Cough Plate Examinations for B. Pertussis*

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THIS paper is a report on one phase of a more general study of the problem of whooping cough control in Grand Rapids, a city of 176,700 population. To be convinced of the importance of such a study in as many centers as possible, it is only necessary to consult morbidity and mortality statistics. Approximately 6,000 deaths from whooping cough are reported every year in the United States. Most of them are among children under 5 years. The importance of whooping cough as a cause of death in this age group is well illustrated by figures taken from reports of the Bureau of Records and Statistics of the Michigan Department of Health.

TABLE I

MICHIGAN DEATHS IN CHILDREN UNDER 5 YEARS
COMPARISON OF COMMUNICABLE DISEASES

1929	*1930*	*1931*	*1932*
250 Whooping cough	186 Measles	183 Whooping cough	193 Whooping cough
211 Diphtheria	171 Whooping cough	151 Tuberculosis	139 Measles
185 Tuberculosis	162 Tuberculosis	70 Diphtheria	111 Tuberculosis
111 Measles	122 Diphtheria	42 Scarlet fever	40 Diphtheria
72 Scarlet fever	53 Scarlet fever	17 Measles	37 Scarlet fever

If whooping cough eventually is brought under control, it will be due to a number of factors—including, no doubt, an earlier diagnosis of the disease and the finding of more cases that occur, conscientious reporting by physicians, adequate isolation of infective cases by health departments, and the development and appropriate use of specific agents for its prevention. Our larger problem, therefore, falls naturally into several divisions, and includes: (1) an attempt to determine the practicability and value of maintaining a continuous cough plate diagnostic service in a community such as this; (2) an analysis of the applicability of the results of such a service in obtaining earlier diagnosis and a more adequate isolation and release program; (3) maintenance of a constant supply of freshly isolated cultures for study and for use in pertussis vaccine; (4) a study of the cultures isolated, including serological and other reactions and, (5) an immunization study.

The data in the present report are concerned particularly with the cough plate as a routine diagnostic procedure in a public health laboratory. In Copenhagen, the method essentially as first used by Chievitz and Meyer [1] has

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been employed since 1916 at the State Serum Institute.[2] In this country, its use seems to have been limited largely to special studies of whooping cough such as those of Sauer and Hambrecht,[3] Lawson and Mueller,[4] Culotta and Harvey,[5] and the demonstration by the Cattaraugus County Health Department, reported by Kline,[6] and to the temporary use for obtaining cultures of *B. pertussis* for study or use in vaccine. As a routine procedure in a public health laboratory, we believe the cough plate method has been quite neglected. In the Western Michigan Division of the Bureau of Laboratories, Michigan Department of Health, Grand Rapids, the cough plate diagnostic service for whooping cough has been available continuously since November, 1932.

EXPERIMENTAL METHODS

Cough plate medium—With few modifications, the original formula of Bordet and Gengou[7] has been used. The potato-glycerine-agar base is prepared in large lots and stored in 200 c.c. amounts. This base medium is melted and blood added as needed, at which time the finished medium is dispensed into glass Petri dishes for cough plates and into tubes for stock cultures. Because of its availability in our laboratory, sheep's blood is used for enrichment—having been found as satisfactory as human blood. To each 200 c.c. of base, 30 c.c. or somewhat less than 15 per cent of blood is added. Although this is much less than recommended by Bordet or reported by other workers, it has given as successful isolation of *B. pertussis* as larger amounts and has therefore been used throughout the study. In several lots of medium, the addition of lactic acid, as suggested by Gardner and Leslie[8] was tried but never with better results. In fact, such a medium had a tendency to darken and give less satisfactory growth.

