THE DIAGNOSIS OF WHOOPING-COUGH

BY

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The clinical diagnosis of whooping-cough in its earliest stage—when recognition would be most valuable in preventing spread of infection—is very difficult, and in the majority of cases quite impossible. Even in its later stages diagnosis is far from simple—for example, in a series of 171 cases sent to hospital as whooping-cough thirty-five, or 26 per cent., were wrongly diagnosed, while thirty-eight out of 136 cases, or 28 per cent., bacteriologically confirmed as whooping-cough had not been heard to whoop before admission and never whooped while in hospital. In view of this difficulty in clinical diagnosis an effort has been made to assess the value of various laboratory methods of diagnosis. These may be divided into (1) specific tests—namely, (*a*) the isolation of *H. pertussis* by the cough-plate method, (*b*) the demonstration of specific antibodies by complement fixation or agglutination, (*c*) the intradermal sensitivity test; and (2) non-specific tests—namely, (*d*) total and differential leucocyte counts, and (*e*) the erythrocyte sedimentation rate. The specific tests mentioned depend on the assumption that *H. pertussis* is the aetiological agent, the arguments in favour of which have recently been reviewed by Gardner (1936).

Cough-plate Method of Diagnosis

This is the only certain method of early diagnosis, and as there are several pitfalls in the technique of preparation of media and exposure of plates the methods are fully described.

THE CULTURE MEDIUM

Potatoes are cleaned and cut into thin slices, 250 grammes of which are added to 500 ml. of tap water and 9 grammes of sodium chloride. The mixture is boiled until the potatoes are soft, whereupon the loss of water in boiling is made up, the whole filtered through linen, and the reaction of the filtrate adjusted to *p*H 7; 60 grammes of agar powder (B.D.H.) are added to 1,600 ml. of tap water and dissolved. This gives a final concentration of 3 per cent. agar in the medium. To the dissolved agar solution are added 500 ml. of the potato extract, 20 ml. of glycerin, and 1 per cent. proteose peptone ("difco"); after mixing, distribute in flasks in 100-ml. amounts, which are autoclaved. The medium is stored in the cold room till required.

Finally, a flask of the glycerin-potato-agar medium is placed in the steamer for one hour, after which it is gently inverted several times and put in a water bath at 55° C. for five minutes, until the agar has a temperature of approximately 70° C. An equal volume of defibrinated horse blood is put in the water bath at 55° C. for two to three minutes to take the chill off. The blood is added to the glycerin-potato-agar, mixed very gently to prevent the formation of bubbles, and the mixture is poured into Petri dishes. The plates, which should not be dried in the incubator, are stored in the cold room and may be used for a period of two weeks. It is most important that the surface of the medium be perfectly smooth and free from pitting, which by reflecting the light gives a passable resemblance to colonies of *H. pertussis*.

This medium is similar to that used by Westwater and Straker (1937), but differs from that of Gardner and Leslie (1932), as it contains 50 per cent. instead of 33 per cent. blood. Plates of this medium when compared with those containing only 20 per cent. blood gave greater numbers of more easily identifiable colonies.

TECHNIQUE OF EXPOSURE OF PLATES

At the start of the series all cough-plates were taken by the ward sisters, but later any of the nursing staff, including probationer nurses, exposed the plates, and very few were spoiled. Two plates were exposed in every case, so that a heavy and a light inoculum could be obtained from each, and quite frequently it was found that one plate was positive and the other negative. Either a natural or an induced spasm of coughing may be used, the latter by tickling the front of the neck over the larynx, giving the patient a drink of cold water, or, as was done in most cases, inserting a spatula into the pharynx. Once the paroxysm was started the plate was held about four inches from the mouth and exposed for a number of expiratory "barks," which varied from fifteen to twenty in a very young infant to four to eight in children aged 2 to 5 years. It is impossible to state any definite number of barks required, as the force of the bark varies greatly, and some experience by the person exposing the plate is very helpful. Young children should preferably be lying down and older children sitting up. If the spasms are short the plate may be exposed on several occasions. Before exposure the surface of the medium should be quite dry, as any condensation moisture may help to spread contaminant growths.

