tions on the care and preparation of milk, the feeding of infants, of schoolchildren, and the wholesome preparation of foods common to that locality. The fact was also disclosed that the bottle-fed baby is a product of the city. Of nearly 900 such children, 545 were raised in small towns of 3,000 and under. Of strictly country-bred children, exactly two were weaned at birth, one because of the death of its mother and the other on account of a "bealing breast."

The average length of breast-feeding was from eighteen to twenty months, though especially unfortunate little victims were kept at the breast for three, four and even six years. This does not by any means imply strict breast-feeding, for the rural child is almost invariably fed, in addition to the breast, a taste of everything its mother eats—"to keep it from having colic." A young mother smiled scornfully when remonstrated with for feeding her baby green grapes—"La!" she said, "he don't think no more o' eatin' them grapes 'n you do o' spittin'."

The proportion of defects varied slightly from those found by medical inspectors of schools, as the children ranged from 3 weeks to 15 years; 541, or 66.6 per cent., being under school age; and enlarged lymph-nodes were proportionately in excess of defective teeth. The East Tennessee child represented a type taller than Holt's standard, 612, or 71.3 per cent., being above Holt's normal, as against 154 below, and 90 corresponding to his standard.

In weight they are nearer normal, 422, or 49 per cent., being below Holt's standard, while 337, or 39 per cent., are above size, and 97, or 11 per cent., normal.

The conference seemed to establish several facts which have been agitating the public mind for a few years past.

1. The medical profession is alive and quite able to care for the children of the country without resorting to hysterical or spectacular methods.

2. It does not need the financial backing of advertising concerns to point the way to its duty. This work may be kept free of commercial entanglement and on the usual high plane of professional activities.

3. The medical profession stands ready to give of its knowledge and efforts to the uplift of mankind, and the little child shall not call in vain.

4. It is to be hoped that the work of raising the standard of our public health may remain where it belongs, in the hands of the government and of the medical profession, and that together they may work out the solution of this most vital phase of conservation.

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PRECIPITATION TESTS IN SYPHILIS *

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Wassermann, Neisser and Bruck applied Bordet-Gengou's phenomenon of complement fixation to syphilis in 1906. Since then, their principles have undergone many refinements until to-day the method of complement fixation stands out as a test *par excellence* for syphilis. The recognition of natural antisheep red cell amboceptor, the provisions against non-specific complement deviation, the use of inactive serum, the refinement

of antigen, the cognizance of anticomplementary effects—all have determined the specificity of the Wassermann reaction.

Owing to the difficulties encountered in a reliable performance of the reaction, methods have been devised for the serodiagnosis of syphilis, having for their main objects simplicity and rapidity. These methods are based on precipitation in luetic serums by various reagents.

R. Kraus,¹ in 1897, discovered the precipitating action of immune serums by bacterial suspensions; but, as the *Treponema pallidum* is so difficult of cultivation, no similar method was used in syphilis to detect antibody. Fornet and Schereschewsky² modified this principle by adding the serums of tabetics (luetie) to those of earlier syphilis and obtained precipitations. They found the reaction specific and explained it by declaring that "antigen" existed in early syphilis serum and "antibody" in later. Several factors are concerned here that affect the specificity of this action which we shall show later.

Elias, Neubauer, Porges and Soloman³ were the first to use a hydrophilic colloid. They employed a 1 per cent. solution of sodium glycocholate and obtained precipitation with syphilitic serums. These authors, however, have failed to demonstrate an absolute specificity in their reaction. Wieder and L'Engle⁴ have used sodium taurocholate, taurin solution and distilled water, but while precipitation occurred there was a complete lack of specificity. Klausner⁵ obtained a precipitum when fresh syphilitic serum was added to distilled water and allowed to incubate for several hours. Our experiences taught us that some non-luetic serums would give positive while some luetic serums would give negative results. Also, we confirmed Hayn's observation that inactivation as well as aging abolished the effect. Sachs⁶ found that the addition of from 1:1,000 to 1:500 normal hydrochloric acid restores this property, but he, likewise, could not determine the specificity of the Klausner reaction. The effect of addition of the dilute hydrochloric acid is of interest in the Wassermann reaction, in which similar results may be had, and is probably due to the acid disturbing the hydroxyl concentration which occurs in inactive serums. Cuorin, used by Teruuchi and Toyada,⁷ and lecithin by Georg Meier⁸ gave precipitation when added to syphilitic serums, but Meier showed that these precipitinogens had a high degree of non-specificity (50 per cent. of non-luetic serums gave precipitations).

