hours, while vibrios were isolated from the untreated patients up to the seventh day (*see* table).

*Discussion.*—In view of the reported inhibitory effect of chloromycetin on *V. cholerœ*, the drug was tried on a series of ten cases of cholera. They were admitted with acute symptoms after an average duration of nine hours in more or less severely dehydrated condition with very low or almost inestimable blood pressure and feeble or imperceptible pulse. The total dosage was about 48 capsules (0.25 gm. each) administered in three days. They received in addition the usual saline treatment like the control group which also consisted of ten cases of similar severity and duration. No difference in the course of the disease or results of the treatment was noticed between the two groups. That there were two deaths against one in the control series is of no statistical importance. Besides, one of them belonged to young age group that stands dehydration badly and had only 3 gm. of chloromycetin. But what was remarkable was the almost complete disappearance of the vibrios within 24 hours of starting the treatment with chloromycetin. This did not make any difference in the ultimate results, but then we must remember that no drug is likely to influence the disease when severe dehydration is present with all its complications (Chaudhuri, 1950). At this stage parenteral fluid is the only procedure that can avert its downward course and this will always be our mainstay in the treatment of cholera. But here we wish to note that chloromycetin by its quick action on the vibrios might be of considerable value as a prophylactic and also against the spread of cholera, though the optimum dose has yet to be determined. For the same reason it is also possible that given in the earliest stage, before dehydration sets in, it may be able to minimize the ill effects and control the course of the disease. It is suggested that trials be made along these lines in a larger series of cases. In our experience neither sulphaguanidine nor formo-cibazol has any marked effect on the excretion of vibrio in cholera patients.

Our thanks are due to Dr. A. K. Dutta Gupta, Superintendent, Campbell Medical College Hospital, for permitting us to carry out this clinical trial and to Messrs. Parke, Davis & Co., for a free supply of chloromycetin.

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## A RAPID METHOD OF IRON HÆMATOXYLIN STAIN FOR PROTOZOA IN TISSUE SECTIONS AND SMEARS

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THERE are several well-known methods of iron hæmatoxylin staining of protozoa in smears and tissue sections, *viz*, Heidenhain's, Mallory's, and Weigert's. In the first two of these methods, the mordant, an iron salt, and the stain hæmatoxylin, are applied separately; and in Weigert's method, the mordant and the stain are mixed up immediately before staining. Though excellent results can be obtained with all the above methods, Heidenhain's method evolved in 1891 is still regarded as the best for permanency and for allowing very satisfactory control over differentiation of the different structures of the cell. But it must be agreed that the method is very time-consuming; up to 48 hours or more may be required for the completion of mordanting and staining, though by the use of the mordant and the stain at a temperature higher than the room temperature, it is possible to shorten the time considerably.

The iron hæmatoxylin stain described in this paper was first introduced by Professor M. N. De at the Pathology Department, Medical College, Calcutta, many years ago. This is an excellent hæmatoxylin stain for general purposes and with slight modification in the process of staining and differentiation, satisfactory results have been obtained in staining certain protozoa in tissue sections and smears.

The hæmatoxylin solution used is ripened with bleaching powder (*cf.* Anderson, 1923) and exposure to sunlight, and the mordant, an acid iron alum solution, is mixed with the stain immediately before use. About ten minutes are required for staining of ordinary tissues. For general purposes, acid alcohol is used for differentiation and the de-staining is quite rapid. After differentiation, the sections are left in running water till a warm blue-black colour develops. The sections are then counter-stained with eosin, dehydrated, cleared and mounted in the usual manner.

It has been found that if the time allowed for the iron hæmatoxylin stain is prolonged and a saturated aqueous solution of picric acid is used for differentiation, the staining of protozoa in tissue sections and smears is more satisfactory and the different structures in the protozoan cell more easily differentiated. Full details of

this modified method of iron hæmatoxylin staining is given below :

*Preparation of the stain*

1. *Stock solution of hæmatoxylin.*—Dissolve 10 gm. of hæmatoxylin in 650 cc. of absolute alcohol. Add 350 cc. of distilled water. Then add 25 cc. of a 4 per cent aqueous solution of bleaching powder. Mix. Expose to sunlight for about a month. The fully ripened solution has a deep brown colour. The solution keeps for months.

2. *Acid iron alum solution.*—Dissolve iron alum 2 gm. in 98 cc. of distilled water and add 2 cc. of concentrated sulphuric acid. The solution is almost colourless or has a very light yellowish tinge.

To one volume of the acid iron alum solution add two volumes of hæmatoxylin solution. Mix. This iron hæmatoxylin stain is prepared immediately before use.