The importance of the salt content of the medium came to our attention early in the study, in our attempt to explain occasional lots of base which partially hemolyzed the blood and gave less satisfactory growth. By the addition of titrated amounts of sodium chloride to such a base, the difficulty was overcome. No reason has been found for the relatively small amount of added sodium chloride in the original Bordet-Gengou formula. The final content was less than 0.5 per cent—a quantity of salt dangerously near the lower level tolerated by red blood cells. By substituting 0.75 per cent sodium chloride for the 0.6 per cent solution used in the original formula, we apparently have given the necessary safeguard against occasional lots with an insufficient salt content and there has been no inhibition of clear-cut hemolysis. This modification has made more certain the bright red plate so necessary to optimum results.

Exposure of plates—As is true for any laboratory specimen, proper collection of the cough plate is important. That successful exposure of the cough plate is not an insurmountable difficulty, however, is demonstrated by the fact that the data presented are based upon the findings in plates exposed by 22 physicians, 3 bacteriologists, 20 nurses and a number of mothers. The exposed plate is held 4–5 inches from the patient's mouth during several expulsive coughs. It is often necessary to induce a cough by tickling the throat with a swab or tongue depressor, by pressing on the larynx, by a drink of ice water, or by some other device.

Examinations of plates and Criteria for the identification of B. pertussis—The plates are examined several times during the first 48 hours of incubation for molds or spreaders which might overgrow the plate. Such colonies are cut out with a sterile needle or scalpel. At the end of 48 hours the plates are examined for colonies of *B. pertussis*

and this is repeated twice a day thereafter. If *B. pertussis* has not been found by the end of 4 days, a negative report is made. The plates are incubated and observed for 2 more days, at the end of which they are discarded. In the rare instance of a positive during that time, an additional report is made.

In a nicely seeded, uncrowded plate, colonies of *B. pertussis* can be recognized macroscopically after 48 to 72 hours with a great degree of certainty. As a source of light for examining the plates the ordinary sub-stage microscope lamp with white ground glass is very satisfactory. The uncovered plate is placed over the lamp and the colonies studied with the aid of a hand lens (10 x). By transmitted light, *B. pertussis* colonies appear smooth, raised, glistening, pearly, and almost transparent while the colonies of gram positive cocci in general appear duller, darkly colored and opaque. This light also reveals the zone of hemolysis which is characteristic of *B. pertussis*—a zone which is not sharply delimited but merges somewhat diffusely into the surrounding medium. In general, it may be said that the colony characteristics of *B. pertussis* on Bordet-Gengou medium are even more distinctive than are those of hemolytic or green producing streptococci on ordinary blood agar.

Confirmation of the identity of selected colonies is based upon several points. The consistency of the growth and the manner of its diffusion into water are very typical of *B. pertussis* and have been noted by Sauer.[9] As a loop of growth touches a drop of water or saline, it spreads and shows at first a momentary clumping but, with very slight agitation, it quickly smooths into a homogeneous suspension. Stained by Gram's method, *B. pertussis* decolorizes readily—much more readily, in fact, than does *H. influenzae*. Viewed microscopically, the small faintly-stained coccoid bacilli are scattered evenly throughout the film occurring singly for the most part, seldom in chains of even two and never observed as pleomorphic threads. Slide agglutination tests have been used frequently for the confirmation of colonies. The colony is emulsified in a drop of saline and a loopful of the suspension mixed with a loopful of antiserum, diluted 1:10, the remainder of the suspension being used as a control. Agglutination of *B. pertussis* is immediate and complete and readily observed with the unaided eye. The dried stained films, when observed microscopically, show the typical arrangement in the control suspension and definite clumps in the test suspension. These simple slide tests have proved reliable and of especial value in identifying suspicious colonies from plates too crowded or darkened by certain types of growth for the distinctive characteristics to be satisfactorily observed. In the case of less satisfactory plates, it is advisable to transplant suspected colonies to fresh medium. The resulting subculture grows sufficiently for identification within the next 24 hours.