Herman and Perutz⁹ modified Elias, Neubauer, Porges and Soloman's reaction and asserted that a precipitation by their method meant syphilis. Remarkable attestations to this specificity have been appearing from then to date.

In 241 cases of syphilis, Thomsen and Boas¹⁰ found the Wassermann reaction to be positive in 86 per cent., while the Herman-Perutz reaction was positive in 48.4 per cent. In 182 non-luetic cases the Wassermann was negative in all, the Herman-Perutz positive in three.

1. Kraus, R.: Wien. klin. Wchnschr., 1897, No. 32.

2. Fornet and Schereschewsky: München. med. Wchnschr., 1907, No. 30, p. 1471.

3. Elias, Neubauer, Porges and Soloman: Wien. klin. Wchnschr., 1908, No. 23.

4. Wieder, Henry S., and L'Engle, Edward M.: Some Studies of the Precipitation Tests for Syphilis, THE JOURNAL A. M. A., Nov. 6, 1909, p. 1535.

5. Klausner: Wien. klin. Wchnschr., 1908, Nos. 7, 11, 13 and 16.

6. Sachs: Semaine méd., June 24, 1908.

7. Teruuchi and Toyada: Wien. klin. Wchnschr., 1908, No. 23.

8. Meier: Berl. klin. Wchnschr., 1908, No. 10, p. 522.

9. Herman and Perutz: Med. Klinik, 1911, No. 2.

10. Thomsen and Boas: Ztschr. f. Immunitätsforsch. u. exper. Therap., Orig., 1913, xvi, No. 4.

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One case was that of a third stage pulmonary tuberculosis, another of venereal ulcer without spirochetes and the third a case of osteomyelitis and sepsis with subsequent death, necropsy showing no syphilis. In 106 serums Jensen and Freilberg¹¹ found sixty-three non-luetic, with the Herman-Perutz reaction negative in all, and forty-three luetic with the Herman-Perutz reaction positive in all tested, corresponding to positive Wassermann reactions. Gammeltoft¹² found it a quick and convenient method for diagnosing syphilis. Of 156 control serums (negative) only one (cancer of the liver) gave a positive precipitation, in which case the Wassermann was negative. Ellermann,¹³ Lade¹⁴ and Kallós¹⁵ (the observation of the latter two having been made recently) came to the same conclusions, that a precipitation means syphilis whenever it occurs.

We have investigated this question by applying this reaction 259 times to 175 serums. We employed the technic recommended by Herman and Perutz. This is, in short, as follows:

Two solutions are used:

Solution 1 (stock solution diluted 1 : 20 with distilled water before use):

Sodium glycocholate	2.0 gm.
Cholesterol	0.4 gm.
95 per cent. alcohol.....	100.0 gm.

Solution 2 (freshly prepared before use):

Sodium glycocholate 2 per cent. in distilled water.

The test was performed by adding to 0.4 c.c. of clear inactive serum (56 C. [100.8 F.] for half an hour) in a 7 mm. bore tube, 0.2 c.c. of Solution 1 and 0.2 c.c. of Solution 2. (We controlled the test by adding 0.4 c.c. of distilled water, instead of reagent, to the serums and for each set of tests adding 0.4 c.c. of distilled water to 0.4 c.c. of the reagent.) The tubes were plugged tightly with cotton and set aside at room temperature for twenty hours, when the presence or absence of precipitation was noted.

By this method Herman and Perutz obtained these comparative results: Of 89 non-syphilitic cases, only one reacted feebly (a serum from a tuberculosis patient), of 134 syphilitic serums, 102 were examined. The Wassermann reaction was positive in 72 and negative in 30, and their reaction positive in 76 and negative in 26.

In our series of examinations, the serums were all controlled by first performing the original Wassermann reaction, but using only one-tenth of Wassermann's amounts. Alcoholic extract of normal guinea-pig heart was employed as antigen, and fixation allowed for four hours in the ice-chest. Readings were made just as soon as the controls (containing twice the amount of antigen and serum used in the test) hemolyzed.

In 95 cases of syphilis in different stages we have found 88 giving a positive Wassermann reaction, 7 being negative. Correspondingly, the Herman-Perutz modification, when used, showed 58 positive, 3 doubtful and 34 negative reactions. In 80 non-luetic serums, the Wassermann reaction was negative in all, but the Herman-Perutz test was positive in 16. Of these, 50 per cent. were serums from chronic nephritics. Other positives were found in pulmonary tuberculosis, arteriosclerosis, chronic arthritis, chronic endocarditis, obesity and a normal condition.

