ent in the particulate fraction is essential for marked immunogenicity(6). Freund's incomplete adjuvant will replace the 'natural' adjuvant for the production of immunity, but attempts to determine whether Freund's incomplete adjuvant would contribute to the resistance lowering action of the ribosomal fraction have been frustrated by the fact that mice will not tolerate Freund's incomplete adjuvant given intravenously.

The way in which the mycobacterial intracellular particulate materials lower resistance is not known. Presumably the cells of the reticuloendothelial system are affected in some manner which makes them less capable of inhibiting the multiplication of virulent cells. This action differs from that of the endotoxins obtained from Gram negative bacteria since *E. coli* lipopolysaccharide under these conditions will not lower resistance of mice to tuberculous infection(11).

Summary. When virulent mycobacteria are mixed with large numbers of viable attenuated mycobacterial cells and injected intravenously into mice the susceptibility to tuberculous infection is markedly increased. By mixing different mycobacterial components, prepared by differential centrifugation of mechanically ruptured attenuated mycobacterial cells, and injecting these into mice, the resistance lowering factor was localized in the intracellular particulate material which sedimented at 144,000 $\times$ *g*. Subfractions, prepared by differential centrifugation, of this particulate fraction, were usually inactive but gained resistance lowering activity when any two were recombined. The particulate fraction is composed of cell membranes, enzymatically active particles and ribosomes.

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1. Kanai, K., Youmans, G. P., J. Bacteriol., 1960, v80, 607.
2. Kanai, K., Youmans, G. P., Youmans, A. S., J. Bacteriol., 1960, v80, 615.
3. Youmans, A. S., Youmans, G. P., J. Bacteriol., 1964, v87, 278.
4. ———, ibid., 1964, v87, 1346.
5. ———, ibid., 1964, v88, 1030.
6. ———, ibid., 1965, v89, 1291.
7. Millman, I., Am. Rev. Respir. Dis., 1961, v83, 668.
8. Youmans, G. P., Youmans, A. S., J. Immunol., 1957, v78, 318.
9. ———, J. Infect. Dis., 1964, v114, 135.
10. Schaedler, R. W., Dubos, R. J., J. Exp. Med., 1957, v106, 719.
11. Youmans, G. P., Youmans, A. S., J. Bacteriol., in press.
12. Youmans, G. P., Karlson, A. G., Am. Rev. Tuberc., 1947, v55, 529.
13. Brodie, A. F., Gray, C. T., Science, 1957, v125, 534.
14. Litchfield, J. T., Jr., J. Pharmacol. Exp. Therap., 1949, v97, 399.
15. Youmans, G. P., Youmans, A. S., Am. Rev. Tuberc., 1951, v64, 534.
16. Sever, J. L., Youmans, G. P., ibid., 1957, v75, 280.

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Detection of Gonococcal Antibody.* (30617)

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Since asymptomatic gonorrhea is a continuing public health problem, especially in females, a serologic test readily applicable to large-scale detection of the disease could be a useful adjunct to presently available culture and fluorescent antibody (FA) procedures. Serologic procedures currently available for detection of gonococcal antibody appear to be nonspecific, especially with respect to meningococcal antibody and/or insufficiently correlated with the presence or absence of human

* Trade names are for identification only and do not represent an endorsement of the P.H.S. or the U.S. Dept. of H.E.W.

infection(1-3). The present investigation describes a new gonococcal antigen preparation employed in a microprecipitin test for detecting human gonococcal antibodies. Results obtained by the new procedure, compared with FA and cultural techniques on both male and female patients suspected of having gonorrhea, indicate a greater specificity and sensitivity than previously obtainable by other serologic tests.

Materials and methods. A virulent *Neisseria gonorrhoeae* culture, designated F62 clonal type 1 by Kellogg(4), was employed in this study because it seemed most likely to reflect the antigenic stimulation responsible for antibody production in the human. The cells were grown on GC Medium Base (Difco) containing 1% of a modified defined supplement composed of cocarboxylase 0.002 g, glutamine 1.0 g, dextrose 40.0 g, and ferric nitrate 50 mg dissolved in 100 ml distilled water and sterilized by filtration (Seitz EK) (4). Plates were incubated under increased carbon dioxide tension (candle extinction) in jars at 35°C for 20-24 hours. The cell crop was harvested with 1.5 ml of distilled water per plate. The harvest was split into 2 parts for serologic comparison of the total antigen mosaic and phenol extract component of the mosaics. One-half of each harvest was exposed to the action of a 200 watt, 10 Kc Magnetostrictive Oscillator for one hour, and this sonicate was arbitrarily designated as the total antigen mosaic. The other half was mixed with an equal volume of 90% phenol (5). This mixture was heated at 65-68°C for 30 minutes, transferred to a separatory funnel and held at 5°C to allow separation of the water and phenol phases. The phenol phase was dialyzed against buffered saline until a negative test for phenol was obtained (6).