Agglutination technic—The details of the method employed are outlined in a separate communication.[10] In brief, it is essentially the technic devised by Noble,[11] and it eliminates the troublesome, nonspecific settling and clumping of *B. pertussis* suspensions which have stood for a few hours. The antisera used for the tests were produced in rabbits by the injection of suspensions of recently isolated cultures and according to the terminology of Leslie and Gardner [12] were Phase I sera.

General plan of collecting plates—Since the cough plate diagnostic service was first offered, a number of physicians, mainly pediatricians, have used cough plates in all cases suggesting whooping cough. During the earlier months, members of the laboratory staff also collected plates as a part of the immuniza-

TABLE II

SUMMARY OF COUGH PLATE EXAMINATIONS

Clinical Diagnosis	Number of Cough Plates		
	Positive	Negative	Total
Whooping Cough Through 4 Weeks	163	63	226
Post-Whooping Cough, 5 weeks to 6 months after onset	2	166	168
Other than Whooping Cough	0	66	66
Clinical Data Not Available	0	35	35
Totals	165	330	495
Unsatisfactory Plates			16

tion study outlined above. The cases included in that series were for the most part in families receiving aid from the city, and therefore visited by city physicians and nurses. As the work enlarged, it became impossible for the bacteriologists to make all the required calls. In the meantime the City Health Department had found the laboratory results useful and in order to continue and enlarge the work, they offered the services of the Bureau of Public Health Nursing. Since this bureau uses the district nursing plan, it was agreed that each nurse secure the desired cough plates from welfare patients in her district. Because of her familiarity with the territory as a whole and with each family as a unit, the plan proved an ideal way of meeting the situation. The nurses, after instruction in technic, have been successful in exposing plates. A further step was taken recently when the City Health Officer, Dr. A. H. Edwards, offered Grand Rapids physicians the services of the nurses for collecting plates from their private patients. At present, then, most of the plates reaching the laboratory are collected by a group of about 25 nurses experienced in the procedure. Copies of all cough plate reports are sent to the City Health Department, as for other communicable diseases.

EXPERIMENTAL DATA

Summary of cough plates—In Table II is a summary of the cough plates submitted to the laboratory between November 1, 1932, and September 1, 1933, classified according to clinical diagnosis.

Whooping cough cases—The cough plates from patients with whooping

TABLE III

WHOOPING COUGH CASES: SUMMARY OF PLATES CORRELATED WITH STAGE OF DISEASE

Week of Disease	Number of Plates			Per Cent of Positive Plates
	Positive	Negative	Total	
1	62	20	82	75
2	55	24	79	70
3	39	8	47	83
4	7	11	18	39
5	2	17	19	11
6	0	12	12	0
Totals	165	92	257	

cough, so diagnosed by the physician, are summarized separately in Table III.

Because of its bearing on the practicability of obtaining cough plates on young children, it may be mentioned that of the positive plates tabulated, 42 were from children 2 years old or less. Of these 42 children, 9 were 5 weeks to 6 months, 3 were 7 to 11 months, and 30 were 1 to 2 years of age. In comparison, there were 23 negative plates from pertussis patients of the same age group, taken during the first four weeks of disease. In other words, approximately 65 per cent of plates obtained from children 2 years of age or less, during the first 4 weeks of disease, were positive.

Comparison with certain other reports—The percentages used in Table III were calculated on the basis of number of plates, without regard to the fact that in certain instances, there were several plates on the same patient. Most percentages recorded in previous tabulations have been based on the number of patients found positive, disregarding the number of examinations made to obtain the result. Calculating our percentages on this basis for the sake of comparison, we include them along with certain previous reports in Table IV.

There is no question that by more often repeated examinations the percentages could be markedly increased. Since our examinations on the 207 cases tabulated numbered 257, it is evident that the number of repeat examinations was not large.