After exposure the plates were incubated at 37° C. In a number of instances they were left lying in the ward for twenty-four hours after exposure, and others were kept in the ice-chest for the same period, but subsequent incubation showed that these procedures did not in any way delay or inhibit the growth of *H. pertussis*.

EXAMINATION OF PLATES

The plates were examined as a general rule after seventy-two hours' incubation, but in many instances the pertussis colony could be identified in forty-eight hours. The ease with which this was done depended almost entirely on a very smooth surface to the medium. Practically all plates were discarded after three days' incubation, since a plate negative on the third day was never found to be positive on the fourth. In no instance did spreading colonies need to be cut out of the medium, as recommended by Gardner and Leslie (1932).

The colonial and morphological appearances of the organism conformed to the descriptions given by many workers. Daylight was preferred to artificial light, and the use of a lens was found very helpful in looking for colonies. Haemolysis did not appear in the medium at seventy-two hours, but when plates were stored for a day or two longer in the ice-chest a zone of clearing was often observed. Of the other colonial appearances which may be confused with pertussis that of *H. influenzae* is most important. The latter was flatter, duller in oblique light, slightly transparent, and occasionally of a yellowish colour.

Subculture.—At least two colonies suspected as being *H. pertussis* were subcultured on the Bordet medium, and after twenty-four hours' incubation the typical growth with a grey sheen like that of aluminium paint was seen. *H. pertussis* in a Gram-stained smear of the culture appears as a small Gram-negative cocco-bacillus, uniform in size, occurring in clumps, and giving the impression of a thumb-print. Occasionally the bacilli appear in paired forms, but very seldom in chains.

Agglutination.—Final identification was carried out by performing "slide agglutination" with a thick suspension of the subculture, and this was further verified by agglutination by Dreyer's method.

RESULTS

All cases in the series were diagnosed as whooping-cough on bacteriological, clinical, or serological grounds. The results in 136 cases, many of which were examined by the cough-plate method on several occasions, are shown in Table I.

TABLE I.—*Isolation of H. pertussis from 136 Cases of Whooping-cough*

	No. of Tests	Positive	Per cent. Positive
1st week	5	5	100
2nd „	31	29	93.5
3rd „	58	55	94.8
4th „	75	33	44
5th „	113	8	7.1
6th „	120	1	0.8
7th „	128	1	0.7
Total	530	132	

The duration of disease was definitely ascertained in almost all cases by personal interview with relatives, and the date of the first cough was taken as the beginning of the illness. The small number of cases examined in the first week is due to the fact that parents do not consult the doctor until the paroxysmal stage has been reached. One case which gave positive plates in both the tenth and eleventh weeks is not included in the table. These findings are comparable with those recently published by Westwater and Straker (1937), who obtained positive plates in 86.5 per cent. of cases in the first week, 84.4 per cent. in the second, 70.6 per cent. in the third, and 50 per cent. in the fourth.

EFFECT OF BRONCHOPNEUMONIA ON EXPECTORATION OF THE BACILLUS

In the series there were sixteen cases of bronchopneumonia. From eleven of these positive cough-plates were obtained, and all were in the third week of illness except two, which were admitted during the fourth and fifth weeks. As the remaining five cases were all admitted after the third week of the illness it would appear that bronchopneumonia does not affect the expectoration of the bacillus. Of these sixteen patients four died, all having given positive cough-plates, and at necropsy in three of them large numbers of *H. pertussis* were cultured from the lungs, trachea, and bronchi; no examination was performed in the remaining case.

DEGREE OF POSITIVITY OF COUGH-PLATES

It was found that of 134 positive plates obtained at various stages of the illness ninety-six, or 71.6 per cent., had an estimated *H. pertussis* colony count of ten or less. This shows the need for careful scrutiny of each plate, especially if it has been over-exposed.