*Method of staining protozoa in tissue sections*

1. Fix in Zenker or suitable fixative.

2. Embed in paraffin and cut sections 4 to 5 microns thick.

3. Remove wax with xylol, xylol with alcohol and then transfer successively to rectified spirit 90 per cent and 70 per cent alcohol and water.

4. Remove mercury with Lugol's iodine, iodine with 0.5 per cent 'hypo' solution in water and wash in running tap water for 5 minutes.

5. Stain with iron hæmatoxylin stain prepared as described above for 15 to 20 minutes.

6. Wash in tap water till the section appears black in colour.

7. Differentiate with a saturated aqueous solution of picric acid. For tissue sections about 1 to 5 minutes or a little longer is required for a satisfactory degree of differentiation. The section is de-stained with picric acid for 1 to 2 minutes, washed in water, and then examined under the microscope using high power to see if the differentiation is satisfactory or not. If the protozoa are still overstained, it is again covered with picric acid and the whole process repeated till the differentiation is satisfactory. The section is then left in running tap water for about 5 minutes to wash out most of the picric acid stain. (70 per cent alcohol and rectified spirit may be used for removing the picric acid stain, but the section has to be brought down to water before the next stage.)

8. Counter-stain lightly with a weak watery solution of yellowish eosin, controlling the depth of staining with the microscope. Wash in water.

9. Dehydrate, clear and mount in balsam.

*Note.*—With a little experience it is possible to differentiate with acid alcohol (1 per cent sulphuric acid in rectified spirit), but the de-staining is rapid and somewhat difficult to control. Acid alcohol is dropped on to the section with the slide held in a slightly slanting position so that the acid alcohol rinses the section and runs down the slide. This is done once or twice and the section is washed in running tap water for a short time and examined under the microscope to see if the differentiation is complete. The process is repeated if necessary and the section is then left in running tap water for 10 minutes to 'blue' it.

Counter-staining with eosin is not essential. The slight yellow colour of picric acid that is left after washing the section serves as a counter-stain and the nuclear details show very distinctly.

With this method of iron hæmatoxylin stain, the cell nuclei appear dark blue-black to black, the nucleoli and the chromatin net-work or granules are well shown.

*Method of staining protozoa in smears*

1. Fix in Schaudinn's fixative for 20 minutes.

2. Rinse in 50 per cent alcohol and then transfer to 70 per cent alcohol to which enough alcoholic iodine has been added to give it a port wine colour. Soak for 20 minutes.

3. Transfer to 70 per cent alcohol, and then to rectified spirit; leave for an hour or longer.

4. Rinse successively in 90 per cent and 70 per cent alcohol and bring down to water.

5. Stain with iron hæmatoxylin solution for 20 to 30 minutes. Wash and soak in tap water till the smear appears dark black.

6. Differentiate with saturated aqueous solution of picric acid for 1 to 6 minutes, controlling the de-staining with the high-power microscope using 10 × eye-piece. When the differentiation is satisfactory, leave in gently running tap water for 5 to 10 minutes to wash out most of the picric acid stain.

7. Counter-staining is not generally necessary.

8. Dehydrate, clear and mount in balsam.

*Note.*—Plasmodia and hæmoflagellates are more readily stained with the Romanowsky stains. Even in tissue sections excellent results comparable with those obtained in smears may be obtained with Giemsa stain as described by Shortt and Cooper (1948).

For smears of leishmanial flagellates, fixation with osmic acid vapour and Schaudinn's fluid followed by the method of staining as described above gives good results. But the time required for differentiation is shorter, ½ to 2 minutes.

This method of iron hæmatoxylin staining, as modified from that introduced by Professor M. N. De, is rapid and allows a relatively easier

control over differentiation of the nuclear structures of protozoa and the stain is more or less permanent, lasting for several years at least. The photomicrographs (figures 1, 2 and 3, plate XLVI) show the results obtained in staining some protozoa in tissue sections and smears.

*Summary*

A rapid method of iron hæmatoxylin stain is described for staining protozoa in tissue sections and smears. The hæmatoxylin solution is matured by the addition of bleaching powder and exposure to sunlight. The mordant, an acid iron alum solution, is mixed with the stain immediately before use. For differentiation, saturated aqueous solution of picric acid is used.

We are thankful to Dr. M. J. Miller and to Dr. G. N. Sen for some of the specimens used for staining.

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EXPLANATION OF PLATES XLIV TO XLVI

Fig. 1.—Note the short left ulna with its styloid process made further prominent by an exostosis and the bent fingers (right middle and ring and left index fingers).