Carrier data—Before we proceed very far with a discussion of whooping cough carriers, we feel the need for definition. For the sake of clarity in our own thinking and discussion, we consider as carriers those individuals harboring *B. pertussis* who, (a) have no symptoms—the healthy carrier, (b) have had whooping cough for more than four weeks—the convalescent or post-whooping cough carrier, and (c) have respiratory symptoms diagnosed other than whooping cough. On the other hand, the patient with an atypical cough not otherwise diagnosed and a positive cough plate, we consider not as a carrier but as an abortive case of whooping cough. The data in Table V include the findings in those individuals who, if positive, are classified as carriers according to this definition.

The only positive cough plates we obtained from 234 examined were two

TABLE IV

COMPARATIVE FINDINGS OF VARIOUS AUTHORS
PERCENTAGE OF WHOOPING COUGH CASES FOUND POSITIVE BY COUGH PLATE METHOD

Year of Report	*Authors*	*Per Cent of Patients Found Positive Stage of Disease in Weeks* 1	2	3	4	5	6	*Total Cases*
1924	Chievitz & Meyer [1]	75	57	61	45	40	9	914
1927	Lawson & Mueller [4]	59	53	33	38	15	6	533
1930	Sauer & Hambrecht [9]	98	—	65	—	0	0	200
1932	Gardner & Leslie [12]	75	67	75	25	0	0	47
1932	Culotta & Harvey [5]	82	56	19	2	0	0	129
1933	Sauer—7 yr. summary [3]	88	—	64	—	0	0	400
1933	Kristensen—15 yr. summary [2]	65	58	52	40	34	8	2,144
1933	Kendrick & Eldering	84	78	83	31	11	0	207

As tabulated here, the week of disease given for one author is not always strictly comparable with that of another. For example, Sauer gives his results in terms of catarrhal and convulsive stages; Kristensen in terms of catarrhal and 1st, 2nd, 3rd, and 4th week of convulsive stage.

TABLE V

COUGH PLATE EXAMINATIONS FOR CARRIERS

Clinical Symptoms	*Number of Plates*	
	Total	*Positive*
Post-Whooping Cough		
5th week	19	2
6th week	12	0
7th week	6	0
8th week	7	0
2 to 6 months	124	0
No Symptoms	13	0
Respiratory—Not Whooping Cough	53	0
Totals	234	2

during the fifth week after the onset of whooping cough.

The second attack—Although a second attack of whooping cough usually is considered an infrequent occurrence, 7 of the cases of this series gave a history of previous pertussis. There is, of course, always the question of the reliability of the history. The second infection in each case was confirmed by a positive cough plate. Mrs. B., age 27, had a severe second attack, and gave a history of a severe case when she was an infant. Mrs. M., age 30, had a moderately severe second attack. Her mother stated that she had had whooping cough when she was a child. Miss R., age 25, a city nurse, had an abortive case which will be described later, and said she had had whooping cough when she was about 5 years old. The other 4 cases were children. D. B., age 4½, had a typical case of moderate severity, and was said by his mother to have had a similar case when he was 1 year old. F. H. and D. H., brothers, age 4 and 5, respectively, had severe attacks and were hospitalized for several weeks, 1 child with a complication of pneumonia. The mother said they had had whooping cough 2½ years before. J. H., age 3, had a severe case. His first attack, only 6 months previous to the second, had been diagnosed by a pediatrician.

The abortive case—If we define as an abortive case an individual harboring *B. pertussis,* having respiratory symptoms but not developing the typical paroxysms, and with an attack not clinically distinguishable as whooping cough, 5 cases would be so classified, 4 of which were children who had mild coughs for 1 to 3 weeks, in homes where other children had typical whooping cough. The remaining abortive case was the nurse, Miss R., cited among the second attacks. She developed what she considered to be a bad cold accompanied by a cough which never became paroxysmal and lasted about a week. As she was interested in the cough plate method she exposed a plate from which *B. pertussis* was isolated. About 6 days later a baby in the house developed whooping cough, and as it had had no other known exposure, it was concluded that the case was contracted from Miss R. These cases serve to emphasize the value of the cough plate in detecting atypical whooping cough, as well as to point out the importance of the atypical case in the spread of the disease.

