A NOTE UPON THE USE OF ANTIFORMIN IN SPUTUM EXAMINATION,

AND ON STAINING METHODS FOR THE DEMONSTRATION OF TUBERCLE BACILLI.

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SINCE the routine examination of sputum as a means of diagnosis for tubercle became a universal practice, it has been recognized that diagnosis might be rendered more precise by some satisfactory method of rendering the sputum homogeneous, and dissolving the non-tuberculous elements therein contained. For this purpose such reagents as caustic alkalis, hydrogen peroxide, etc., were tried with a certain measure of success. The procedure of Ellermann and Erlandsen (1908), which consisted in autodigestion of the sputum in the presence of dilute sodium carbonate, and subsequent treatment with dilute sodium hydrate, was shown by careful workers (Jørgensen, 1910) to give results superior to those obtained with antiformin, but, nevertheless, the introduction of this latter reagent must be regarded as the chief recent advance in this branch of study. Antiformin is a proprietary substance which is made up of equal parts of caustic soda 15 per cent., and liquor sodæ chlorinatae. It was first used in breweries for cleansing vats and for general disinfection. It is a very powerful solvent of organic substances, rapidly causing the disintegration and solution of such materials as pus, mucus, connective tissues, faeces, and hair. Waxes and waxy substances alone resist its action. Uhlenhuth and Xylander (1909) studied its solvent and its germicidal powers, and published the description of a method for its application to the investigation of tuberculous material. They found that, whereas other bacteria dissolved in the reagent as rapidly as sugar in water, tubercle bacilli retained their vitality in a 20 per cent. solution for twelve to twenty-four hours, and continued to exhibit their characteristic form and staining reactions for eight days.

Uhlenhuth made use of a 20 or 25 per cent. dilution of antiformin. This was added to sputum in equal bulk. The mixture was well shaken and incubated at 37° C. for six to twelve hours. Then after centrifugalization, the deposit was examined for tubercle bacilli in the ordinary way.

Lorenz (1911) used the same strength of antiformin, but heated the mixture over a flame, thus rendering the sputum more rapidly homogeneous. Haserodt (1909) shook up the sputum and antiformin mixture, after it had become homogeneous, with ligroin, and placed the mixture in a water bath at 60° C. The lighter fluid rising to the top, carried with it the tubercle bacilli, which collected at the zone of junction between the two layers of fluid. For this method no centrifuge is required.

When rapid concentration of tubercle bacilli in sputum is required, Hammerl's method (1909) is satisfactory. One part of sputum is shaken up with two parts of a 1 per cent. solution of caustic potash in strong ammonia. The mixture is incubated for half an hour, being shaken up from time to time. Then, after the addition of one part of acetone, to lower the specific gravity of the liquid, it is centrifugalized and the sediment examined. This method I have tried in a number of cases with good results. By this means, however, tubercle bacilli are rapidly killed, and therefore the method cannot be used for the purpose of obtaining pure cultures of tubercle. Antiformin, which does not kill tubercle bacilli if used in sufficient dilution, is well adapted for such cultural purposes.

In the routine work of the Lister Institute, the sample of sputum is mixed with an equal quantity of a 30 per cent. solution of antiformin, and the mixture is incubated overnight at 37° C. After centrifugalization, the supernatant fluid is poured away and replaced by an equal bulk of normal saline solution. This is shaken up with the deposit, and the mixture again centrifugalized. Films from the deposit thus washed adhere better to the slide than those prepared after one centrifugalization only.

The antiformin is diluted for use with distilled water. It was suggested by Beitzke (1910) that the use of tap water for this purpose might introduce a fallacy, owing to the occasional presence in such water of acid-fast bacilli. Organisms of this type have been found on water taps at the Lister Institute.

Results Obtained with the Antiformin Method.

Since March, 1911, all sputa received at the institute and found negative on direct examination have been treated with antiformin in the manner described above and further investigated. The number of examinations has been relatively large, and it has therefore been thought that statistics based upon these data would possess sufficient value to justify this note.

The number of sputa received for investigation during thirteen months has been 2,273. On direct examination 622 were found to be positive. The remaining 1,651 specimens, which gave negative results on direct examination, were treated with antiformin, and further investigation showed the presence of tubercle bacilli in 9 cases. The percentage of cases found positive on direct examination was thus 27.36, and the percentage of all positive cases 27.76. The cases found positive only after they had been treated with antiformin amounted to 0.4 per cent. of the total number of sputa investigated, and to 0.54 per cent. of those found negative on direct examination. The figure 0.54 may be described more concisely as the improvement percentage.

Comparison with Other Observations.

