Staff Meeting Bulletin
Hospitals of the » » »
University of Minnesota

Diagnosis and Treatment Of Whooping Cough

Volume XIV | Friday, October 16, 1942 | Number 3

STAFF MEETING BULLETIN
HOSPITALS OF THE . . .
UNIVERSITY OF MINNESOTA
Volume XIV | Friday, October 16, 1942 | Number 3

INDEX

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William A. O'Brien, M.D.

I. LAST WEEK

Date: October 9, 1942

Place: Recreation Room
Powell Hall

Time: 12:15 to 1:00 p.m.

Program: "Adamantinoma of Tibia"
J. C. McCartney
G. R. Dunn
E. T. Evans

Discussion
Leo G. Rigler

Present: 93

Gertrude Gunn,
Record Librarian

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II. MEETINGS

1. SEMINAR IN PATHOLOGY

Congenital vs. acquired stenosis.
Drs. M. J. Shapiro and A. J. Hertzog.

Monday, October 19, 1942, at 12:30 p.m., 104 Anatomy Building.

Visitors welcome.

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2. THE MINNESOTA PATHOLOGICAL SOCIETY

The University of Minnesota Medical School
Institute of Anatomy

Tuesday, October 20, 1942, 8:00 P.M.

- -

Tetanus — Dr. A. B. Baker

Sequels in equine encephalomyelitis — Dr. H. H. Noran

- - -

Business meeting 7:45 to 8:00 P.M.

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III. REMARKS BY DEAN DIEHL

In the procurement and placement program, the response of the medical profession has been excellent; but there is an increasing need for physicians for the next year. Personnel must be released as much as possible for war service. There must be curtailment of our activities, research, special studies, etc.; they must be sacrificed for the time being. A minimum essential staff approved by Washington is all we can expect. Graduate training of specialists must take second place to the needs of the armed forces and civilian population. The number of residencies must be reduced to a minimum. No individual will be considered essential after two years of training. Internships will be kept at one year; English internships are now 3 months. The Army and Navy will allow a one year internship before going into service provided it is taken within a year after graduation.

Medical care for the civilian population in fast growing war centers is a problem. Young men rejected by military forces are urged to practice in these industrial areas as their contribution to the war effort. The medical profession has been allowed to meet its own problems. If they cannot do so, the government will step in.

IV. DIAGNOSIS AND TREATMENT OF WHOOPING COUGH

Allan J. Hill

Whooping cough cannot be traced beyond the middle of the 16th century when it was first described by Baillou in 1578. Further contributions regarding its clinical aspects were made in the latter part of the 17th by Sydenham, Willis, Ettmuller, and Harris. In the middle of the 19th century the disease entered America. Prior to the advent of modern bacteriology, treatment was largely of the folk medicine type, bizarre examples of which were the use of horse urine, sweet milk boiled in donkey's excreta, purges, or pushing the children downstairs.

Pertussis attacks annually about 300,000 children in the United States and kills 1 out of every 39 of these. The average mortality in infants under 3 months of age is 50%, in those in their first year 25%, and in those in their second year 10%.

In the age group from birth to 5 years, pertussis is a far more common disease than measles or scarlet fever, and during this period of life is responsible for more deaths than diphtheria, scarlet fever, measles, or tuberculosis. The death rate from it exceeds that from measles and scarlet fever combined, and is 50% greater than that from tuberculosis. The average age of children attacked is 4 years, with the peak incidence between 2 and 3 years.

Epidemics of moderate severity will attack 222 out of every 100,000 individuals in a community. The attack rate (number of cases of pertussis developing in a certain number of individuals in a population without reference to exposure) varies with investigator and epidemic from 4% to 25%, the average figure being between 12% and 16%. The communicability rate (the incidence of the disease in those with definite exposure) varies with the type of exposure. In previously unexposed populations the communicability rate approaches 100%, regardless of the age of the individual. Striking examples of this were the epidemics in the Faroe Islands. In countries where pertussis is endemic, those children exposed by casual contact in other households develop the disease in about 56% while those with prolonged intimate contact in their own homes develop it in 65% to 96% of the exposures, the average figure being somewhere between 80% and 90%. The communicability rate for institutions is usually on the higher side. The variations in incidence in a particular community will alter the number of exposures to a particular child, and may also play a role in altering the resistance of a particular individual. Since by far the greatest concentration of organisms in droplets of sputum coughed up by the child occurs during the first week, with a rapid decline to a negligible number present after the sixth week, the duration of the disease in the child with pertussis who exposes others is also important.

The Organism and Its Products

Hemophilus pertussis was first seen in the sputum from a child with pertussis by Bordet and Gengou in 1900. The organism was first cultured by them in 1906 on a special medium composed of 100 Gm. of potato in 200 cc. of 4% glycerine solution containing 3% sodium chloride, 5 Gm. of agar, and 50% defibrinated rabbit or human blood. The organism is coccoid in its young virulent form, and bacillary in its old culture phases. A capsule is formed by the young strains. Because of the interstitial type of pneumonia and the intranuclear inclusions occasionally found in the bronchial epithelium in fatal cases, Rich and McCordock in 1932 expressed doubt that the organism was the true cause of whooping cough. Later investigators, however, by means of the instillation of bacterial filtrate and filtrate-free H.pertussis into the respiratory tracts of nonimmune individuals, have shown H.pertussis to be the true etiologic agent. The filtrate may produce the coryza but not the paroxysms characteristic of the disease. Sprunt in his as yet unpublished work has apparently shown that the interstitial pneumonia may be due to the

toxin, and that this pneumonia may be prevented by passive protection with pertussis antitoxin. The value of vaccines made from the virulent phase of the organism and the high incidence of positive cough plates early in the disease would seem to add strong circumstantial evidence of the etiologic relationship.