Time required for diagnosis—The time required to complete a laboratory diagnosis is an important practical consideration. In Table VI the positive results for 10 months are tabulated in terms of days required to make the report. The information was obtained

TABLE VI

INCREASE IN SPEED OF DIAGNOSIS DURING PROGRESS OF STUDY

Relative Period of Study	Per Cent of Positive Plates Reported on Various Days After Their Receipt						Total Positive Plates
	2	3	4	5	6	7+	
First 6 Months	3	43	27	13	5	10	109
		73			28		
Subsequent 4 Months	23	53	15	8	2	0	53
		91			10		

by comparing the entry date with the date of report on the filed laboratory blanks.

A practical point brought out in this tabulation is the lessening of the time required for diagnosis as the study progressed and greater facility was gained. With greater experience, there is increased facility in recognizing colonies of *B. pertussis* as soon as they appear and it becomes necessary only in relatively few instances to wait for subcultures for differentiation. During the latter part of the year, 76 per cent of all positive reports were made within 3 days, and 91 per cent within 4 days. We believe this compares favorably enough with certain other accepted diagnostic procedures.

Serological grouping of the cultures isolated—The maximum titers observed for the various antisera used in the agglutination tests ranged from 1:1,000 to over 1:4,000, in terms of final dilution in the 0.2 c.c. rapid test; or from 1:5,000 to over 1:20,000, expressed as equivalent titers—that is, in terms of the usual 1 c.c. test but based upon actual quantities of serum and antigen in the mixtures. For the sake of closer comparability with agglutination titers as usually given, the results of agglutination tests with 149 cultures are expressed in Table VII as equivalent titers.

All the Grand Rapids cultures, the 2 from Copenhagen, those from Sauer, the Lapeer, and the Phase I cultures

TABLE VII

SUMMARY OF AGGLUTINATION TESTS WITH 149 CULTURES OF B. PERTUSSIS

Number of Cultures	Designation of Cultures	Titers with Phase I Serum
136	Isolated at Grand Rapids	
	94 — to 14th day of disease	1:2,500 to 1:20,000+
	40 — 15th to 28th day of disease	1:2,500 to 1:20,000+
	2 — Over 28th day of disease	1:15,000 & 1:20,000
4	Old Laboratory Cultures (1911–1922)*	Negative (1:20)
6	Received from Lansing Laboratory	
	2 — Copenhagen	1:20,000
	1 — Lapeer, 1931	1:15,000
	1 — Sauer	1:15,000
	1 — Phase I Harvard	1:15,000
	1 — Phase III Harvard	Negative (1:20)
3	Received from Dr. Sauer, Evanston, Ill. recently isolated cultures	1:20,000

* Received through the courtesy of Parke, Davis & Co., Detroit.

from Harvard, were agglutinated to a significant titer by "Phase I" serum, while the 4 old laboratory cultures and the culture labeled Phase III were not agglutinated. From the tabulated data, it is seen that the Grand Rapids cultures were isolated at all stages of the disease—94 during the first 2 weeks, 40 during the third and fourth weeks and 2 after more than 4 weeks of disease. This suggests that, at least in their agglutination response, the cultures of *B. pertussis* did not change during the course of the disease. The agglutination of all these 144 cultures by "Phase I" serum is in harmony with the findings of Leslie and Gardner [12] with 20 recently isolated strains.