Fig. 2.—Note the protuberances at the upper end of left arm and near both knees.

Fig. 3.—Note the multiple exostosis at the upper ends of both humeri. The pedunculated one on the left side pointing towards the middle of the shaft.

Fig. 4.—Note the short right ulna and the exostosis at its lower end giving an appearance of cavitation in the bone because of overlapping of the cancellous exostosis in the metaphysis. Note also the exostosis at the bases of some of the phalanges.

Fig. 5.—Note small exostoses along the crest of the ileum on the side (better visible in the original skiagram) and lack of tubulation in the necks of the femora.

Fig. 6.—Note the large pedunculated exostosis arising from the posterior surface of femur and another one giving an appearance of cavitation of lower metaphysis. On the right side note the exostoses from the lower end of femur and the large cauliflower-type exostosis at the upper end of fibula from which a biopsy was taken.

Fig. 7.—Photomicrograph of biopsy specimen (low power × 130). Note the hyaline cartilage, the transitional zone and the cancellous bone with one of its septa enclosing fatty bone marrow cells.

Fig. 8.—Same as 7. Magnification × 430 (high power).

# A Mirror of Hospital Practice

## A CASE OF HYDRONEPHROTIC KIDNEY

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A HINDU female child, aged 9, was admitted to the hospital for enlargement of the abdomen.

*Duration.*—Since childhood.

*Past history.*—Three months after birth, a swelling of the size of an orange was noticed in the left lumbar region. A needle was put in with no result. Subsequent treatment with electrotherapy produced no change in its size.

*Progress.*—The swelling went on increasing gradually without giving rise to any symptoms pertaining to any system except heaviness in the abdomen.

*Examination :* (1) *Local inspection.*—Generalized enlargement of abdomen, more on the left flank (figures 1 and 2, plate XLVII).

(2) *Palpation.*—Elastic feel of the abdomen with no definite outline of the enlargement made out. Tenderness. Fluid thrill +.

(3) *Percussion.*—No shifting dullness. Dullness +.

(4) *Auscultation.*—No sounds heard.

(5) *P. V. examination.*—Not made.

(6) *Urine and blood examinations.*—Nothing particular.

*Operation*

Right paramedian incision was made extending from just below the costal margin to a point four inches below the umbilicus. After incising posterior rectus sheath, large tense swelling was visible. Peritoneum was difficult to find because it was pushed to the right so much by the tumour that incision directly opened up the extraperitoneal space and the incision exposed the lateral side of the descending colon.

On exploring the tumour, 5 cc. fluid, watery and odourless, was aspirated. On separating the tumour from the surrounding structures, mesenteric cyst was suspected but no connection with mesentery was found. The tumour was getting the blood supply from aorta at the site of renal arteries. Then the tumour burst all of a sudden and about 3 pints of fluid came out. On examination, both ovaries, liver, spleen and right kidney were normal but left kidney was not found at all. On further dissection downwards, tumour was found connected with a cord-like structure following the course of ureter

METAPHYSIAL ACLASIS : J. N. BERRY. (O. A.) PAGE 387
(For description *see* page 402)

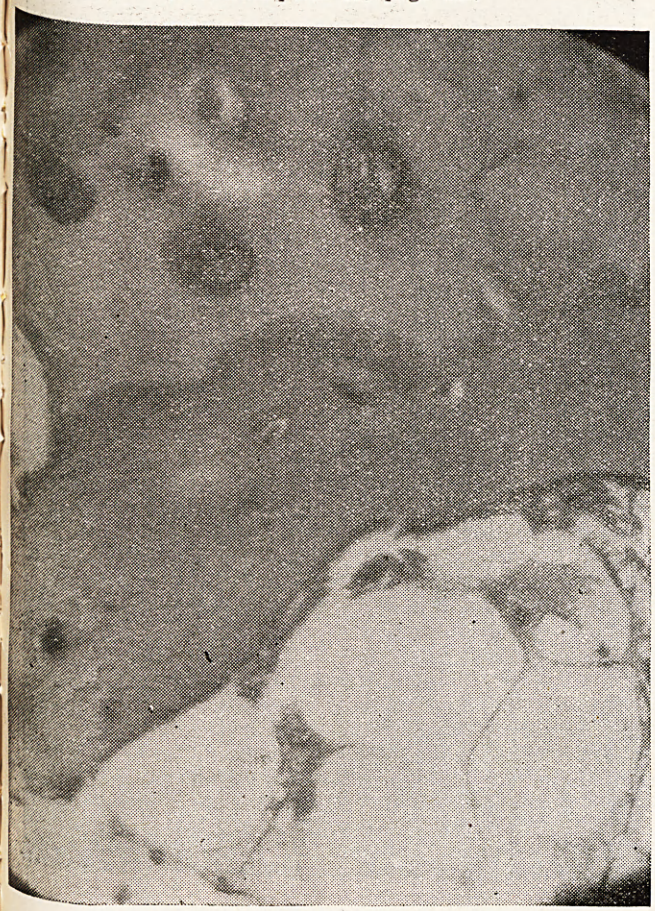

Fig. 8.