Toomey and his associates claim that the smooth-colonied coccoid form of the organism is necessary to initiate the disease in man and is the resistant phase of the organism. They believe that the rough-colony, fixed bacillary form produces a mucoid material which is responsible for the characteristic whoop. This mucoid material when injected into rabbits results in the production of antibodies specific for old strains of the organism. When used therapeutically in whooping children this is said to modify and occasionally abort the whoop.

Two other bacilli have been found in a typical case of whooping cough. These are B.bronchosepticus described by Brown in 1926, and B.parapertussis first isolated by Bradford and Slavin in 1937, and later by Kendrick and Eldering in 1938. Incubation periods in such instances are similar to that of genuine pertussis, but the disease course is somewhat milder and shorter. Parapertussis occurs in children already immunized against pertussis and also in those who have previously had the latter disease. H.pertussis is separated from B.parapertussis by cultural methods and by specific agglutination and mouse protection reactions. These antibodies persist for as long as 40 months. Rabbit antiserums against B.parapertussis show a degree of agglutinative cross reaction which varies with the intensity of immunization. This is a result of the presence of a common minor antigen in both organisms. Convalescent human serums also show some degree of cross reaction, and the adult human serums from hyperimmune donors show a much greater degree of cross reactivity, doubtless because of the much greater intensity of immunization. The usual single course of immunizing injections does not yield this. The toxins of B.parapertussis are immunologically the same as those of H.pertussis, but are found in only one-tenth the concentration occurring in H.pertussis Phase I.

Hemophilus pertussis was first divided by means of agglutinogenic properties into four phases by Leslie and Gardner in 1931. Phases I and II are present in smooth-colonied, young, relatively pathogenic forms obtained directly from the whooping patient. Phases III and IV are relatively harmless and are found in rough colonies grown on media containing little or no blood. This work has been amply substantiated by Lawson in 1939 and Flosdorf and co-workers in 1941. Types A and B of Krumwiede, Mishulow, and Oldenbusch demonstrated in 1923 evidently corresponded to Phases IV and III respectively of Leslie and Gardner. Bordet and Sleeswick in 1910 has separated out two types, the first corresponding to Phases I and II, the second to Phase IV. Toomey and his co-workers in 1935 were unable to confirm Leslie and Gardner's work. The only means of determining whether or not the organisms are in the virulent Phase I is to test their surfaces serologically, using Phase I and Phase III antiserums. Phase I antiserum will react with Phase III organisms; but phase I organisms will not react with Phase III antiserum. Consequently both antiserums must be used to establish that any given organism is in Phase I. Phase I antiserums do not react with Phase IV organisms. Phase II organisms are rare.

The virulent Phase I organism can grow on the Bordet-Gengou medium, but not on any liquid medium. The probable minimum requirement of blood in the culture medium for the organism to stay in Phaae I is 15%. Sheep or human blood may be preferable to horse blood in the nutrient medium. On media containing horse blood virulence and typical morphology are lost relatively early. This probably does not apply to the ability to produce specific agglutinins in the rabbit. On sheep and human blood Phase I characteristics are maintained for 85 generations, about a year. This work has been amply substantiated experimentally and clinically by the use of Phase I

organisms maintained by culture for the production of effective vaccines. Sauer's contention and that of earlier workers that the Phase I organism must be isolated directly from the active cases of the disease to make effective vaccines were apparently the outcome of manufacture by commercial houses of vaccine on media unsuitable for maintenance of Phase I characteristics.

Two toxins are present in all phases of H.pertussis. One is extremely thermolabile, being completely destroyed at a temperature of 56°C. for one-half hour; the other is thermostable and only partially destroyed at a temperature of 100°C. for one hour. The thermolabile toxin possesses strong necrotic properties in the skin. One milligram injected intravenously is lethal to rabbits weighing 2000 Gm.

If pertussis toxin plays an important role in whooping cough, one would expect to find antitoxin in the serums of patients recovering from the disease. Yet antitoxin is found in only a small percentage of children convalescing from whooping cough. Because whole killed Phase I vaccines contain no thermolabile toxin, no antitoxin is found after the injection of Sauer's vaccine in the usual doses nor in the large doses used to develop hyperimmune human serum. Bordet and Gengou were of the opinion that the toxin is responsible for the changes in the bronchial epithelium which account for the clinical features of the disease. Sprunt has shown that passive protection with pertussis antitoxin can prevent interstitial pneumonia. Strean and his co-workers and Joslin and Christensen have maintained that the thermolabile toxin has sufficient antigenicity in man to justify the use of a toxoid. But this is as yet unproved in a clinical trial in a study comparable with that of Kendrick and Eldering.

The variant nonvirulent phases of the organism also contain the same toxins but they are present in only about one-tenth the amount present in Phase I. Broth filtrates of organisms grown in liquid media have been shown by Wood to contain the thermolabile toxin. Much greater yields are obtainable from the bacteria themselves by means of mechanical disruption and extraction. Killed whole organisms contain the thermostable, but not the potent thermolabile toxin.

