth disease; a case study of airborne disease.

15.20 hr J.C. Zadoks (Wageningen): Long range transmission of phytopathogens

16.20 hr The limitations of human aerobiology (by a Streptococcus).

16.45 Closing of the symposium