The tubes used in the microprecipitin technique had an average inside diameter of 2 mm and an overall length of 35 mm. Approximately 0.04 ml of Seitz (EK)-filtered serum and 0.04 ml of antigen were used in a single test. The tests were incubated for 30 minutes at 23-25°C and read for a precipitation band at the interface using an oblique light against a black background.

The agar gel diffusion technique employed was essentially that of Feinberg(7), using 1% Oxoid "Ionagar" No. 2, 1% sodium azide, and 0.003% of the chelating agent—sodium ethylenediamino tetraacetate. Wells for the serum and antigen were cut with Feinberg agar gel cutters. FA and cultural procedures for the detection of *N. gonorrhoeae* were the same as previously described by White and Kellogg(8).

Human sera presumed to be free of gonococcal antibody were obtained from 22 laboratory personnel (20-45 years) and a group of 40 teenage boys (14-18 years). Through the assistance and cooperation of the Fulton County Health Dept., Atlanta, Ga., sera were obtained from males with signs and symptoms of gonorrhea, males having a history of repeated reinfection with *N. gonorrhoeae* and female contacts of males having gonorrhea. Reactive control serum was prepared by immunizing rabbits (6-8 lb, New Zealand Whites) with *N. gonorrhoeae* F62 type 1 cells harvested in buffered formalin-saline (3% formalin in Bacto-hemagglutination buffer), washed 3 times with Bacto-hemagglutination buffer (BS), and adjusted to a concentration of 10× MacFarland No. 10 in BS. The same procedure was followed in preparing antisera against other species of *Neisseria*. These cell suspensions were divided into aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 ml and frozen at −20°C. Starting with 0.5 ml, these aliquots were inoculated in order on alternating third and fourth days. One week after the last inoculation the rabbits were exsanguinated and the sera frozen at −20°C. Sera from 11 meningococcal carriers were obtained through the courtesy of Dr. J. D. Thayer, Veneral Disease Research Laboratory.

Results. The phenol extract antigen of *N. gonorrhoeae* cells formed a precipitation band in the microprecipitin test with gonococcal hyperimmune rabbit antiserum but not with rabbit hyperimmune antisera against other *Neisseria*. The total antigen mosaic (sonicate) and the phenol extract antigen were compared on rabbit hyperimmune antisera against *N. gonorrhoeae* and *N. meningitidis* serogroups A, B, C, D, in gel diffusion studies (Table I).

TABLE I. Number of Precipitin Bands by Gel Diffusion.

Antigen	Sera: Normal rabbit	Gonococcal type 1	*N. meningitidis* serogroups A	B	C	D
F62–type 1 sonicate	0	6–7	2	2	3–4	2–3
F62–type 1 phenol extract	0	1	0	0	0	0

The human serum control groups, consisting of sera from 22 laboratory personnel, 40 teenage boys, and 11 meningococcal carriers, were serologically nonreactive in the microprecipitin test. Reactive serologic tests were obtained in 70 and 75%, respectively, of the two groups of infected males as seen in Table II. *N. gonorrhoeae* infections were detected in the males of each experimental group through FA and cultural procedures. Of the 197 females named as contacts of men having gonorrhea, 136 (69%) were shown to have gonococcal infections by FA or cultural procedures, and 81 (60%) of these infected females were serologically reactive. Only 17 (17%) of the serologically reactive specimens were not supported by FA or cultural detection of *N. gonorrhoeae.*

Discussion. A phenol extract antigen was obtained from *N. gonorrhoeae* type 1 cells and used to detect antibodies in an overall 62% of the serums of both males and females shown to have gonococcal infections by FA and cultural procedures. The 55 (40%) females who were shown to be infected but serologically nonreactive may indicate undersensitivity in the test, or that the infection had not yet initiated a detectable immunologic response. No information is now available on the time sequence of infection and related immunological response in gonorrhea. The reactive serologic results obtained in the absence of detectable *N. gonorrhoeae* may reflect a situation where self-treatment, after antibody response initiation, had reduced the number of *N. gonorrhoeae* to levels undetectable by either FA or cultural procedures.