In rabbits the production of circulating antitoxin can be demonstrated after the injection of living organisms or their extracts. In cutaneous tests on rabbits with the toxins of H.pertussis, a primary toxic reaction is observed in normal animals which lasts for a week or longer. The thermolabile toxin produces necrosis. In immunized animals, the prolonged primary toxic reaction is not shown. The presence of circulating antitoxin in the serum of immunized animals can be demonstrated by neutralization tests in the skin of normal rabbits, but no antitoxin can be demonstrated when convalescent or hyperimmune human serums are used. Strean's and Weichsel's results of cutaneous tests with the thermolabile toxin in human beings would indicate the presence of some fixed antitoxin.

Phase I agglutinogen should be present in any preparation for active immunization against pertussis. According to evidence now available there would appear to be little justification for expecting protection from the disease by active immunization with the toxins only. Passive treatment or protection with rabbit serum containing antitoxin as well as antibacterial antibody, however, might possibly be expected to be of greater value than serum containing antibacterial antibody alone. The major thermolabile toxin is not present in Phase I vaccines made from whole killed organisms. Nevertheless, successful active protection has been obtained by the use of this vaccine which contains only agglutinogen and the minor thermostable toxin. The excellent results which have been obtained with properly prepared whole killed Phase I vaccines by Sauer, Kendrick and Eldering, Miller and Faber, Singer-Brooks and many others would make one loathe to substitute untried reagents. Yet the work of those using a detoxified antigen should not be dis-

carded without further clinical trial.

Serum obtained from infants treated with immune rabbit globulin may show no parallelism between agglutinative power and mouse protective ability. Those with high agglutinative power may have low mouse protective value. This is to be explained not on the variation in the antibody content of the serum but on the variation in the content of thermolabile toxin contained in the highly lethal organisms used in the mouse protection tests. Since animals are sensitive to the thermolabile toxin, one should not expect a high agglutinative power in the serum used for protection to save them. Thus agglutinative titer of convalescent serum rather than the mouse protective power parallels recovery.

Further evidence that the toxins are inadequately antigenic in man is the lack of cross protection between pertussis and parapertussis. Since the agglutinogens of these organisms differ, but not their toxins, recovery from either disease can hardly be a result of the production of antitoxin. This explains the observation of Silverthorne and Cameron that highly potent agglutinating and mouse-protecting rabbit serum fails to neutralize "pertussis toxic material" in the skin of normal rabbits. This serum is obtained by injecting rabbits with killed intact Phase I organisms which contain no thermolabile toxin. On the other hand, a high-agglutinating rabbit serum containing little or no mouse-protective antibody did neutralize this material. This latter serum is obtained by injection of disrupted Phase I organisms containing thermolabile toxin. From the foregoing discussion it is evident why the results from skin tests have been conflicting.

Hyperimmune human serum, high in agglutinative power, has been shown to be very effective in the treatment and prevention of pertussis. It is especially valuable in infants under 7 months of age in whom the antibody response to disease is poor. The value of hyperimmune human serum in the passive protection and treatment of parapertussis has not as yet been determined. Because of intensive immunization with Phase I whole bacterial vaccine, high heterologous titers are produced through the agency of the common minor antigen. On this basis there is a possibility of cross protection as a result of the use of such serum.

Skin Tests

The reason for the nonspecificity of the earlier skin tests has only recently become apparent, largely through a better understanding of the antigens of the organism. Workers who used washed Phase I vaccines made from whole killed organisms had somewhat better results as regards a clear-cut reaction. Those who used disrupted organisms (endoantigen) had more specific but less clear-cut reactions, while those using stock vaccines had almost no results at all. At best the tests were not more than 75% to 85% specific.

Finding a method for separating the agglutinogen from toxic components, Flosdorf and Kimball used the Phase I agglutinogen in intradermal tests. Five to ten units of agglutinogen (about 10 to 20 gamma) were used in a volume of 0.1 cc. No primary toxicity was observed with this material either in rabbits or in human beings, including normal babies 8 to 14 months of age. In children vaccinated as long as 6 years previously, in convalescent children, and in hyperimmunized adult donors an immediate response was observed. Reactions may appear either one-half hour or twenty-four hours later. The reaction is characterized by induration with or without erythema.

Strean and his co-workers have prepared toxin free from agglutinogen. In normal animals or human beings this material produces a primary toxic reaction lasting a week or longer. In immunized animals the primary toxic reaction is not shown. Weichsel and his co-workers using a broth filtrate obtained an impure material which gave positive skin tests in convalescents. It apparently produced a skin hyper-

sensitivity which could be passively transferred. The nature of this material has not as yet been determined. Detoxified pertussis antigen acts as a desensitizing agent against Weichsel's material.

Toomey used his mucoid material in skin tests and claimed a high degree of specificity. No other workers have as yet reported results from use of his material.