A RAPID METHOD OF IRON HÆMATOXYLIN STAIN FOR PROTOZOA IN TISSUE SECTIONS AND SMEARS : P. C. SEN GUPTA, K. C. BASU MALLICK AND B. BHATTACHARYA. (O. A.) PAGE 400

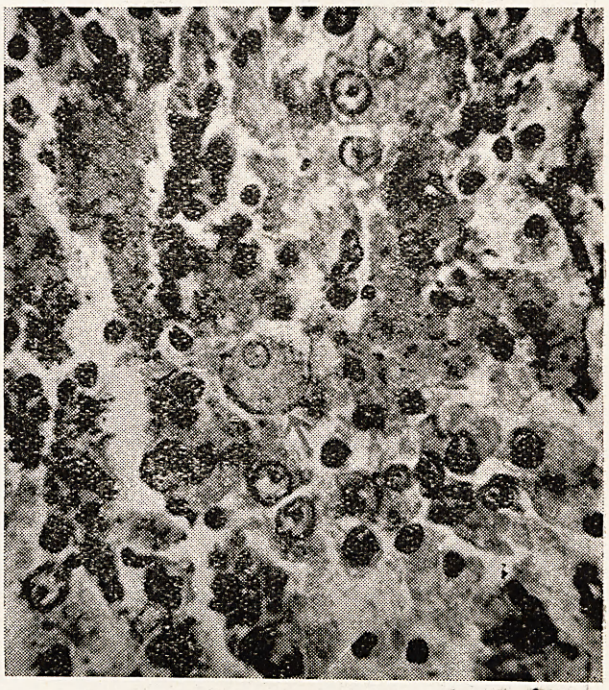

Fig. 1.—*E. histolytica* in liver abscess of a cat

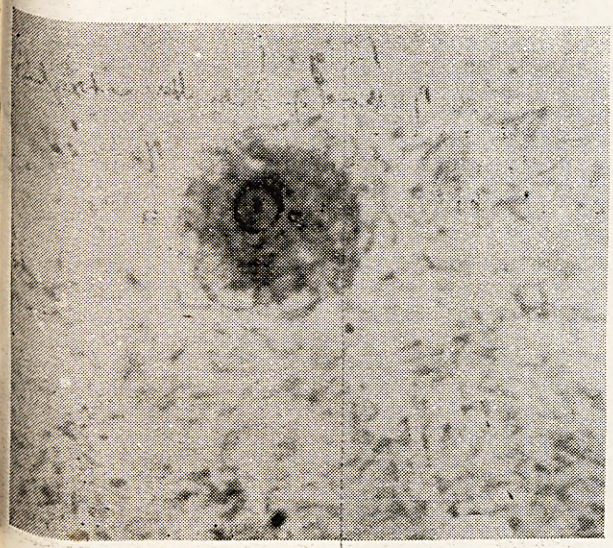

Fig. 2.—*E. histolytica* in fæcal smear.

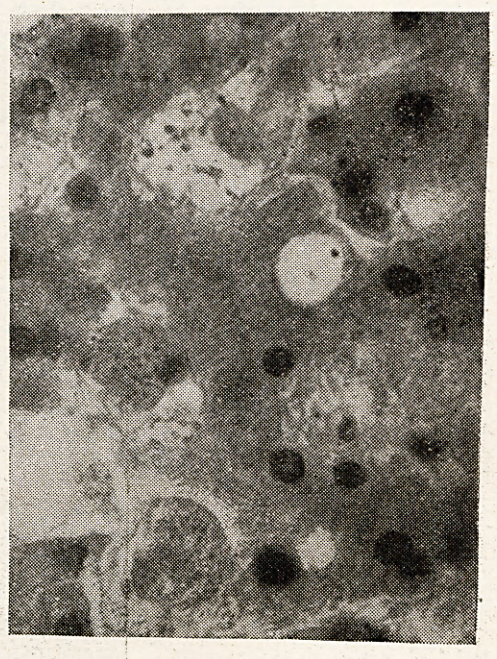

Fig. 3.—*Leishmania donovani* in a section of liver from a case of kala-azar.