Nucleated Red Cells.—Nucleus almost black, with sharp outline; extranuclear portion, grey.

Blood Plates.—Deep ruby-red with spiky margins, frequently showing a pale-blue peripheral zone surrounding the red centre.

Bacilli and Micrococci.—Speaking generally, these stain evenly blue, but, by prolonging the period of staining, and subsequently decolorising with absolute alcohol, many interesting differences may be noted with different organisms by which structural details are brought out, not generally observed by other staining methods.

Malaria Parasites.—The body of the parasite stains blue and its chromatin ruby-red; in the case of the tertian parasite Schüffner's dots are well marked in the containing red blood corpuscle. The coloured plate accompanying the article on Romanowsky staining, previously referred to, might have been drawn from specimens stained by this method, the appearances being identical.

For most clinical work in connection with blood examination for malaria parasites, census of leucocytes, etc., where a permanent preparation is not wanted, it is quite sufficient to examine the films in water with a $\frac{1}{4}$ or $\frac{1}{8}$ -inch objective. The staining is so sharp and clear that it is unnecessary to use an oil-immersion lens except to determine the presence of bacteria or the detailed structure of parasites or cells.

Postscript.

While this note was in preparation for publication articles on the same subject, by Dr. Karl Reuter, appeared in the *Munch. med. Woch.* of July 30th, 1901, and in the *Centralbl. f. Bakt.*, vol. xxx, 6, of August 26th, 1901. While it is evident that Dr. Reuter has been working on similar lines, and has also succeeded in isolating the active staining ingredient in Romanowsky's method and applying it as a single stain, I may briefly indicate a few material points in which his method differs from mine. (1) He employs as a solvent absolute alcohol. (2) A separate process of film fixation occupying "at least one hour" is necessary before staining. (3) The staining process in itself "occupies two to three hours." (4) More elaborate precautions are necessary to avoid precipitation on the film.

NOTE.

I have found that by treating Louis Jenner's blood stain with distilled water, in a similar manner to that described for the stain in question in this note, the results are greatly improved, the usual pale-blue nuclear staining being intensified to a deep Oxford blue, while the granular staining is as well marked.

THE VALUE OF NEISSER'S STAIN IN THE DIAGNOSIS OF DIPHTHERIA.

By R. M. BEATON, F. FOORD CAIGER, AND
WALTER C. C. PAKES.

(Preliminary Communication.)

SINCE Neisser published the account of his differential stain for the bacillus of diphtheria many bacteriologists have used it, but they appear to differ in opinion as to its value in the diagnosis of this organism. There seems to be no doubt that the great majority of the bacilli obtained from a young and pure culture exhibit the differential staining described by Neisser, but a doubt exists whether the stain is altogether satisfactory when used for mixed cultures such as are obtained from the throats of patients suffering from diphtheria.

We determined therefore to make a series of observations under the conditions which so frequently holds nowadays, namely, when the bacteriologist is not also the physician, in order to test the value of the stain in the diagnosis of diphtheria. Each of the cases was diagnosed independently at the South-Western Hospital by Mr. A. W. Daniel and confirmed by one of us (F. F. C.) from a consideration of the whole of the clinical evidence, and by the others of us at Guy's simply by the presence in the cultures of bacilli having the characteristic staining reactions described by Neisser, and the clinical and bacteriological evidence was subsequently compared.

Swabbings were taken with every necessary precaution from the throats of all cases certified as diphtheria which were admitted into the South-Western Fever Hospital; tubes of Loeffler's blood serum were inoculated, and both swabs and culture tubes were sent to the laboratory at Guy's Hospital without having been previously incubated.

When the cultures and swabs reached the laboratory, the swab was examined at once, and the culture was placed either in the incubator or the ice chest and subsequently in the incubator; the reason for this latter proceeding was that it was desirable to examine the cultures after eighteen hours incubation at 37° C. At the end of this time preparations of the growth were made and stained.

The method of preparation adopted was the usual one, the films being fixed by heat. After fixation, the films were stained for two minutes in the methylene-blue solution, washed well in water, stained for two minutes in the watery vesuvin, dried and mounted in canada balsam. It will be noticed that in our experiments we have not adhered to the method of Neisser as regards the time necessary for staining in each of the fluids. The reason for this alteration was that we found that, even when dealing with pure cultures of the Klebs-Loeffler bacillus, the bacilli did not always show the characteristic appearance after being stained for a few seconds as recommended by Neisser. When the films were stained for two minutes, however, we found that the bacilli invariably exhibited the characteristic appearances.

All the specimens were examined by two of us separately (R.M.B. and W.C.C.P.), and the bacteriological diagnosis arrived at independently of each other. If, upon comparing notes, we agreed, the diagnosis was made accordingly. When, as happened on three or four occasions, there was any discrepancy in the verdict, the films were again examined by each of us, and fresh specimens were made and examined. The bacteriological diagnosis of each case was, therefore, that arrived at by both of us.