The Opsonocytophagic Index

Serums containing opsonins facilitate phagocytosis of organisms by blood leukocytes. The number of organisms phagocytosed by a leukocyte give an index of the level of opsonins present in various serums. Three factors exert an appreciable influence on the phagocytic capacity of the newborn's blood leukocytes to take up H.pertussis. These are: 1. the phagocytic power of the mother's blood, 2. previous pertussis in the mother, and 3. artificial immunization of the mother during the latter part of pregnancy with Phase I pertussis vaccine. While pertussis in the mother 6 months to 7 years previously or immunization of the mother during the last 6 weeks of her pregnancy does influence the opsonocytophagic index in the infant, these factors do not influence the titer of opsonins in the mother's blood. This high titer in the infant persists for about 2 months after birth, after which it rapidly falls. If the mother has not had pertussis or immunization recently, the opsonocytophagic index of the child remains negative from birth to about 18 months of age. Nonspecific opsonins appear in the blood after high fever or after diseases of the upper respiratory tract. These nonspecific opsonins reach such a level during later childhood that the opsonocytophagic index loses its value. Earlier, however, those children showing negative, weak, or moderate reactions easily contract pertussis.

During pertussis an increasing opsonocytophagic index is found shortly after the first acute catarrhal period. A high titer is maintained for 2 months after the onset of the disease. The reaction becomes weaker, to remain, however, at relatively high levels for 6 to 9 months. It then becomes weak for 1 to 2 years, and finally disappears.

During immunization with Phase I vaccines the titer increases in the first week to reach a maximum level 2 months after vaccination. The titer declines slightly for the next 6 to 9 months, falling thereafter to relatively low levels for 1 to 2 years. Six months after vaccination 7 to 13% of immunized children have low titers, one year after vaccination 20 to 22%, 1½ years 50%, and 2 years after 60%. Krueger's endoantigen produces no such response. The index also varies slightly with other potent Phase I vaccines, and is low after the use of old strains as vaccines. The test is, therefore, a useful method of estimating the potency of H.pertussis antigens. It has also shown that children under 3 months of age respond poorly to immunization with vaccine, whereas after this period the response is more satisfactory. The test has also been instrumental in demonstrating that a stimulating dose of pertussis vaccine from 6 months to 5 years after the first course of injections tends to renew protection against the disease. It has also been of assistance in showing that vaccine combined with alum or combined with alum-precipitated toxoids produces satisfactory immunity. Administration of human hyperimmune serum increases the opsonocytophagic titer in the pertussis patient.

The Complement Fixation Test

The complement fixation test does not give a true assay of therapeutically effective antibodies unless the test antigen is of whole organisms washed free of metabolites and the products of disintegrated or lysed organisms. The serum (hemolytic amboceptor), sheep red cells, and complement are used in a manner similar to other more familiar modifications of this reaction. In 90% of normal children the test is negative irrespective

of a previous history of pertussis. Regardless of a previous history 87% of normal adults also give a negative reaction. In tests using cord blood, specific antibodies are shown to pass through the placenta. The agglutination test is more sensitive than the complement fixation reaction only when proper preparation of the antigen by freezing and thawing and removal of the thermolabile toxin is used for the former.

In very young infants, the test is regularly negative. It is fairly commonly so up to the end of the first year of life. In older children the complement fixation reaction may be relied on for diagnosis during the acute catarrhal period before the whoop develops. Ninety-six per cent are positive during the first week of the whoop. The highest percentage of positive reactors is reached during the 5th to 8th weeks. Falling gradually the reaction remains positive from 5 to 8 months following the appearance of the cough. Thus in general, one will find positive complement fixation reactions in 80% of cases of pertussis above 1 year of age and after the second week of the disease. Children and adults, with or without a previous history of pertussis, coming in contact with pertussis cases but not acquiring the disease, develop a positive complement fixation reaction in a fair percentage of cases. In some hands, however, the complement fixation test has not proved to be an entirely satisfactory test of immunity developing after pertussis. The development of bronchopneumonia during the course of whooping cough delays the appearance of the reaction or else gives a negative one.

Huenekens was the first to use the complement fixation reaction to control the dosage of fresh vaccine. He first showed by use of this test the differences in immunizing power between fresh Phase I vaccine and stock vaccines made from organisms in older phases. The complement fixation reaction begins to appear about one week after the first dose of vaccine. The maximum reaction appears about one week after the last of three injections. The test becomes negative in from 2 to 4 months. Mishulow's vaccine (new York City Laboratory vaccine), containing more of the toxins than Sauer's vaccine, gives a more marked and prolonged reaction than does the latter. Three to 6 years after the first course of vaccine, a stimulation dose will change the reaction from negative to positive. Because those with incomplete fixations may contract a mild form of the disease, and because maximal responses under the usual course of vaccination occur in only 61% of the cases, higher doses of the vaccine have been recommended. Alum treatment of the vaccine gives a good complement fixation reaction. It is believed that administration of toxoids combined with pertussis vaccine produces a higher antibody titer than giving the vaccine and toxoids separately. Using this test as a basis for their opinion, Sauer and Tucker have concluded that vaccination is most successful when injections are given at three-week intervals sometime after the 7th month of life. Storing of Phase I pertussis vaccine at a low temperature does not impair its ability to induce the production of complement-fixing antibodies.

Agglutination Reactions

The agglutination reaction in pertussis is unsatisfactory unless the test antigen is properly prepared. The separation of the agglutinogen by Flosdorf and Kimball would seem to simplify the problem. Daughtry-Denmarck, Powell and Jamieson, and Mishulow have recently described bedside agglutination tests in which a drop of serum and a drop of antigen are sufficient. The tests are completed in 3 minutes and can be performed on glazed cardboard for permanent record. Mishulow has found slide agglutination reactions to be more quickly performed and fully as accurate as the tube method.