ATYPICAL COLONY APPEARANCE

In the majority of cases positive plates gave typical colony appearances, but five children were found to be exceptions. Four of these were infected by an unsuspected case of whooping-cough which had been admitted to a scarlet fever ward. All gave much larger colonies, whiter than normal, and not so glistening as typical *H. pertussis* colonies. Subcultures from these colonies gave a luxuriant growth, less glistening than usual, and failed to show the typical aluminium-paint appearance. No explanation has been found for this atypical colony.

RELEASE PLATES

Although more attention has been paid to the cough-plate as a diagnostic method, it follows that it may have value as indicating the cessation of infectivity. As the certainty with which the bacillus can be recognized increases the duration of stay in acute fever hospitals could be greatly cut down in cases where further convalescence could be carried out at home or in a convalescent institution if the patient were shown to be non-infective.

CARRIERS

The question of carriers of *H. pertussis* has for long attracted the attention of public health workers. In the present series 110 cases were examined on the day of discharge or shortly before, and in no instance was a carrier found, two plates being taken from each case. One case in particular may be mentioned. A boy of 8 years was admitted with mild faucial diphtheria at least ten weeks after the start of a mild but definite clinical attack of whooping-cough, and this history was supported by serological findings, his complement-fixation test being strongly positive (+++). While in hospital he had a very occasional slightly spasmodic cough, and, as a matter of interest, cough-plates were taken, and two colonies of *H. pertussis* were isolated and subcultures agglutinated to the maximum titre of the known serum. A week later a plate with one colony was obtained, but in the twelfth and subsequent weeks there were no further positive plates. No cross-infection was present in the ward, in which there had been one week's exposure, and the suggestion was put forward that *H. pertussis* isolated from late cases might possibly be avirulent. To test this, virulence tests were made by Dr. J. C. Cruickshank at the London School of Hygiene on several strains of *H. pertussis* isolated in the fifth, sixth, and seventh weeks of infection as well as in the early stages. These were carried out by injecting mice intraperitoneally and noting the day of death. No relation was noted between the degree of virulence of the organism and the severity or duration of the illness in the patient.

Complement-fixation Tests

This diagnostic procedure is of very little value as a means of early diagnosis, as the test does not become positive until the third or fourth week or later. It may, however, be employed to establish the diagnosis in late or atypical cases, when the period of expectoration of the bacillus is past. In hospital practice it has a practical value, too, in ascertaining whether a child with a cough of long standing, suspected of being whooping-cough, should be admitted to a whooping-cough ward or not. In this series the test was carried out bi-weekly in 123 cases of whooping-cough.

Technique of Test.—This was performed in a manner similar to the gonococcal complement-fixation test, using a virulent strain of *H. pertussis* as antigen (2,000 million organisms per ml.), and 2, 3, and 5 M.H.D. of complement. Sera fixing less than 2 M.H.D. were recorded as negative.

In the series of cases under review all, except four patients diagnosed as having whooping-cough on bacteriological and clinical grounds (including haematological examination), showed a change from a negative to a positive complement-fixation reaction, or, if already positive on admission, became more strongly so while in hospital. Three of the exceptions were aged 3 weeks, 4 months, and 3 years, and although all had typical attacks of whooping-cough and gave positive cough-plates, none showed antibody in their sera, although they were examined repeatedly until the tenth week. The fourth patient, aged 7 months, died of bronchopneumonia during the sixth week of illness, and fixation tests were repeatedly negative, but as several other cases of bronchopneumonia showed delay in the appearance of antibodies until the sixth week or even later this result is inconclusive. At necropsy a heavy growth of *H. pertussis* was obtained from the lungs and trachea. All four cases had typical leucocytosis with lymphocytosis.

RESULTS OF COMPLEMENT-FIXATION TESTS IN 123 CASES OF WHOOPING-COUGH

In 123 cases of whooping-cough 383 complement-fixation tests were carried out at various periods, the result being shown here in graph form (Chart 1).