Strong¹⁶ likewise found that 10 per cent. of non-specific serums gave a positive precipitation.

We attempted to obtain more reliable results by trying various modifications in technic. One series of serums was set aside in the ice-chest instead of being kept at room temperature, but no increase in precipitation was noted; two serums of fourteen, however, positive at room temperature, became negative in the ice-chest. Another series was incubated, but the results were far from satisfactory. This temperature offered a good field for the development of bacteria, molds, etc., which interfered with precipitations. Then again, great variations were found between the precipitinogenic Solution 1, when it was diluted by the addition of distilled water drop by drop, or when water was added *en masse*. Analogously to the results in the Wassermann reaction, many more positives were obtained by the former method. The results obtained by using active or inactive serums almost corresponded; but, since some of the positives obtained in active serums were non-specific and since these became negative in inactive serums, it appears best to use inactive serum under all circumstances. In fact, the modification of Herman-Perutz in all details gave the most constant results.

We attempted to substitute the precipitinogen by using an alcoholic extract of normal guinea-pig heart (Wassermann antigen), but there was a uniform pre-

Curve of precipitation of the Herman-Perutz reaction.

cipitation in normal as well as luetic serums. This is due to the presence of alcohol, which has the power to coagulate the albumins present in serum. Others have obtained precipitations by using various fractions of syphilitic liver antigens, yet have not obtained a desideratum for the rapid diagnosis of syphilis.

RATIONALE OF PRECIPITATION REACTIONS

We were struck by the constancy with which all serums, luetic or non-luetic, became clouded when these tests were performed. Indeed, only such as gave a distinct flocculation could be regarded as positive. This reaction is a colloidal one. There is a throwing of the sols into gel formation. It is represented by cloudiness of the serum. In fact, particles measuring 100 microns will give rise to haziness, yet the individual masses will be invisible, unless the ultramicroscope is called into play. The reactions are purely quantitative, not qualitative.

If, on an ordinate, we were to plot normal serum, serum from nephritis, tuberculosis or conditions in which cholesterol or the albumins have been found to be increased, and then syphilis; and on an abscissa we were to plot the degree of precipitation found by using the Herman-Perutz reaction, we should obtain a curve with its maximum point at syphilis and descending

11. Jensen and Freilberg: Berl. klin. Wchnschr., 1912, No. 23.
12. Gammeltoft: Deutsch. med. Wchnschr., 1912, No. 41.
13. Ellermann: Deutsch. med. Wchnschr., 1912, No. 5.
14. Lade: Deutsch. med. Wchnschr., 1913, No. 15.
15. Kallós: Deutsch. med. Wchnschr., 1913, No. 39.

16. Strong: Jour. Med. Research, 1911, xxv, No. 1.

therefrom to normal serum, but never reaching normal serums at zero precipitation.

Other factors appear to interfere with precipitation tests. We have seen alcohol by itself in dilutions from 20 per cent. to 95 per cent. produce a gel formation. Again, serum is contaminated easily and, owing to consequent changes in reaction and surface tensions, spontaneous gel formation may occur. Elias, Neubauer, Porges and Soloman have shown that sodium glycocholate in 1 per cent. dilutions may give very light precipitations in normal serums.

CONCLUSIONS

The Herman-Perutz reaction or precipitation by hydrophilic colloids in general should be regarded as only partially specific. That is, only a heavy precipitation should be considered positive, and then only in a presumptive sense. A diagnosis of syphilis should not be made therefrom, but further investigations of such a serum is in order, especially by means of the Wassermann reaction.

The Wassermann reaction is by far the more reliable of the two.

There is no absolute parallelism between precipitation and complement fixation. The degrees of similarity should not warrant the theory that antigen precipitates antibody, thus binding complement.

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THE LIBERATION OF FORMALDEHYD FROM HEXAMETHYLENAMIN IN PATHOLOGIC FLUIDS *

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In a previous publication¹ it was shown that the decomposition of hexamethylenamin in the living normal organism takes place only in acid urine and gastric juice, that it depends on the excess hydrogen ion $[H^+]$ concentration² of the fluid, and that in those body fluids which are neutral or truly alkaline (possessing $[H^+]$ concentrations of 7.0 or less), no liberation of formaldehyd from hexamethylenamin takes place. It is conceivable, however, that the reactions of certain pathologic fluids may be truly acid. This might be true of those fluids which exhibit pronounced degrees of stasis, putrefaction or those of clinical "acidoses." With this in view, we have investigated the distribution of hexamethylenamin and free formaldehyd, and the reaction of these fluids, according to the methods described in our previous paper.