A positive agglutination test may be obtained on the 4th week of the disease. The titer remains high for several months then rapidly decreases, although an occasional patient may show a positive reaction for 2 years. Individuals, with or without previous vaccination, will frequently show an increase in the agglutination titer on exposure to the disease. Hyperimmune and convalescent human serums

contain agglutinins in high titer. These serums given during the course of the disease will raise the antibody titer in the patient. Immune rabbit serum, however, may decrease this titer.

Vaccination produces a maximum agglutination response 2 to 3 weeks later. The response is usually found to have decreased 3 months after vaccination and more so after 5 to 8 months. It is maintained, however, at relatively high levels for from 1 to 3 years. Mishulow's vaccine gives a little better agglutination response than does Sauer's. There is a slight nonspecific rise 2 to 3 years after vaccination which may represent exposure to, and transient carriage of, H.pertussis. Stimulation doses of vaccine after preliminary immunization will usually cause a marked rise in the agglutination titer. There is a good agglutination response to vaccination with alum-treated or combined vaccine and alum-precipitated toxoids.

Mouse Protection Test

When inoculated intraperitoneally with virulent Phase I H.pertussis, 17% of mice die. When 5% aqueous solution of hog mucin or 4% starch solution is included with the organisms, 88% of the mice die. After controls have been established, the mice can be protected to a varying degree with various antigens and with antibodies contained in immune serum.

Mouse-protective antibodies in the serums of patients with whopping cough develop rapidly and consistently beginning at about the third week of the disease. They reach their maximum titer a few weeks later, then fall off much as do the titers of the other antibodies described above. The protective power of convalescent and hyperimmune human serums and of immune rabbit serum bears no relation to their agglutinin titer. This is due to the variation in lethal thermolabile toxin content of various Phase I organisms used to test protection. Mishulow and associates have shown the development of mouse-protective antibodies in immune and non-immune persons in contact with cases of pertussis. From these studies they concluded that a history of pertussis cannot be relied on. "Head colds" do not increase the mouse-protective titers.

After immunixation of children with the usual doses of Phase I vaccine, protective antibodies appear in their blood 3 weeks after the last injection, reach their maximum level in $1\frac{1}{2}$ to 3 months, and persist for from $1\frac{1}{2}$ to 4 years. Stimulation doses of vaccine give an increase in mouse protection of the serum. Combined alum-treated vaccines give as good protection as single vaccines, and increased dosage gives even better protection.

The mouse protection test has thrown light on many facts about vaccines and other methods of treatment. Intact smooth-colonied organisms in Phase I make better vaccines than disrupted, dissociated, or intermediate forms of the organism. To produce effective vaccine organisms are better killed by phenol or cresol than by formalin, merthiolate, ether, or heat. Washing of vaccines in saline or distilled water removes only a negligible amount of the effective antigen. The use of detoxified pertussis antigen or of antigens administered by the intranasal route has not received unqualified approval by studies of mouse protection. Sulfanilamide and sulfapyridine have no mouse-protective power. Sulfadiazine has good protective power.

Cough Plates and Nasopharyngeal Swabs

Cough plates are positive in 75% of the cases in the first week of the disease, 70% in the second, 60% in the third, 30% in the fourth, 15% in the fifth, and 5% in the sixth. Nasopharyngeal swabs have greatly increased the percentage of positive cultures. When both swab and cough plate are used simultaneously, the percentage of positive cultures is increased about 15% to 20% over the above figures. The nasopharyngeal swab aids in the recovery of the organism more certainly than does

the cough plate, particularly in children under 3 years of age. Throat cultures are ineffective. Frequently the nasopharyngeal produces the organisms in pure culture. Bronchopneumonia does not affect the expectoration of the organism.

Sedimentation Rate

The erythrocyte sedimentation rate is within normal limits in pertussis. But since 30% of all forms of bronchitis exclusive of pertussis also have a normal or retarded sedimentation rate, the test has little or no value.

Phase I Vaccine

Before 1917 vaccines were made from stock cultures of organisms not in Phase I, and were used in small doses of one-half to one billion organisms. Huenekens in 1917 and 1918 showed by means of the complement fixation test that commercial stock vaccine was inferior to fresh vaccine. Vaccine only 2 to 3 months old immunized 12½% of children vaccinated, vaccine 2 to 4 weeks old immunized 25 to 75%, while fresh vaccine immunized 94 to 100%. He also showed that 3½ billion organisms as a prophylactic dose was far more satisfactory. Barenberg found shortly thereafter that this dose of fresh vaccine was satisfactory in prophylaxis and he claimed that a dose of 5½ billion organisms ameliorated the disease. During the Faroe Island epidemics doses of 22 billion organisms were used and reported by Madsen in prophylaxis and prodromal treatment. Madsen cited two deaths occurring in newborns upon the second injection of vaccine. Convulsions in these two cases with death, and in three other somewhat older infants under two years of age with recovery have been the only severe reactions reported.