It is also possible that these unsupported reactive serologic results reflect a cross-reactivity of the antigen with antibodies against related or unrelated organisms. Hyperimmune rabbit antisera against other *Neisseria* were nonreactive; however, comparable human antisera would be those obtained from persons convalescing from meningococcal infections. Such antisera were not available to us and would not constitute an epidemiologic problem. Such a problem could be posed by a meningococcal carrier having a demonstrable titer. In our case a limited number of proven meningococcal carriers were all found serologically nonreactive with the phenol extract antigen. Furthermore, it has been stated (9) that with meningococcal meningitis "whereas, the formation of antibodies as a result of the disease can be demonstrated, the formation of antibodies in the serum of known 'carriers' has not been conclusively demonstrated." Recent work(10-12) indicates that cross-reactivity with antibodies to unrelated organisms is a possibility; however, such a situation should have been detectable in the control sera. In view of the available information, the authors feel that the use of the phenol extract antigen in this microprecipitin

TABLE II. Microprecipitin Test with Human Sera.

Specimen category	No. specimens	Serology: Reactive		Nonreactive	
Controls					
Laboratory personnel	22	0		22	
Teenage boys	40	0		40	
Meningococcal carriers	11	0		11	
Patients		+ FA or + culture	−FA and −culture	+ FA or + culture	−FA and −culture
Males (signs & symptoms of gonorrhea)	20	15	0	5	0
Idem + history of repeated reinfection	10	7	0	3	0
Females (named contacts of males having gonorrhea)	197	81	17	55	44

test provides a relatively specific, though somewhat insensitive system, for detecting gonococcal antibody in human sera. Although it could be a useful adjunct to present laboratory procedures and may have value as an epidemiological tool, it does not provide information concerning the presence or absence of an infection in a human. Current research is directed toward the development of a procedure that will distinguish between present and recent infection.

Summary. An antigen which is suitable for detecting gononoccal antibody is obtained from *Neisseria gonorrhoeae* by phenol extraction. The results of employing this phenol extract antigen in a precipitin test, with 3 groups presumed free of gonococcal infection and 3 groups demonstrated to have gonococcal infection, indicate that it can detect gonococcal antibody in human sera.

1. Labzoffsky, N. A., Kelen, A. E., Can. J. Microbiol., 1961, v7, 715.

2. Scherp, H. W., Annual Rev. Microbiol., 1955, v9, 319.

3. Wilson, J. F., J. Path. Bact., 1954, v68, 495.

4. Kellogg, D. S., Jr., Peacock, W. L., Jr., Deacon, W. E., Brown, L., Pirkle, C. I., J. Bact., 1963, v85, 1274.

5. Kabat, E. A., Mayer, M. M., Experimental Immunochemistry, Charles C Thomas, Springfield, Ill., 1961, p740.

6. Feigl, Fritz, Spot Tests in Organic Analysis, Elsevier Publishing Co., New York, 1960, p129.

7. Feinberg, J. G., Intern. Arch. Allergy, 1957, v11, 129.

8. White, L. A., Kellogg, D. S., Applied Microbiol., 1965, v13, 171.

9. Schoenbach, E. B., The Meningococci. Bacterial and Mycotic Infections of Man, J. B. Lippincott, Co., Philadelphia, Pa., p547.

10. Kunin, C. M., Beard, M. V., Halmagyi, N. E., Proc. Soc. Exp. Biol. and Med., 1962, v111, 160.

11. Whang, H. Y., Neter, E., J. Bact., 1964, v88, 1244.

12. Grados, O., Ewing, W. H., Proc. Am. Soc. Microbiol., 1965, M-82, 65th Annual Meeting.

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Biliary Excretion of Chelated Iron.* (30618)

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The biliary route is not usually considered a major pathway for physiologic elimination of iron(1,2,3). It can be estimated that in man less than 0.01 mg per day of the daily iron loss arises from bile excretion(4). However, there are some pathologic situations in which the iron content of the bile increases. In hemolytic anemia, for example, the biliary iron in humans increases 5-fold(4). In experimental hemolytic anemia induced by acetylphenylhydrazine, the bile iron in dogs is elevated 10-fold from a normal output of 0.2 mg/day(5).

Our earlier studies provided some suggestion that a suitable chelate ligand could markedly enhance the biliary excretion of parenterally administered iron(6). We have also shown that uptake of iron by the reticulocyte in an *in vitro* system can be sharply modified by the nature of the iron chelate(7). The present study was designed to further investigate, *in vivo*, these possibilities for modification of iron distribution.

Material and methods. Normal adult albino rabbits of both sexes in the weight range of 2-4 kg were maintained on common laboratory food pellets, and water *ad lib.* Food was withdrawn 18 hours before experiments. Following induction of anesthesia with intraperitoneally administered dial-urethane, the abdomen was opened by a midline incision and the hepatic bile duct was isolated by blunt dissection. The duct was cannulated with a

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