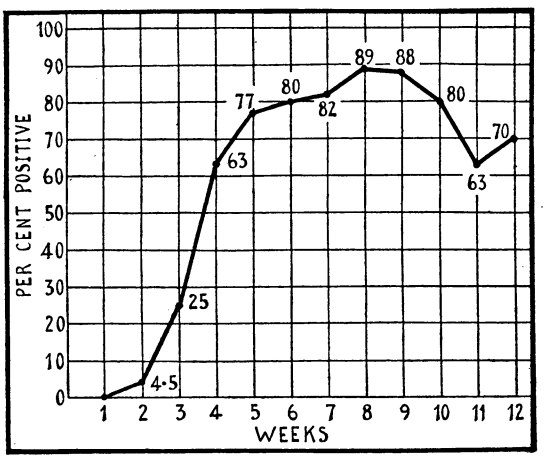

CHART 1

It is seen from the chart that the reaction becomes positive at approximately the third week, and increases steadily till at the seventh and eighth weeks the great majority of cases give positive results, after which it falls off slowly. In general, as in other infections, the antibodies tend to disappear after the acute phase of the disease, but they may persist in low titre for a considerable time or be stimulated to reappear by another infection, as will be shown later.

These results may be compared with those of Chievitz and Meyer (1916), who found that 25 per cent. of 259 cases tested gave positive results in the second week, and that during the fifth, sixth, and seventh weeks all cases examined gave positive reactions (100 per cent.), with a gradual fall to 89 per cent. at the twelfth week. This relatively high incidence of positive reactions early in the disease may be due to the fact that in many cases they did not inactivate the serum, which they found gave a definite positive when inactivated serum gave only a feebly positive reaction.

DEGREE OF POSITIVITY

The above graph (Chart 1), however, gives no indication of the degree of positivity, and an effort is made below (Table III) to do this. In Table II the method of reading results of the tests is shown.

TABLE II

	M.H.D. 2	M.H.D. 3	M.H.D. 5	Serum Control 2	Result
Degree of haemolysis	C	C	C	C	Negative
	Tr	C	C	C	Doubtful ±
	0	Tr	C	C	Positive +
	0	0	Tr	C	„ ++
	0	0	0	C	„ +++

M.H.D.=Minimum haemolytic dose. 0=No haemolysis. Tr=Trace of haemolysis. C=Complete haemolysis.

TABLE III (*Total Tests, 383*)

Degree of Positivity	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	24
− ..	2	18	20	11	6	3	5	1	1	2	3	2	1	1	0	0
± ..	0	3	11	9	7	5	3	5	3	2	3	0	1	1	1	0
+ ..	0	1	11	31	34	15	11	18	2	7	7	4	3	2	0	2
++ ..	0	0	1	3	10	11	19	15	14	7	3	4	0	0	1	0
+++ ..	0	0	0	0	0	1	2	2	4	2	3	0	0	2	1	0

It will be noted that out of seventeen cases fixing 5 M.H.D. of complement (+++) fourteen were between the sixth and eleventh weeks, the remaining three occurring in the fourteenth and fifteenth weeks (two of these from the same case); while of eighty-eight cases fixing 3 M.H.D. of complement seventy-nine did so between the fifth and eleventh weeks, so that it would appear that the peak of antibody formation occurs at approximately the seventh to eighth week, which is a result closely allied to the curve of appearance of the antibodies. It may therefore be claimed that, if one wishes to verify the diagnosis in a doubtful or atypical case of whooping-cough, serum should be tested at this period to obtain the most certain proof of recent infection.

Does a Positive Complement-fixation Test indicate a Recent Attack of Whooping-cough?

