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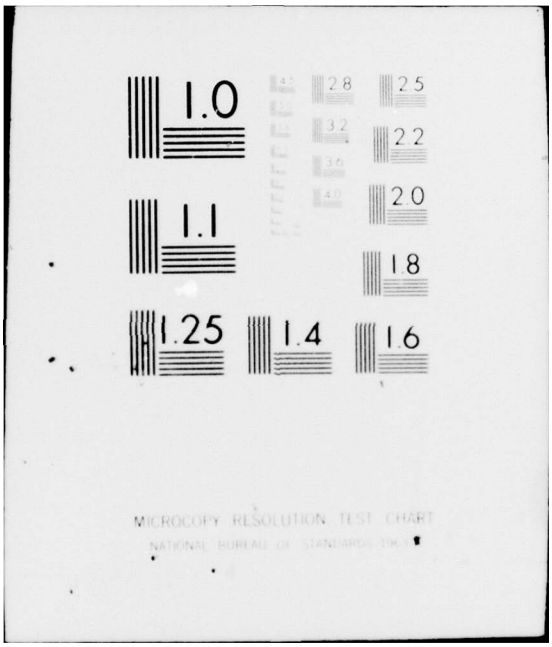
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FINAL REPORT

Serological Diagnosis of Gonorrhea

by

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1 December 1976

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a) Summary of research accomplished.

An antigen was extracted from T-1 phase of Neisseria gonorrhoeae strain F62 which was grown in a medium containing ^{14}C-glucose. This antigen was extracted with sodium deoxycholate and was purified partially by fractional ethanolic precipitation and centrifugation (1,2). This ^{14}C-labeled antigen was used in an attempt to develop a radioimmunoprecipitation assay which could detect antibodies in human sera specific for N. gonorrhoeae. The ^{14}C-antigen was incubated with samples of sera from patients with gonorrhea and the radioactive antigen which had bound to antibodies was precipitated as an immune complex by rabbit antiserum to human immunoglobulins (2).

This procedure appeared to offer promise for detecting antibodies to N. gonorrhoeae in human sera. In general, it was found that acutely infected (longer than 7 days) patient's sera and convalescent (cured less than 6 months before) patient's sera reacted more strongly with the gonococcal antigen than did control sera from presumably uninfected individuals (3,4). Sera from male gonococcal infected patients could be assigned correctly as positive by the radioimmunoassay (RIA) procedure 93% of the time. Well documented male negative control sera were called negative in radioimmunoassays in 75% of the cases. This gave a total correct diagnosis rate of negative and positive males by RIA of 90%. Sera from females was correctly assigned as positive or negative in 89% of the samples tested. The combined correct diagnosis rate for sera from males and females was 90% (4).

It was found that female negative control sera demonstrated a lower percentage of false positive reactions than did sera of males. Also, sera from females had a lower background reactivity with the ^{14}C-labeled gonococcal antigen than did sera from control male subjects (4).

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The chemical, physical, and biological properties of the gonococcal specific antigen were studied (4,5). It was found that the specific antigenic activity was destroyed by boiling for 5 min, by digestion with proteolytic enzymes, and by periodate oxidation. Antigenic activity was not destroyed by heating at 56 C for 1 hr, by treatment with 0.1 N acid or alkali, by saponification, or by digestion with ribonuclease or deoxyribonuclease. It was found that the specific antigen was not contained in T3 phase gonococci or in an endotoxin preparation of T1 phase gonococci. A fraction containing specific antigenic activity was eluted in the void volume from Sephadex G-200. Fractions containing specific antigenic activity were found in the regions of 40% and 10% sucrose following ultracentrifugation in a sucrose gradient. A model for the structure of the gonococcal specific antigen was proposed to account for the above findings. It was hypothesised that the gonococcal specific antigen was a low molecular weight, linear or loosely folded glycoprotein. Large, apparently stable, aggregates of the antigen appeared to occur spontaneously (5).