Sauer has popularized the use of fresh vaccine. His vaccine is made by isolating freshly expectorated organisms from acute pertussis cases. These organisms are grown for 48 hours on Bordet-Gengou medium containing 20% human placental blood. The colonies are then scraped off, suspended in physiologic saline containing 0.5% phenol, stored in the refrigerator for one week, and then diluted with saline and phenol to contain 10 billion organisms per cc. Fresh vaccine is made up every few months from freshly isolated strains. The injections are made in both arms weekly, 1 cc., 1½ cc., 1½ cc. in each arm, to total 80 billion organisms. Reactions seldom exceed a local induration 4 to 5 cm. in diameter, some tenderness persisting 48 hours, and occasionally a nodule persisting a week or two. There is no enlargement of the axillary lymph nodes. The temperature varies from normal to 102°F for 8 to 12 hours. The leukocyte count rises during the period of vaccination to levels of 12,000 to 16,000, occasionally to 30,000. He has found no instances of the Arthus phenomonon and believes that immunity is not complete until 4 months have elapsed after the last injection. He does not advocate his vaccine for treatment of the active disease. He found that vaccination in infants less than 3 months of age is less effective than in those over 7 months. In these his failures have been about 6% in his series of 2500 children so immunized.

Other Phase I vaccines have been prepared. Kendrick's vaccine differs from Sauer's only in the use of 15% sheep blood and 1:10,000 merthiolate instead of phenol. Doull's vaccine contains formalin, has been grown on 30% human blood, and is washed in distilled water. Mishulow's vaccine is grown on a 30% sheep or horse blood infusion, or chocolate agar, and contains toxic filtrates. Some workers have found slightly greater protection with Mishulow's vaccine, others slightly worse than with Sauer's. There is probably no difference between fresh and stored vaccines of Phase I organisms. Flosdorf has advocated the use of master strains of broad specificity maintained in a stabilized dessicated form for the production of vaccines.

With the exception of certain workers, the reports on the use of prophylactic Phase I vaccine have been favorable. The duration of immunity is apparently about 2 years. The communicability rate in a vaccinated population for all types of

Phase I vaccine prophylaxis lies between 8 and 35%, the average range being 13 to 17%. The communicability rate for the unvaccinated population is between 80 and 90%. All are agreed that the disease is milder and shorter in vaccinated individuals. Those of this group of workers who have tried the vaccine in treatment have found its effect negligible. Some believe that treatment with vaccine makes the clinical course more severe. The vaccine can be given intracutaneously, subcutaneously, or intramuscularly. Incutaneous injections can be smaller in volume and still produce good immunity, but in general are too painful.

Antibody and clinical studies have suggested that the total dose be raised to 140 billion organisms, best given in the double-strength vaccine of 20 billion organisms per cc. Daughtery-Denmarck believes that the 40 billion alum-treated vaccine gives the best results. The effectiveness of the vaccine, she claims, is not determined by the number of organisms but by the rate of absorption.

The well-known decline in antibody titer and the failure of the vaccine to protect 8% of children exposed 2 to 3 years after vaccination and 57% exposed 4 to 5 years later indicates the desirability of giving a stimulating dose of vaccine 2 years after vaccination or just prior to the child's entering school. The preferable dose is 20 billion organisms.

Harrison, who advocated the use of alum-treated vaccine, used it in one dose, Later certain of his co-workers gave it in two doses. Alum-treated vaccine has been used by others combined with diphtheria and/or tetanus toxoids. Laboratory and clinical studies still being accumulated appear to show that the immune response is better with mixed antigens than with single antigens given in separate courses. Dosage intervals have lengthened to 3 weeks and even 4 weeks. The first injection is best given in the seventh month. Vaccination of mothers in their last 2 months of pregnancy with 3 injections of 20 billion organisms each at two-week intervals may tide the infant over the difficult first 6 months.

VACCINE DOSAGES

Type of Vaccine	Single or combined Vaccine	Strength of Vaccine in billions per cc.	Total immunizing dose of Vaccine
Plain	Single	10	14 cc.
Plain	Single	20	7
Plain	Single	40	4
Alum-treated	Single	20	2.5
Alum-treated	Single	40	1.5
Alum-treated	Plus diphtheria toxoid	20	8
Alum-treated	Plus diphtheria and tetanus toxoids	20	2.5

Endoantigen - Undenatured Bacterial Antigen

Krueger believed that the protein antigen of H.pertussis was denatured by the addition of various sterilizing agents in the preparation of vaccines. He therefore disrupted the organisms in a ball mill, filtered, and adjusted the final concentration of the filtrate so that 1 cc. contained the product of 10 billion organisms. The earlier and some of the later reports on the use of this antigen in prophylaxis in doses equivalent to 40 to 80 billion organisms were rather favorable. But in general later reports have shown that this antigen produces no change in the communicability rate from that of the controls. Failure in prophylaxis amounted to somewhere between 40 and 90%, probably nearer the latter.

The impression concerning the use of the antigen in treatment is a little more favorable. If the antigen is begun early in the incubation or catarrhal period and is given in doses equivalent to between 100 and 300 billion organisms, the whoop may be somewhat modified. But this dose in the case of infants should be given with extreme caution. The possible mechanism of this action, whether desensitization or the production of antitoxin, if either, has not as yet been explained. In fact, Miller and Singer-Brooks under carefully controlled conditions failed to duplicate the results of other workers in treatment. They emphasized again the variable course of pertussis. Too often it has not been appreciated that the peak of frequency of the cough occurs between the 8th and 15th days of the whoop, thereafter decreasing. There is an average daily variation of 4 in the number of paroxysms, and in at least 10% of the cases more than 9 occur.