Other observers have obtained much higher percentages of improvement with the use of antiformin than that shown by our figures. Shortly after the introduction of the method, Meyer (1909) published a report upon the examination of 114 samples of sputum. Fifteen were found positive on direct examination. The remaining 99 samples were treated with antiformin, and further investigations revealed the presence of tubercle bacilli in 14 cases. Other contributions to the literature of this subject, too numerous to be cited in detail, give improvement percentages ranging from 10 to 35. Schneider (1912) has recently made a comparative study of some of the principal modifications of the antiformin method, and has obtained such improvement percentages as 17.5 and 22.8. He recommends the use of 15 per cent. antiformin, and that the examination be made as soon as possible after the material has become homogeneous. For the demonstration of bacilli, when very few are present, he considers the method of Lorenz the most satisfactory. Upon methods involving the use of ligroin and allied substances he reports unfavourably, being of opinion that such materials have a detrimental effect upon the staining powers of acid-fast bacilli.

In order to reconcile the results obtained at the Lister Institute with those quoted above, it is necessary to consider the procedure that is followed here. Care is taken in the first instance to select a suitable portion of the sputum for examination. The film prepared from this is examined, if need be, for four minutes. In the majority of cases tubercle bacilli, if present, are discovered within the first half-minute, but in a number of cases they are only detected after three or four minutes' search. Moreover, in two of the nine cases in which they were not found until after antiformin treatment, reference back to the original film showed the presence of one or two bacilli. The slides prepared from material which has been treated with antiformin are examined with no less care than the original film. Here bacilli, if present, were usually found in large numbers, but in one instance they were only discovered after a protracted search. In this connexion it is interesting to note the work of Meyer, who investigated the twenty-four hours' sputum of a number of sanatorium patients, and found in some cases such small numbers as two, four, or six bacilli.

No paper previously published upon the subject deals with such a large number of specimens as the present one, and the results of those observations appear to show that the greater the care expended upon the examination of the direct film, the less will be the apparent gain with the use of antiformin. This does not detract from the value of the antiformin method, as an improvement percentage as low as 0.54 involves an approximation to complete precision, which is of importance when the number of specimens examined is large.

SUMMARY.

By means of treating with 25 per cent. antiformin 1,651 samples of sputum found negative as a result of the direct examination, it was possible to demonstrate the presence of tubercle bacilli in 9 cases, that is to say, in 0.54 per cent.

of cases. This figure, though small in comparison with those obtained by other workers, is large enough to justify the use of this reagent when a large number of specimens are examined. The discrepancy between these results and others is possibly due to the detailed care which is expended upon the direct examination.

STAINING METHODS.

A comparative study of the efficacy of certain staining methods for tubercle as applied to sputa has been carried out with a series of about 200 sputa. The methods dealt with have been those of Much, Herman, and Gasis.

Much's Method (1907) is a modification of Gram's method. Of the three different processes described by Much the following has been used. The fixed film is treated for a few minutes with hot carbol-methyl-violet solution (10 c.cm. of hot saturated alcoholic solution of methyl violet in 100 c.cm. of 2 per cent. carbolic acid). The slide is washed, and treated with Lugol's iodine for one minute, 5 per cent. hydrochloric acid for one minute, 3 per cent. hydrochloric acid for ten seconds, and a mixture of acetone and absolute alcohol in equal parts to complete the decolorization. One per cent. neutral red may be used as a counterstain.

Herman's Method (1908).—The stain, a 3 per cent. solution of crystal violet in 95 per cent. alcohol, and the mordant, 1 per cent. ammonium carbonate solution, are kept in separate bottles, and mixed for use in the proportion of 1 to 3. The fixed film is stained with this mixture over a small flame for a few minutes. The slide is washed, transferred to 10 per cent. nitric acid, and then to absolute alcohol. Herman does not use a counterstain, but in the present investigation a 0.3 per cent. chrysoidin solution has been found of service.

Gasis's Method (1907).—Corrosive sublimate crystals amounting to the size of a pea are boiled in about 5 c.cm. of a 1 per cent. eosin solution (eosin 1 gram, absolute alcohol 5 c.cm., distilled water 95 c.cm.). The hot stain is filtered on to the slide, and staining over the flame is allowed to proceed for two minutes. The slide is washed and then decolorized with alkaline potassium iodide solution (potassium iodide 1 gram, sodium hydrate 0.5 gram, 50 per cent. alcohol 100 c.cm.) and with absolute alcohol. Acid methylene blue is used as a counterstain.