GENERAL DISCUSSION

Practicability of the cough plate method—It is with considerable interest that we have analyzed the results of the first year of continuous cough plate diagnostic service. Is the procedure practicable? Before starting this study, we were well aware of the difficulties held up as a warning to any workers who might be tempted to take the cough plate seriously. As in any diagnostic procedure, there are certain difficulties to overcome in mastering the technic. After a year's experience, however, we believe the difficulties have been overemphasized. Great stress has been placed by various writers upon the differentiation of *H. influenzae* and *B. pertussis*. With a properly balanced medium and increased experience in examining the cough plate, this problem tends to vanish. The necessity of securing a constant, uniform and adequate supply of blood has been met under our particular conditions by the use of sheep's blood. *B. pertussis* is easily isolated on a medium enriched with sheep's blood, it grows well in mass culture, it is antigenic, and it produces toxic symptoms in animals. With respect to the time required for diagnosis, we have shown that an unreasonably long period is not necessary, and it is entirely possible that future studies of the growth requirements of the organism will materially shorten the present minimum period. An important aid in making the procedure practicable in our particular community is the coöperative plan with the City Health Department already outlined. In brief, there are no insurmountable difficulties surrounding the cough plate technic and we believe it deserves a more prominent place than it now occupies in public health laboratory procedure.

The value of negative cough plate findings—Particularly in studying the period of infectivity, we wish to know how much dependence can be placed on negative findings. It is generally true of diagnostic procedures that negative results do not carry the same weight as positive ones. We do rely on them to an extent, however, in various communicable disease release procedures. Whether negative cough plates can be used as an indicator of the probable state of infectivity of a patient at the end of his isolation period is under study. The Michigan Department of Health regulation states that, "Patients shall be isolated for 3 weeks after development of the characteristic cough." The city of Grand Rapids requires 4 weeks. During this fourth week of isolation, we are securing 2 cough plates at least a day apart. We are hopeful that this study will afford information that will help to answer the question as to whether the cough plate has a place in the release of whooping cough. It is safe to say that the value of negative plate findings increases in direct proportion to excellence in technic of obtaining plates and facility in laboratory diagnosis.

The period of infectivity and the post-whooping cough carrier—Any discussion of whooping cough is limited by the difficulty of exactly establishing the

stage of disease and the impossibility of achieving entirely adequate definition of terms. Under the conditions of our own study, we have been able to determine with sufficient accuracy the date of onset and we have, therefore, expressed the stage of disease in terms of the day or week after onset of symptoms. We realize that designations by different workers of the week of disease cannot be strictly comparable. For example, many clinicians place the date of onset of a disease at the time when symptoms are severe enough for a physician to be called or for the patient to go to bed, whereas epidemiologists set the date of onset as the time when the first indication of illness is noted. However, keeping the limitations in mind, we believe a comparison of the results of different workers with respect to the period of infectivity as indicated by cough plate findings, has meaning and yields considerable information.

By referring to Table IV it is seen that aside from the reports of Chievitz and Meyer and of Kristensen, relatively few positive findings have been reported after the fourth week of disease. The findings in general point toward the first 4 weeks of disease as including the infective period in most instances. Our positive findings suggest that most whooping cough patients are actively infectious during the first 3 weeks, a certain percentage remain so during the fourth week and most of them are non-infectious after the fourth week.

As found in other communicable diseases, there are occasional individuals who harbor the infecting organism for longer than the average period. Just how important these individuals are as a reservoir of infection in whooping cough and whether they can be located by the culture method remains to be worked out. If it were true, as suggested by several authors, that the organisms lose their virulence during the course of the disease, the carrier would present no problem; but convincing data on this point are lacking. It seems likely that the early case offers the greatest opportunity for exposure and is the most potent source of infection in whooping cough. However, we cannot disregard the significance of even a small per cent of post-whooping cough or convalescent carriers. To go a step further and translate the findings with respect to the period of infectivity into practicable communicable disease regulations is a problem for coöperative study between health department and laboratory and one which will require time.

Indirect results of cough plate study —Besides the more direct results which we believe are being obtained by the routine plate service, there are less tangible effects which play a part in the general effort toward whooping cough control. The members of the community in general are reminded of the disease and its importance and they are impressed with its communicability. As a physician recently expressed it, "Grand Rapids is whooping cough conscious." The district nurses are on the alert for suspicious cases. With the nurses available for collection of plates, more physicians are making use of the cough plate service, and incidentally they are reporting their cases to the Health Department. This increased emphasis upon whooping cough has the general effect of encouraging early recognition and better isolation.