While carrying out this investigation it was observed that eighteen cases which had given a positive complement-fixation reaction eventually became negative, and it was thought that the fixation test might be found to be absolutely specific for the acute stage of whooping-cough. To verify this a series of tests were carried out on patients who had a previous history of whooping-cough. These patients were for the most part cases of scarlet fever and diphtheria, some acute, others convalescent, and at all ages from 2 to 30 years. The results obtained were as follows: of eighty-eight cases examined, sixty-six gave positive complement-fixation tests; but of these, only four fixed 3 M.H.D. (++), the remainder (sixty-two) only fixing 2 M.H.D. of complement. It may be that the febrile period through which these patients passed stimulated the reappearance of antibodies, but pyrexia could not be held responsible as several with positive results had very little elevation of temperature, while others with a marked degree of pyrexia gave negative results. In this series also the parents were not interviewed, except in a few cases, and it is possible that the histories of whooping-cough (given to nurses) were in some instances inaccurate. In five cases with a history of whooping-cough a year or less previously, three gave negative and two positive complement-fixation reactions. On the other hand, all the four cases giving ++ comple-

ment-fixation reactions (3 M.H.D.) had had whooping-cough at least three years previously.

Gardner (1936) in a recent publication states that, "like all specific antibodies, however, their presence only proves that the subject has recently been under the stimulus of the specific antigen, not that he is suffering from the present infection," and in 1934 Bennholdt-Thomsen claimed that adults in contact with whooping-cough acquired a strongly positive complement-fixation reaction without showing any clinical symptoms. He found that eight persons with a negative or feebly positive complement-fixation reaction were strongly positive after being in contact with cases of whooping-cough for periods varying from fourteen to seventy-four days. An attempt was made to verify this during the present study, but without success. Of fourteen persons tested before commencing work in the whooping-cough wards only two gave positive fixation reactions. When tested again after periods of contact varying from thirty-one to 120 days only two showed any change. One, previously negative, became weakly positive after forty-two days' contact, and the other changed from weak positive to positive after eighty-five days' contact. In addition to these results it was found that my own blood was negative after nine months' contact, and when examined after twelve months gave a doubtful positive (±) reaction, while a sister in charge of a whooping-cough ward for sixteen months failed to show any antibody after this period. This short series helps to establish the specificity of the test.

EFFECTS OF BRONCHOPNEUMONIA AND AGE ON COMPLEMENT-FIXATION TESTS

It has been pointed out by Paton (1937) that negative complement-fixation reactions were found in cases with severe attacks of whooping-cough or where there were complications such as bronchopneumonia, but in most of his cases the blood was tested only once. In this series twelve cases of bronchopneumonia were tested at intervals, and on an average the reaction was found to become positive at the sixth week, which indicates delay in the appearance of the antibodies; but as one of the cases in question did not give a positive result until the twelfth week, the average time of appearance of antibodies in the remaining eleven would be the fifth week. Of three cases

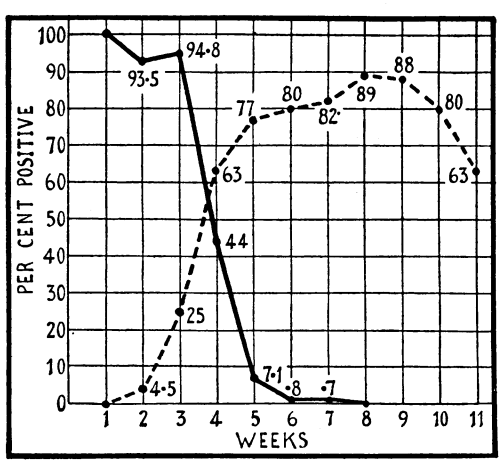

CHART 2.—The continuous line represents cough-plates, and the broken line complement-fixation tests.

of bronchopneumonia that ended fatally all gave negative reactions at the seventh week (aged 3 months), third week, and second week respectively.

Age in the present series appeared to have little effect, except in one child aged 1 month and in another aged 4 months, both of whom failed to give positive complement-fixation reactions at any stage; but in contrast to this seven other children, all aged 3 to 4 months, gave positive complement-fixation reactions, although the appearance of antibodies was slightly delayed. Only two of these gave ++ complement-fixation reactions (3 M.H.D.), the remaining five sera fixing only 2 M.H.D. Sauer (1934) mentions the difficulty in immunizing very young children, and other workers have experienced difficulty in demonstrating antibodies in infants.

The accompanying graph (Chart 2) shows how the antibodies appear as the expectoration of bacilli ceases.