Much claims that by the use of his method it is possible to demonstrate tubercle bacilli which are not shown in preparations stained by the Ziehl-Neelsen method. This granular non-acid-fast type of tubercle bacillus has been investigated by a number of workers, who have demonstrated its presence in tuberculous matter where no acid-fast bacilli of the ordinary type were to be found. Much also describes granules, visible in preparations stained by his method, but not seen in Ziehl-Neelsen slides, which are capable of causing tuberculous disease when inoculated into guinea-pigs.

Herman's method has been in use for a number of years, and its author claims that it is superior to the Ziehl-Neelsen method. He lays stress upon the advantage of using ammonium carbonate as a mordant, and states that in preparations stained by this process there are seen not only the bacilli which can be shown by the Ziehl-Neelsen method, but also the granules and granular bacilli described by Much.

Gasis's method differs essentially from the foregoing in that it is based upon the alkali-fast and not the acid-fast property of these organisms. It is stated that the alkali-fastness is due to a nucleo-protein and not to a waxy component of the bacillus. The method was first introduced for the purpose of demonstrating tubercle bacilli in the urine, and the claim was made that in urinary sediments so stained, morphological characters distinguishing these organisms from smegma bacilli were rendered obvious.

Application of Staining Methods to Sputum Examination.

A series of 206 sputa were investigated by the methods of Ziehl-Neelsen and Herman. One hundred specimens were examined also by Much's method, and in 48 of the cases Gasis's process was also employed.

No. of Specimens.	Positive Results with			
	Ziehl-Neelsen.	Herman.	Much.	Gasis.
206	51	51	—	—
100	30	—	29	—
48	13	—	—	9

If the Ziehl-Neelsen figures be regarded as expressing 100 per cent. of accuracy, it is found that the other methods—Herman's, Much's and Gasis's—give respectively the figures 100, 96.66, and 69.23, indicative of their relative accuracy.

Gasis's method is the most unsatisfactory. The reagents are unstable, and require to be freshly prepared at the time of use, the staining process is troublesome and requires careful watching, and the results are uncertain, as the figures show. It possesses, however, considerable scientific interest, due to the peculiar biochemical reaction which underlies it. Bacilli stained by this means have a rose pink tint. The outline is well defined, and there is frequently to be seen some degree of granularity and of irregular vacuolation.

Much's method is not suitable for the direct examination of sputum, owing to the fact that a large number of Gram-positive cocci and bacilli other than tubercle take up the stain. This renders the process of search so troublesome that the routine use of the method for sputum diagnosis is not to be recommended. Tubercle bacilli so stained possess a highly granular character, and assume at times a streptococcal type, while free granules of the Much type are occasionally to be seen. The examination of the present series of films from sputum did not, however, afford any independent evidence of the existence of a type of tubercle bacillus demonstrable by this method which would not be shown more satisfactorily when stained according to Ziehl-Neelsen. The impression derived from a study of these specimens was rather that the marked granularity of tubercle bacilli on the Much-stained films was an artefact due to the nature of the staining reaction employed.

Herman's method is the only one of the three which can be compared with that of Ziehl-Neelsen for ease, utility, and efficiency. The necessity for keeping the solutions of ammonium carbonate and of crystal violet separate until they are required for use introduces a slight complication, which leaves the Ziehl-Neelsen method as the easiest, as it certainly remains the most efficient process at our command. It is largely a matter of individual taste whether it is more desirable to search for violet bacilli upon a yellow ground, as with slides stained by Herman's method, or for red bacilli on a blue ground, as with Ziehl-Neelsen; but when the eye has become trained to one method, it requires to be educated afresh before equally satisfactory results are obtained with slides stained otherwise. Bacilli stained according to Herman present interesting morphological characters. Their outline is often irregular and granularity is well marked. Metachromatic spore-like granules were frequently noted, and branching forms were observed on two occasions.

An interesting account of various methods of staining tubercle bacilli, describing the earliest processes, which possess now a purely historical interest, and giving a comparative study of the efficiency of the recent methods, has recently been published by Böhm (1912). This author considers the method of Gasis interesting but unreliable, and finds the Ziehl-Neelsen method superior to all others.

The results detailed above show that the Ziehl-Neelsen method, which has stood the test of time, remains the most suitable of all for the detection of tubercle bacilli in urine. Next to it comes the method of Herman, almost as simple, quite as easy of execution, and giving equally good results. Much's method is not suitable for direct investigation of sputum, and the method of Gasis is both difficult and unreliable.

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THE Port of Karachi is to be opened, in addition to Bombay, for the embarkation of pilgrims to Hedjaz, from the current year. A camp is to be constructed for the accommodation of pilgrims and a Protector of Pilgrims will be permanently appointed.