Pertussis Antigen Detoxified

This material is made by growing Phase I organisms on Bordet-Gengou medium for a few days, then transferring to beef heart infusion broth for 4 to 6 days, and detoxifying the collected filtrate with 0.5% formalin for 4 days at 40°C. The filtrate produces skin necrosis in rabbits and probably contains the thermolabile toxin. The addition of formalin to this filtrate probably forms a toxoid.

For prophylaxis the antigen is given in 1½ cc. doses every other day for 4 doses. Investigators of this material are not in complete accord as to its value. Favorable reports suggest that with the above dosage protection is possible in 90% of the children immunized, while with smaller doses it is less certain. Protection during the incubation period of the disease is said to be about 65% effective. This is better than any agent other than convalescent serum. The immunity is said to last from 5 months to 2 years. Results in the mouse protection test are equivocal.

In treatment with this antigen there is again considerable difference of opinion. The recommended doses are 1 to 2 cc. daily, maximal dose the first day, then 1 to 1½ cc. every or every other day thereafter to total 6 to 12 cc. It is claimed by certain investigators that beneficial responses are obtained in between 56 and 90% of children so treated. Others using even higher than the recommended doses under excellent control, fail to find the disease altered.

Inasmuch as this antigen may contain the toxoid of the thermolabile toxin, and as toxin may play a role in the disease in man, it is possible that this antigen may prove to be more promising than simple Phase I vaccine. Sprunt observed that the thermolabile toxin is responsible for the interstitial pneumonia and that passive protection with the antitoxin prevents this pneumonia. This justifies further investigation with this material. It is unfortunate, however, that previous studies involving the use of pertussis antigen detoxified have not been carefully controlled.

Intranasal Instillation of Antigen

Disrupted organisms have been used to prepare filtrates for intranasal admin-

istration. The filtrate is adjusted so that 1 cc. contains the products of 20 billion organisms. Prophylaxis with one-quarter to one cc. given every other day for 4 or 5 doses is said to result in the protection of between 40 and 50% of children so treated. Mouse protection tests show this form of administration to be only 50% as effective as Sauer's vaccine by the usual route. In treatment one-half to 1 cc. given every day for 5 to 10 doses is believed to improve 40 to 80% of the cases. Better results may be obtained in the first week of the paroxysmal stage. The results with the material used subcutaneously are a little better.

Sulfonamides

Lawson and others have shown the ineffectiveness of sulfanilamide and sulfapyridine in mouse protection tests. This has been confirmed clinically. Sulfapyridine is somewhat more effective in pertussis pneumonia even though only one-fourth to one-half of cases had pneumococci in the sputum. Sulfathiazole used in pertussis pneumonia has lowered the case fatality in infants under one year from 25 to 6%, and in those from one to two years of age from 10 to 3%. Low blood concentrations of the drug of 2 to 3 mgm% were sufficient. The average case required 4 days for the temperature to return to normal. The drug was well tolerated. There are no reports in the clinical literature as yet as to the results with sulfadiazine. Sulfadiazine does give good mouse protection.

Miscellaneous

Toomey has claimed that his mucoid material is effective in lessening and occasionally aborting the whoop. His work has not been repeated by other investigators. Vitamin C is probably ineffective in treatment. Favorable reports with the vitamin have been recorded only on cases well in the third week of whoop. Bacteriophage for H.pertussis discovered by Sauer and Hambrecht has not as yet been put to clinical use. One has the impression that airplane rides at high altitudes for treatment of pertussis belong to the Middle Ages. Holinger, Basch, and Pemchor have reported the effective combination of steam and carbon dioxide inhalation to reduce the viscosity of sputum. They have also named ammonium chloride, potassium iodide, ipecac, and emetine-HCl as the most effective expectorant drugs.

Immune Serum

Human hyperimmune serum is prepared by repeated vaccination of young healthy adult donors at monthly or quarterly intervals over a year's period. These donors have had pertussis in childhood. The agglutination titer reaches levels of 1:2560. The bloods after the end of a year are pooled, and the serum separated by the lyophile process. Rabbit serum is prepared by intensive vaccination with increasingly large doses of living organisms, the agglutination tier reaching 1:2560 to 1:20,480. Rabbit serum may be concentrated by globulin fractionation, and, as mentioned above, contains antitoxin, while human hyperimmune serum does not.

Users of these serums have obtained excellent results in producing passive immunity after exposure. The results have been particularly gratifying in young infants who develop antibodies poorly. Ten to 40 cc., usually 20 cc., intramuscularly protects 60 to 90% of the cases. When the disease does develop after administration of serum, it is mild in 80% of the cases, moderate in 20%, and is almost never severe. Human hyperimmune serum whether given intramuscularly or intravenously has proved somewhat disappointing in treatment, particularly in the presence of pneumonia. In treatment 10 to 20 cc. are given intravenously every or every other day until 60 to 80 cc. have been given. But while the disease may not be shortened with serum treatment, it is milder, and the incidence of complications is far less. Rabbit serum may prove to be preferable to human immune serum in treatment if the thermolabile toxin is shown to play a role in the disease in man.

Summary

1. Whooping cough is due to Hemophilus pertussis. The organism exists in 4 serologic phases of which the smooth-colonied virulent Phase I is the most important in initiating the disease and in the preparation of effective vaccines. Atypical cases of whooping cough may be due to parapertussis and bronchosepticus organisms. These differ in their agglutinogen from H.pertussis; but B.parapertussis, at least, forms identical toxins and is related through a minor antigen.