Agglutinins in the Blood

Fifty different sera, most of which gave well-marked complement-fixation reactions, were tested for agglutinins, and the conclusion come to was that the agglutination test was unreliable and useless as a method of diagnosis.

Skin Tests

A series of fifty-eight cases were tested by the intradermal injection of 0.1 ml. of a killed suspension of *H. pertussis* (2,000 millions per ml.), but results were equivocal, and this method was abandoned.

Non-specific Tests

BLOOD COUNTS

There is considerable controversy in the literature regarding the value of white cell and differential blood counts as an aid to the early diagnosis of whooping-cough.

In the present study 118 patients were examined. White cell and differential blood counts of all cases were done on admission, and in several instances were repeated before discharge. The normal standard for children employed was that of Still (1927) (Table IV), most of the counts being done at a relatively late stage of the illness, usually after the second week.

TABLE IV

	Birth	6 months	2 years	5 years	10 years
Total leucocytes ..	24,000 to 62,000 per c.mm.	15,000 per c.mm.	13,000 per c.mm.	11,000 per c.mm.	9,000 per c.mm.
Polymorphonuclears	78 to 80%	35%	40%	55%	60%
Lymphocytes ..	20 to 22%	57%	52%	37%	32%

One case only was examined during the first week of illness, a girl of 5½ years whose count showed 10,600 leucocytes per c.mm., with a differential count of 55 per cent. polymorphs and 37 per cent. lymphocytes, which may be regarded as normal. During the second week sixteen cases were examined, and all, except two with leucopenia, showed a definite leucocytosis, with a relative and absolute lymphocytosis.

The highest counts with lymphocytosis predominant were obtained in the third and fourth weeks. The average white cell and differential counts at three age groups are shown (Table V) for this period of illness (third and fourth weeks).

A control series of thirty-two cases of bronchitis, mainly of a mild nature, were examined, the majority of the cases being in the age group 2–5 years (Table V).

TABLE V

	Age	Leucocytes	Polymorphs	Lymphocytes	No. of cases
Whooping-cough	0–2 yrs	30,800 per c.mm.	23%	65%	28
	2–5 „	31,000 „	29%	66%	23
	5+ „	28,000 „	34%	63%	12
Bronchitis ..	2–5 „	15,000 „	59%	37%	32

From these figures it will be seen that at all age groups in children the average leucocyte count during the third and fourth weeks of whooping-cough is 30,000 per c.mm., while the differential count shows a well-marked lymphocytosis in each age group. It will be noted that the series of controls also shows a very mild degree of lymphocytosis, but the total white cell count is approximately only half that found in the cases of whooping-cough. Only four cases under 6 months of age were examined, and in contrast to the findings of Begg and Coveney (1935) all showed marked lymphocytosis but only a mild degree of leucocytosis.

From this peak at the third and fourth weeks the fall to normal was found to be very gradual. The total leucocyte count was the first to change, and in the majority of cases of uncomplicated whooping-cough had returned to normal at approximately the sixth or seventh week; but the relative lymphocytosis persisted for a longer period, and in 50 per cent. of cases examined was still present during the ninth and tenth weeks.

Erythrocyte Sedimentation Rate

This test was carried out in ninety-five cases of uncomplicated whooping-cough, employing the Westergren method. The results showed a retardation of the sedimentation rate in the second and third weeks, with a slight acceleration in the later stages. Since, however, 29 per cent. of twenty-four cases of acute bronchitis also showed a retardation of the erythrocyte sedimentation rate, the test would appear to be of little value by itself, but, as suggested by Gold and Bell (1936), when used in conjunction with total and differential leucocyte counts in cases with a suspicious cough the test is of definite value.