Extracts of the gonococci were prepared as described previously, except that non-radioactive glucose was used in the growth medium. Preparations of these gonococcal antigens could be labeled with ^{125}I by a very mild diffusion method (2-5). It was found that when these externally labeled gonococcal antigen preparations were used in the radioimmunoprecipitation assay procedure to detect gonococcal antibodies in human sera, the results shown in Table 1 were obtained. Sera from culturally positive males gave positive reactions in the RIA in 79% of the cases; well documented negative male sera reacted negatively in 21 out of 32 samples (66% correct). Sera from gonococcal positive females reacted positively in 81% of the samples tested, and negative

female sera reacted negatively 73% of the time. This gave an overall diagnosis rate with the ^{125}I-RIA of 77% correct, 13% incorrect, and 10% indecisive.

In an effort to answer the criticism that the serum component reacting with gonococcal antigen might have been C-reactive protein and not specific antibody (5), the following experiments were performed. The RIA reactivities of selected sera were compared with the reactivity of these sera in a latex-agglutination test for C-reactive protein (CRP). However, no correlation was observed between the RIA reactivity and CRP reactivity of 62 sera tested. Therefore, CRP was not being detected in our assay (5).

A cooperative blind study, to determine the efficiency of the RIA on sera from cases of gonorrhea of short duration, was initiated with Dr. M. C. Shepard at Camp Lejeune, N.C. (1-3). Serum samples were collected from gonococcal infected individuals and from control (non-infected) individuals. The histories of the patients were sent to Dr. G. T. Strickland, NMRI, Bethesda, MD. The diagnosis of these 600 coded sera based on RIA results was as follows: 301 (50.2%) demonstrated positive reactivity; 207 (34.5%) were diagnosed as negative; and 92 (15.3%) gave indecisive RIA reactivity (5). The results of the assays on the individual sera were sent to Dr. Strickland for examination of correlation of the RIA with the patients' histories. Unfortunately, the results were not available in time to be included in this FINAL REPORT.

Several other phases of the investigation were in progress and were terminated when the contract expired. The nascent results which were just being obtained are summarized below:

Experimental methods were being worked out to examine the qualitative nature of the antibodies in human sera which reacted with gonococcal antigen in the RIA (5). Hopefully, this information would have been used to modify

the assay procedure so that an indication of the duration of the infection could have been gleaned from the test results. Also, it was hoped that vaginal and seminal secretions could have been tested for secretory antibodies to the gonococcus. However, none of these experiments had, as yet, yielded meaningful results.

On a limited scale we had initiated studies to determine the feasibility of differentiating cultures of virulent N. gonorrhoeae from related organisms by pyrolysis-gas-liquid-chromatography (5). Preliminary studies indicated that this technique could be used to determine whether or not certain gram negative diplococci, cultured from patient exudates, but which did not ferement glucose were virulent, but atypical gonococci or were atypical saprophytic Neisseria (or related genera).

Table 1. Summary interpretation of reactivity of sera with ^{125}I-labeled gonococcal antigen in radioimmunoprecipitation assay.

Sex of patient	Patient history	Number tested	RIA diagnosis Diagnosis	No.	Percentage correct
Male	positive	72	P[a]	57	79
			N[b]	7	
			I[c]	8	
	well doc.[d] negative	32	P	8	66
			N	21	
			I	3	
	ill doc.[e] negative	66	P	31	39
			N	26	
			I	9	
Female	positive	58	P	47	81
			N	6	
			I	5	
	well doc. negative	96	P	14	73
			N	70	
			I	12	
	ill doc. negative	5	P	0	80
			N	4	
			I	1	
Total of male and female	positive	130	P	104	80
			N	13	
			I	13	
	well doc. negative	128	P	22	71
			N	91	
			I	15	
	positive and well doc. neg. cumulative	258	correct	195	76
			incorr.	35	
			Indecis.	28	

[a] P-positive
[b] N-negative
[c] I-indecisive (within 2 standard deviations of the mean dividing positive and negative)
[d] Well doc.-well documented negatives (good culture methods and history)
[e] Ill doc.-ill documented negatives (no culture and poor history)

b) Index of technical reports.