Through the courtesy of the various staffs of the Lakeside, City and Charity hospitals of Cleveland, the following pathologic fluids were obtained from patients suffering with different clinical conditions. Usually a dose of 60 grains of hexamethylenamin was given about one hour before the fluid was obtained.

* From the Pharmacological Laboratory, Western Reserve University Medical Department.

1. Hanzlik, Paul J., and Collins, R. J.: Hexamethylenamin: The Liberation of Formaldehyd and the Antiseptic Efficiency under Different Chemical and Biological Conditions, *Arch. Int. Med.*, November, 1913, p. 578; abstr., *THE JOURNAL A. M. A.*, Nov. 29, 1913, p. 2012.

2. Of the two forms for this sign, $[H^+]$ and $[H]$, the former has been preferred by *THE JOURNAL*; the latter was the form used in the author's previous paper, mentioned above.

URINES

In twelve urines from persons suffering from acute, chronic and hemorrhagic nephritides, the mean value for the $[H^+]$ concentration was about 6.5; that is, they were truly acid, and formaldehyd was present at all times together with hexamethylenamin. The average time of appearance of hexamethylenamin was seventeen minutes, that is, about the same as normal. In two patients with cystitis, the urines contained hexamethylenamin and free formaldehyd; the $[H^+]$ concentration of several specimens ranged from 6.3 to 6.51; that is, they were truly acid. In another person with cystitis whose urine was alkaline ($[H^+]$ concentration, 7.08), hexamethylenamin was present, but no free formaldehyd. These data indicate that the behavior of hexamethylenamin in urines of various pathologic conditions is about the same as in urines of normal persons, since it is the reaction which is the essential factor in determining the decomposition.

OTHER BODY FLUIDS

Eighteen cerebrospinal fluids obtained from the following conditions; cerebrospinal lues, general paresis, diabetes, alcoholic neuritis, neurasthenia, cerebral arteriosclerosis, leprosy, tabes, epilepsy, pneumococcic meningitis, tetanus and chronic gastritis, showed $[H^+]$ concentrations from 6.98 to 7.3; that is, they were neutral or slightly alkaline, and hexamethylenamin was present in all, but no free formaldehyd. Ten specimens of fresh bile from a patient who was ill with typhoid and had received hexamethylenamin for about four months and was operated on subsequently for cholelithiasis, showed the presence of hexamethylenamin in all of the specimens and typhoid bacilli at the beginning and end of the experiment; no formaldehyd was present in any of the freshly collected specimens and the $[H^+]$ concentration ranged from 6.98 to 7.18; that is, the bile was practically neutral or slightly alkaline. No bactericidal action had been exerted by the presence of the hexamethylenamin. Three specimens of bile from another patient operated on for cholelithiasis gave practically the same results so far as the reaction and formaldehyd liberation are concerned. On standing, some of the specimens became truly acid and showed the presence of free formaldehyd. Two specimens of edema fluid from the extremities showed the presence of hexamethylenamin, but no free formaldehyd, and their $[H^+]$ concentrations were 7.1. In a child with tuberculous pleurisy, hexamethylenamin but no free formaldehyd was found in the chest fluid, and the $[H^+]$ concentration was 7.1. An ascitic fluid showed hexamethylenamin, but no free formaldehyd; the $[H^+]$ concentration was 7.42. The bloods of four diabetic patients showed the presence of hexamethylenamin but no free formaldehyd; their $[H^+]$ concentrations ranged from 7.3 to 7.45; that is, they were slightly alkaline.

The pathologic data indicate that these body fluids contained no free formaldehyd. It would be contended, therefore, that no bactericidal action could have been expected. In our observations, the average and maximum acidity were no higher than in the corresponding normal fluids. With urines, it appears that the average acidity is higher in pathologic than in normal conditions; but the maximum acidity so far recorded is not higher in pathologic cases than the maximum acidity of some normal urines.

CONCLUSIONS

The decomposition of hexamethylenamin in pathologic fluids obeys the same laws as in normal circulat-