Practical Application of the Various Diagnostic Methods

The cough-plate has been demonstrated to be the only sure method of establishing the diagnosis at the onset, and the figures show that whooping-cough may be *diagnosed with certainty in over 94 per cent. of cases during the first three weeks of the disease.* By this method the abortive case can be recognized and isolated, thus removing an important factor in the dissemination of infection. The negative cough-plate also plays an important part in deciding when a child is free of infection, and it may render great service to the administrator of a hospital, institution, or boarding school. In the cough-plate the practitioner has at hand a means by which to diagnose and thus control the spread of disease in the home, and a method of determining when isolation should cease. The difficulties of this method of diagnosis should not present a serious obstacle to its more general use. Any modern bacteriological laboratory could prepare a suitable medium in a suitable container. The use of the aluminium plate is to be recommended for institutions, etc., where carriage or postage is necessary. The time required for diagnosis (three days) will probably be shortened as improvements in media are devised, and, in any case, it is no longer than is usually necessary for the isolation of the typhoid bacillus from faeces. If the time and attention given to devising methods and media for the isolation of the latter organism during the past twenty years were now transferred to the problem of the isolation of *H. pertussis*, it is probable that the bacteriological diagnosis of whooping-cough would soon become a simple routine procedure.

The complement-fixation test has definite limitations, and is of no value in diagnosis at the time when it would be most useful—that is, in the early stages of infection. Its main value lies in corroborating the diagnosis of abortive or atypical cases, where the test may be found to have changed from negative to positive, or, if already positive, to become increasingly so, reaching a maximum degree of positivity at the seventh to eighth week. A strongly positive complement-fixation reaction at this period is almost conclusive evidence of recent infection with *H. pertussis.*

When bacteriological facilities are not available the total and differential leucocyte count is valuable in cases where there is a *suspicious cough*, as it has been shown that a leucocytosis and lymphocytosis is an almost constant finding from the second week of the disease until almost the end of the stage of decline. This method has definite value, and could be employed in dispensaries and out-patient departments of general hospitals, and would be extremely helpful in diagnosis.

Summary

An attempt has been made to assess the value of the various methods available for the early diagnosis of whooping-cough. The methods are: (1) *Specific tests*—(*a*) cough-plate method, (*b*) complement-fixation tests, (*c*) intradermal tests; (2) *Non-specific tests*—(*d*) total and differential leucocyte counts, (*e*) erythrocyte sedimentation rate.

SPECIFIC TESTS

(*a*) *The cough-plate method* is the only means of certain diagnosis in the earliest stages of the disease. In a series of 136 cases at all stages, 100 per cent. were found to give positive plates in the first week, 93.5 per cent. in the second week, 94.8 per cent. in the third week, and 44 per cent. in the fourth week, after which there is a rapid fall to 7.1 per cent. in the fifth week, and less than 1 per cent. in subsequent weeks. The value of the cough-plate in the diagnosis of atypical and abortive cases is stressed and its value to public health administrators mentioned. Emphasis is also laid on the negative cough-plate as a criterion of the termination of infectivity.

(*b*) *The complement-fixation test* is shown to be of little value except as a means of corroboration of the diagnosis, and occasionally as an indication of immunity. In a series of 123 cases the reaction was found to become positive in 25 per cent. during the third week of illness, and reached a maximum during the eighth week, when 89 per cent. of the cases gave positive fixation reactions.

(*c*) *The intradermal test*, using a suspension of *H. pertussis*, is found to be valueless in diagnosis or as a method of determining susceptibility or immunity.

NON-SPECIFIC TESTS

(*d*) *The total and differential leucocyte count* is found to be valuable from the second week onwards, almost all cases examined showing marked leucocytosis and both absolute and relative lymphocytosis. During the third and fourth weeks the leucocyte count averaged 30,000 per

c.mm., with a differential count showing 65 per cent. lymphocytes and 28 per cent. polymorphonuclears.

(*e*) *The erythrocyte sedimentation rate* is shown to be slightly retarded or normal in almost all uncomplicated cases of whooping-cough in the early stages, with a slight increase in the later stages of the illness.