1) Rudbach, J. A. STATUS REPORT No. 1, Serological diagnosis of gonorrhea, Contract N00014-74-A-0013-0001, Task No. NR 136-958, 22 February 1974.

2) Rudbach, J. A., M. K. Luoma, and W. R. Cross. ANNUAL REPORT NUMBER 1, Serological diagnosis of gonorrhea, Contract N00014-74-A-0013-0001, Task No. NR 136-958, 31 August 1974.

3) Rudbach, J. A. and M. K. Luoma. STATUS REPORT No. 2, Serological diagnosis of gonorrhea, Contract N00014-74-A-0013-0002, Task No. NR 136-958, 26 February 1975.

4) Rudbach, J. A., M. K. Luoma, and E. C. B. Milner. ANNUAL REPORT NUMBER 2, Serological diagnosis of gonorrhea, Contract N00014-74-A-0013-0002, Task No. NR 136-958, 31 August 1975.

5) Rudbach, J. A., M. K. Luoma, and E. C. B. Milner. STATUS REPORT No. 3, Serological diagnosis of gonorrhea, Contract N00014-76-C-0268, Task No. NR 204-004, 1 March 1976.

c) Index of publications.

Luoma, M. K. and J. A. Rudbach. A radioimmunoassay to detect antibody specific for Neisseria gonorrhoeae in human sera. A. S. M. Abstracts, pg. 40 (1975).

Luoma, M. K., W. R. Cross, and J. A. Rudbach. Radioimmunoassay for quantifying antibody to N. gonorrhoeae in human sera. Brit. J. Vener. Dis. 51: 387-391 (1975).

Milner, E. C. B. Characterization of an antigen of Neisseria gonorrhoeae. Master of Science Thesis, University of Montana (1976).

2 manuscripts are currently in preparation: one describes the modified RIA, which employs ^{125}I-labeled antigen for assaying antibodies to the

gonococcus; the second is a description of the physical, chemical, and biological characteristics of the gonococcal antigen.

d) Conclusions drawn from research data.

With a sensitive and specific radioimmunoassay technique it was possible to detect antibodies in the sera of 90% of males and females which have been infected with gonorrhea for more than 7 days or have recovered from a case of gonorrhea less than 6 months previously. The assay was on the verge of being modified and simplified to the point at which it would be feasible, economically and technically, to be put to use in clinical laboratories. The gonococcal specific antigen used in this assay was a low molecular weight glycoprotein without a highly folded tertiary structure.

e) List of major accomplishments.

1) An extract was prepared from virulent strains of Neisseria gonorrhoeae which contained a gonococcal specific antigen.

2) The gonococcal specific antigen was characterized as a small glycoprotein.

3) The gonococcal specific antigen was labeled, biosynthetically, with ^{14}C.

4) A radioimmunoassay (RIA) was developed with this antigen, and this assay, with 90% accuracy, was capable of detecting antibodies stimulated during human gonococcal disease.

5) The gonococcal-specific antigen was successfully labeled, externally, with the easily quantifiable isotope, ^{125}I.

6) The RIA was modified so that the gonococcal antigen labeled with ^{125}I could be employed in the assay.

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An antigen specific for Neisseria gonorrhoeae was extracted with a surfactant from T-1 phase cells of strain F-62; this antigen was purified partially by fractional ethanolic precipitation and centrifugation. The antigen was characterized as a low molecular weight glycoprotein. With a ^{14}C-labeled preparation of this gonococcal antigen, a radioimmunoassay was developed for the serodiagnosis of gonorrhea. When 382 human sera from acutely infected and well documented negative control subjects were assayed by this procedure, 90% were diagnosed correctly, 5% were diagnosed incorrectly, and 5% of the

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20 results were indecisive. The assay procedure was modified in that the antigen was labeled with a more easily quantifiable isotope, ^{125}I. In this latter system, 77% of the sera were diagnosed correctly, 13% were false positive or negative, and 10% of the results were indecisive.