SUMMARY AND CONCLUSIONS

1. Cough plate diagnostic service for pertussis has been available continuously for about a year at the Western Michigan Division Laboratory of the Michigan Department of Health, Grand Rapids. It is proving a practicable procedure under the conditions existing there.

2. During the past 4 months of this service, 23 per cent of the positive

diagnoses have been made within 48 hours after the plates have reached the laboratory, 75 per cent within 72 hours, and 91 per cent within 4 days.

3. The laboratory findings are being used by the City Health Department in obtaining earlier diagnosis of pertussis and thereby, it is hoped, more effective isolation of cases in their most infective stage. The possible applicability of the results to the problem of release is under study.

4. In agreement with the reports of most other authors, *B. pertussis* could be isolated from relatively few patients after the fourth week of disease. A patient with whooping cough of more than 4 weeks' duration, who can be shown to harbor *B. pertussis*, is defined tentatively as a post-whooping cough or convalescent carrier.

5. One hundred thirty-six cultures isolated from whooping cough patients, at times ranging from before onset to the 35th day of disease, were found to fall into the same serological group.

REFERENCES

1. Chievitz, J., and Meyer, A. H. Recherches sur la coqueluche. *Ann. de l'Inst. Pasteur*, 30:503, 1916.

2. Kristensen, Björn. Occurrence of the Bordet-Gengou bacillus. *J.A.M.A.*, 101, 204 (July 15), 1933.

3. Sauer, Louis W. Whooping cough: Résumé of a seven years study. *J. Pediat.*, II, 740, 1933.

4. Lawson, G. M., and Mueller, Mary. The bacteriology of whooping cough. *J.A.M.A.*, 89:275, 1927.

5. Culotta, C. S., and Harvey, D. F. Whooping cough. I, Early Diagnosis by the cough plate method. *Yale J. Biol. & Med.*, 5:69, 1932.

6. Kline, E. K. Whooping cough plates in a Public Health Laboratory. *A.J.P.H.*, XXIII:493, 1933.

7. Bordet, J., and Gengou, O. Le microbe de la coqueluche. *Ann. de l'Inst. Pasteur*, 20:731 (Sept.), 1906.

8. Gardner, A. D., and Leslie, P. H. Early diagnosis of whooping cough by the cough-droplet method. *Lancet*, 9 (Jan. 2), 1932.

9. Sauer, L. W., and Hambrecht, L. Whooping cough: Early diagnosis by the cough plate method. *J.A.M.A.*, 95:263, 1930.

10. Kendrick, Pearl. Rapid agglutination technic applied to *B. pertussis*. *A.J.P.H.*, XXIII, 12:1310 (Dec.), 1933.

11. Noble, Arlyle. A rapid method for the macroscopic agglutination test. *J. Bact.*, XIV:287 (Nov.), 1927.

12. Leslie, P. H., and Gardner, A. D. The phases of Haemophilus pertussis. *J. Hyg.*, 31:423 (July), 1931.

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Nutrition and Mental Outlook

"INDEED, the effect of nutrition on human physiology is so marked that one might even attribute an association between the unrest and distrust seen in the world today and the dietary which is directly affected by the economic situation. The hungry man is proverbially an angry man, and consequently discontented. It is common experience that the temperament and general outlook on life is governed by one's diet, its digestion, and its effect on the liver. The jaundiced view of life is only an extreme expression of varying degrees of nutritional deficiency or excess. Our food refineries, tinned foods, rapid consumption of meals which we do not take time to enjoy or digest, may be affecting the mental outlook and destroying that peace and contentment to be found in adequately nourished individuals, particularly the beer-drinking, beef-eating Britisher."—J. O. Murray, M.D. The Public Health Outlook on Nutrition, *Pub. Health*, Feb., 1934, p. 164.