On several occasions we found that blue granules were present in bacteria which appeared to us to be definitely cocci. We had to satisfy ourselves, therefore, in order to arrive at a positive diagnosis, that the bacteria in which were the blue granules were definitely bacilli.

In several cases we have found Klebs-Loeffler bacilli present in only small numbers in comparison with those of other bacteria. We therefore made series of subcultures in order to determine whether the bacilli retained the property of staining in this particular manner when they were in an obvious numerical inferiority, and therefore under a disadvantage as regards ease of growth.

The cultures which we chose for these experiments were those containing only Klebs-Loeffler bacilli and cocci, generally staphylococci. These were subcultivated daily and examined. Each subculture, when the staphylococcus aureus was present, was found to contain fewer Klebs-Loeffler bacilli than the culture from which it was made, and finally this bacillus disappeared altogether. The bacilli became increasingly difficult to find, but when they were found they showed the granules as well marked as in the case of a pure culture. We conclude, therefore, that under the conditions of our experiment the Klebs-Loeffler bacillus will show the blue granules, even when it is growing with difficulty owing to the synchronous growth of other bacteria.

Our observations are concerning 100 cases, with the following results:

Clinical and bacteriological diagnosis both positive	53
Clinical and bacteriological diagnosis both negative	17
Clinical diagnosis positive, bacteriological diagnosis negative	25
Clinical diagnosis negative, bacteriological diagnosis positive	5

The clinical and bacteriological diagnoses, therefore, were in accord in 71 cases and differed in 30. Of the cases, 5 in number, in which the clinical diagnosis was negative and the bacteriological positive, one had the appearance of a follicular tonsillitis, another was a case of scarlet fever with yellow spots upon the tonsil, another was suffering from measles and pronounced laryngeal stridor; another, admitted on the fifth day of illness, showed an ulceration of the tonsils which suggested the recent separation of membrane. So that in 4 out of these 5 cases the condition of the throat was compatible with the presence of diphtheria, and in one instance only in this series of 100 cases did the Neisser test warrant a diagnosis of diphtheria when the clinical evidence was entirely

negative. This, considering the well-known fact that Klebs-Loeffler bacilli are occasionally to be found in the throats of healthy persons, is eminently satisfactory.

The most disappointing feature about our series is that on 25 occasions we failed to find Klebs-Loeffler bacillus on the swab or culture taken from cases which were clinically diphtheria. Some of these cases were very typical, several of them dying of cardiac failure; others, however, were less distinctive. The fallacies of a negative examination are obvious.

First, our inability to find the bacilli on the coverslip does not necessarily prove their absence from the culture in which they may not be very numerous.

Secondly, insemination of the culture media may have failed.

Thirdly, the bacilli may not have been transferred from the throat to the swab. In purely laryngeal cases it is quite possible that the bacilli may be present in the throat in very small numbers, or be absent. In pharyngeal cases—and especially in those cases where other bacteria are present in large numbers—only a very few Klebs-Loeffler bacilli may be transferred to the swab.

Fourthly, though the bacilli may be transferred to the swab, they may be incapable of growth, as when a swab is taken directly after the throat has been treated with an antiseptic.

Lastly, there is the general fallacy attaching to a single negative result. For this reason it cannot be too strongly urged that where there are definite clinical symptoms pointing to a diagnosis of diphtheria the value of a single negative bacteriological examination is practically *nil*; it is not until several negative results have been obtained that one is justified in believing that the disease is not diphtheria. The importance of this can hardly be too strongly insisted on.

Concurrently with the examination of this series, two of us have been examining a second series consisting of about 40 cases, in which we have examined preparations from the cultures after staining by methylene blue as well as by Neisser's method. On two occasions Neisser's stain enabled us to arrive at a positive diagnosis with ease when there was some difficulty with the methylene-blue specimens; but in both cases we were satisfied, after careful examination, that the Klebs-Loeffler bacillus was present in the methylene-blue preparations. One of the two cases was undoubtedly diphtheria clinically. In no case did we find that Neisser's stain showed bacilli having the staining reaction of the Klebs-Loeffler bacillus, when at the same time the methylene-blue specimens failed to shew Klebs-Loeffler bacilli.

One of the most satisfactory features about our series is that out of the 48 cases in which the bacillus of diphtheria was found in the culture we were able to diagnose 18 by the microscopical examination of the coverslips made directly from the swab.

We feel convinced that if coverslip preparations were made directly from the throat without the loss of any time, this number of positive results would be increased. On many occasions it is highly desirable to make a diagnosis at as early a moment as possible, and our observations show that a positive diagnosis was possible from the examination of the swab in 18 per cent. of all the cases, and in 37.5 per cent. of the cases of proved diphtheria. If more care were expended in the taking of the swab, so that a really good coverslip preparation could be made from it, we think that a higher percentage of cases would yield an indisputable result. Of course, a negative result would be of little or no value, and it is always well to confirm the result, even if positive, by subsequent examination of the culture.