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HUMAN OIL IN THE TREATMENT OF ADHERENT SCARS

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Adherent scars are always a problem, and especially so when adherent to bone or cartilage. Some five years ago, when confronted with two cases of extensive adherent scars in the neck due to *x*-ray treatment given for exophthalmic goitre, I first tried the experiment of using human oil. In these cases the attenuated and parchment-like skin of the neck was adherent to the cartilages of the larynx and the trachea. All the subcutaneous tissue of the neck and the infrahyoid muscles had disappeared, and there was absolutely no independent movement of the skin over the trachea and laryngeal cartilages. These patients were in a miserable condition, and had travelled over the Continent and visited numerous clinics in search of relief for their sufferings. As may be imagined, swallowing was difficult and painful, as the skin and the trachea were densely adherent. The only form of treatment appeared to be the introduction of some substance between the adherent skin and the trachea, cricoid, and thyroid cartilages. Excision was out of the question, and *x*-ray treatment could not cure a condition it had produced. I experimented with various types of oil, including different grades of paraffin, but none of these gave satisfactory results and all were very painful.

Knowing that human fat was fluid at body temperature—for if an incision is made into the abdominal wall one can see the fat globules when some of the fine trabeculae which hold them imprisoned are cut through—some fat from the omentum was removed at laparotomy and investigated, and it gave good results.

Preparation of the Oil

My colleague, Dr. Creed, assisted me greatly in the isolation and sterilization of the human oil. The procedure we adopt is as follows: A portion of omentum is removed from a person who is undergoing an operation such as the removal of the appendix or gall-bladder. The excised portion of omentum is placed in a sterile vessel and taken to Dr. Creed's pathological laboratory. It is thoroughly washed with distilled water to free it as completely as possible from serum and blood, and then placed in a specimen jar half filled with distilled water and plugged with cotton-wool. This is heated to 120° C. in the autoclave for half an hour. After this treatment most of the fat will be found floating free on the surface of the water as a clear yellow oil. While still warm it is pipetted off with a sterile capillary pipette and distributed into sterile 1 c.cm. ampoules. A little is also inoculated into broth culture medium to check its sterility. Each ampoule, after sealing, is placed in a short wide test tube, which is plugged with wool and sterilized in the autoclave. The ampoules are kept in these plugged tubes so that their exterior remains sterile and can be safely handled by the surgeon.

Technique of Treatment

The human oil is liquid at body temperature but is quite solid at temperatures below that, as can be seen in Fig. 1. The tube on the right contains solid human oil, but that on the left has been heated to body temperature and is fluid of a clear amber colour. The oil is put up in 1 c.cm. ampoules, as it is never safe to inject a larger quantity at one time. The ampoule is warmed in water until the oil is slightly above body temperature; the neck of the ampoule is then broken off and the oil drawn up into a warmed 5 c.cm. Record syringe. The reason for using a 5 c.cm. syringe instead of a 2 c.cm. one is that more pressure can be exerted when the injection is given, and this is a most important point. When the oil is in the syringe the scar tissue is carefully cleaned with spirit, and a site is selected just at the outer border of the scar tissue and the syringe insinuated between the adherent bone and the contracted and adherent skin (Fig. 2). The oil is forced in between these two structures, not more than 1 c.cm. being injected at any one place. The scar tissue is actually raised off the adherent bone or cartilage. Several different areas can be injected at one sitting, and the patient does not experience any more pain than is produced when an intramuscular injection is given. About twenty-four hours after the injection the site becomes somewhat red and hot; this condition persists for about twelve to twenty-four hours and then gradually disappears. Injections can be given at fortnightly intervals, and by degrees the adherent scar is made to float upon the underlying structure. In the case of badly adherent scars due to *x*-ray burns, as many as twenty to twenty-five injections may sometimes be required. It is very important that the injections should not be too frequent and that not more than 1.5 c.cm.

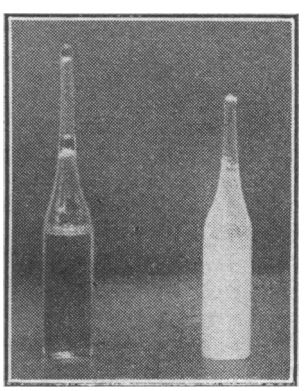

FIG. 1.—Solid human oil in the right-hand ampoule; liquid human oil in the left-hand ampoule.