2. The thermolabile and thermostable toxins of H.pertussis are probably not important antigenically in man, although the potent thermolabile toxin may be responsible for pertussis interstitial pneumonia. The agglutinogen of Phase I is apparently the important antigen, and the mucoid substance of Phase IV may be responsible for the whoop.

3. Skin tests are emerging out of the unsatisfactory stage. The preparation of the pure Phase I agglutinogen will probably produce a satisfactory skin reaction.

4. The opsonocytophagic, complement fixation, and agglutination reactions, and the mouse protection test are fair to good tests of immunity. Immunity develops during the course of the disease in the second to fourth week, and disappears in six months to two years. Immunity appears about three weeks after vaccination, reaches a maximum in one and one-half to three months, and persists for one and one-half to four years.

5. Phase I vaccine is valuable in prophylaxis but not in treatment. Total dosages should probably be in the neighborhood of 100 to 140 billion organisms. Stimulation doses on exposure or two years after vaccination keep up the immunity. Vaccine concentrations of 40 billion organisms per cc., and alum-treated and combined vaccines may prove to give the best prophylactic results. Dosage interval during vaccination should probably be three weeks, and vaccination should not be begun before the seventh month of life.

6. Krueger's undenatured bacterial antigen is probably ineffective either in prophylaxis or in treatment. Intranasal antigen is somewhat more satisfactory.

7. The status of pertussis antigen detoxified is not yet clarified. It may be valuable in the production of antitoxin or in the desensitization of patient to metabolic products of the organism.

8. Carbon dioxide, steam, sulfathiazole and probably sulfadiazine are effective therapeutically.

9. Hyperimmune human and rabbit serums are of definite value in the induction of passive immunity after exposure, especially in infants under 7 months of age, and may also be valuable in treatment. Rabbit serum may prove to be especially valuable because of its antitoxin content.

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V. GOSSIP

Maxine Kennedy, who covered the University Hospitals and Medical School for Minnesota Medicine for new items, has gone to Smith College to join the Waves. Miss Kennedy was employed by the Bruce Publishing Company to pick up news items for several of their publications. A cum laude graduate of the course in journalism of the University of Minnesota, she showed excellent skill and judgment in reporting her personal finds. This section in Minnesota Medicine has grown to be one of the most readable parts of the publication. Her successor has not been selected...The Twin City medical profession has been extended a special invitation to hear Thomas Francis, Jr., of the School of Public Health, University of Michigan discuss influenza. Physicians of World War I vintage appreciate the seriousness of this disease and trust that we may not see another epidemic. Younger physicians will find it difficult to realize how serious the last epidemic was...The Clarence Martin Jackson lectureship established by the Phi Beta Pi fraternity is one of the outstanding events of the year. It illustrates what a group of medical students can do in thanking one of our great teachers in an appropriate way. Dr. Jackson who has been in poor health for some time is remembered by students at Missouri as well as at Minnesota. One of his former students there objected to an item which appeared about him in one of our magazines which brushed off his Missouri experience with a word... James Hamilton, administrator, New Haven Hospital, New Haven, Conn. will speak at the dinner which will honor the 500,000th subscriber to the Minnesota Hospital Service plan. This is the organization sponsored by our hospitals which has so successfully met the problem of pre-payment for hospital service. The entire state is covered and a healthy reserve is laid up for the rainy day. Outstanding hospital and medical leaders from all over this country will be present to wish the Minnesota Hospital Service Association continued success...Two physicians in attendance at the course in Cardiology at the Center for Continuation Study were talking about their college days. Both belong in the group over 45 that are so anxious to be back at school these days. In referring to the game with Nebraska Saturday, one physician said that he played against Minnesota as a member of the Nebraska team 42 years ago. He recalled every incident of the game. The other one listened intently and finally he recognized the other physician as one who must have played against him when he was with the Iowa State team...A sign in the office of Postgraduate Medical Education reads "Wanted - physicians over 45 years of age." Several undertakers have eyed the sign wishing that they might spot one of their ads beneath...A study of man hour contributions to the medical courses at the Center for Continuation Study revealed that surgery had made the greatest contribution last year. Medicine and radiology were second. Physiotherapy was well out toward the top because of the large number of Kenny courses which have been offered. It has been estimated that nearly 500 physicians, nurses, and physical therapy technicians will have been initiated in this method of treatment before January 1, 1943. As the program started in April of this year, it represents quite a sizable contribution...The 20th year class of the Medical School will hold its reunion dinner on Friday of next week. Next Friday being homecoming day we will have as our special guests at the staff meeting luncheon members of the University of Minnesota Medical School alumni and associates. The official homecoming program will start Wednesday evening with a lecture by Dr. Francis and will end Saturday afternoon with the football game with Michigan. This is a more extensive program than usual and will be held at the Center for Continuation Study for the first time. Homecoming dentists will also stay at the Center and have their program in the dental school...The fine weather we are having has prolonged the picnic season for many of our staff. Bill Peyton is the proud owner of a charcoal stove which broils on both sides at the same time. He has become a master in its use and has been generous in lending it to his friends. He has a stock of recipes of all kinds, and his sourdough pancakes were the talk of the town last year...Edward S. Murphy, ophthalmologist, Missoula, Montana, one time member of our dept. of ophthal., has reentered military service as a lieutenant colonel...