As a result of our observations, we feel that Neisser's stain is a valuable one in several ways:—

First, a positive diagnosis is rendered more certain for those who cannot be considered as experts, since it is often easier to diagnose the Klebs-Loeffler bacillus after staining by Neisser than after staining by ordinary methylene blue, as we think that for the inexperienced the bacilli after staining by Neisser's method, are much more characteristic than after staining by methylene blue.

Secondly, a trustworthy positive result may be obtained from the microscopical examination of a preparation made

direct from the swab, and this method is, we think, not only more reliable when Neisser's stain is used than when the ordinary methylene blue is employed alone, but is more obvious.

Thirdly, the use of Neisser's stain does not appear to introduce any fallacy not found in the application of other methods.

TWO SUCCESSFUL CASES OF OPERATION FOR PERFORATED GASTRIC ULCER.

By E. COLLINGWOOD ANDREWS, M.A., M.D., B.Sc.,
Surgeon to the Hampstead Hospital.

It appears to me that it is still desirable to publish the details of successful operations for gastric perforation, even if the only purpose served is to impress once again the necessity for prompt diagnosis and early treatment.

I cannot doubt that both the patients whose cases are here recorded largely owe their lives to the immediate and accurate judgment of the medical men who first saw them. In the search for the perforation, minute flakes of recent lymph are most suggestive indications as to the locality of the lesion, and should be carefully looked for. In both cases a considerable quantity of food was present in the stomach, and in neither was it possible to empty the organ through the pyloric opening. The constant welling-up of semi-digested food through the perforation was a considerable impediment to the introduction of the first few sutures. Another difficulty was the fixation of the stomach by old and firm adhesions.

It will be observed that in the first case irrigation with saline solution was employed. It is my practice, in dealing with the perforation of a viscus, to irrigate if the food is widely diffused, but to content myself with careful sponging if the escape is confined to a limited area. I must also express a decided preference for Halsted's suture, at any rate for the second layer of stitches.

For the notes of the first case I am indebted to Mr. Geoffrey Hobbs, the resident medical officer at the Hampstead Hospital.

CASE I.—C. D., aged 23, a housemaid, had suffered from "indigestion" for some months, but had never vomited blood. For a fortnight the indigestion had been worse, and on the day of admission (February 7th) to the Hampstead Hospital, after a substantial lunch of cold mutton and suet pudding, the patient was carrying up the tea-things, when she suddenly felt intense pain in the stomach, and fell down collapsed. Dr. William Boulting saw her soon after, diagnosed the condition as gastric perforation, and ordered her removal to the hospital. Dr. Andrews saw the case soon after admission, and found the abdomen motionless, board-like, and exquisitely tender, especially in the region of the stomach. There was marked diminution of the liver dullness, a feeble pulse of 120, and all the symptoms of general collapse. Immediate operation was considered necessary, and at 6 P.M. Dr. Andrews made an incision 4 inches long in the middle line between the costal angle and the umbilicus. On opening the peritoneum gas escaped, and the anterior surface of the stomach presented in the wound. To examine the surface it was necessary to separate extensive adhesions, and towards the cardiac end a collection of recent lymph and particles of semi-digested food were noticed. Continuing the search in this direction the perforated ulcer was found near the cardiac orifice on the lesser curvature, the aperture being rather smaller than a threepenny-piece. The peritoneum round the perforation was united by two layers of Lembert sutures, the area around carefully sponged, [and the whole of the peritoneal cavity well flushed with saline solution. A gauze drain was inserted into the upper portion of the wound, and a large drainage tube in the lower angle.]

February 8th. Patient has vomited since the operation, but has had some sleep. She is fed by rectal injections of peptonised milk and beef-tea every four hours. Pulse 120, temperature normal.

February 9th. Patient has been sick once at 10 A.M., but had a good night. Wound was dressed and gauze drain removed. There is only a slight serous discharge, and the abdomen is less distended. Complaints of pain under left shoulder blade. Temperature normal. Pulse 100.

February 10th. Has passed a good night. Discharge still serous. Abdomen less distended and moving with respiration. Drain tube removed. Pulse 100.

February 12th. Had a free action of the bowels after oil injection. Milk given by mouth. Pulse 90.

February 15th. Progress continued and stitches taken out. Patient commenced farinaceous food. Henceforward recovery was uneventful.

CASE II.—M. C., a gentleman aged 30, was seized with violent abdominal pain while riding on an omnibus, and on reaching home fell down in his dining room. The last meal of pork, potatoes, etc., had been taken some three hours earlier. Dr. McLelland was called to the patient, and diagnosed a perforated gastric ulcer. On seeing the patient some two hours later I found the pulse 124, and almost imperceptible, the face pale and sunken, and skin cold and clammy. There was a history of chronic dyspepsia, chiefly occurring before meals. The abdomen was rigid and distended, and no liver dullness could be elicited. Dr. Hale White and my father (Dr. James Andrews) concurring in the diagnosis, I decided to operate forthwith. A median incision was made in the epigastric region