

A NEW MICROSCOPICAL DIAGNOSTIC METHOD AND SOME SIMPLE METHODS FOR STAINING LIQUID BLOOD.¹

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IN a recent publication² we discussed certain strings of granules which can be found by means of appropriate staining in a percentage of the red corpuscles in health and disease. We showed that they are the remains of the nucleus of the original cell and called them *chromolinin granulations*. In health they occur roughly in about 1 per cent. of the corpuscles, but in a few pathological conditions which we studied they occurred much more frequently. Thus, in a case of tropical abscess of the liver they were found in 6·6 per cent. of the corpuscles, in a case of chronic malarial anæmia in 11·5 per cent., while in ankylostomiasis, kala-azar, and rats with trypanosomes and spirochætæ they were detected very frequently. We inferred that an excessive percentage of corpuscles with the granulation was indicative of the discharge of the red corpuscles into the blood stream at an earlier stage in their development than usual, due probably to processes of blood destruction and regeneration which, so far as we know, cannot be estimated by other methods. We now write to call the attention of clinicians more pointedly to the phenomenon in the hope that a study of it in many diseases will prove useful for diagnosis and treatment.

Probably the simplest method for demonstrating the granulation is the glass-rod method of liquid staining described by one of us hereafter. A polychrome saturated solution of methylene blue in 0·5 per cent. salt solution should be used. In about a quarter or half of an hour many of the red corpuscles are found to contain minute blue points, often lying in strings, either few in number or so numerous as to fill a large part of the corpuscle. The granules look like small particles of stain adhering to the surface of the corpuscle; but, of course, are not really of this nature. True nucleated red corpuscles can be easily distinguished by the fact that the nuclear membrane is still intact.

To estimate the percentage of granular red corpuscles as quickly as possible the following procedure is the best. The whole area of the field should not be examined, but the corpuscles should be passed rapidly in review one by one across the centre of the field and be counted as they pass, and as each granular corpuscle comes up an assistant should score a mark on a piece of paper. In this way nearly a hundred corpuscles may be scrutinised by the minute. In order to avoid serious error of "random sampling" at least a thousand corpuscles should be examined; but if the percentage of granular corpuscles is over 5 of this first thousand corpuscles we should examine another thousand, or if it is over 10, 15, &c., we should examine another two or three thousand, &c.

Many of the granular corpuscles, when stained with polychrome methylene blue, will often be found to contain after half an hour, in addition to the blue granules, one or more large deep crimson dots. These are the centrosomes of the original cell, as shown in our paper already referred to. It is possible that an estimate of the percentage of granular corpuscles which contain them will also prove to be of assistance in diagnosis. Nissle has independently just called attention to what appear to be the same bodies but does not give drawings.³

Some Simple Methods for Staining Liquid Blood, by Professor RONALD ROSS.—The staining of dried films is now being abandoned more and more for cytological work, and it may therefore be worth while to describe briefly some methods

for making sufficiently thin stained films of liquid blood. Several such are given in text-books of hæmatology. One of the oldest, which was suggested by Soulié in 1890,⁴ but was probably known long previously and has certainly been often re-discovered since, consists in spreading and drying a film of alcoholic solution of a stain on a glass slide and then placing over it the cover-glass charged with a droplet of blood. The elements soon take up the colour and beautiful preparations can be made, though there is often much granular matter and the blood does not always spread well.

1. *Glass-rod method*.—The plan of mixing the droplet of blood with aqueous solutions of stain in various ways has, of course, been largely used. The difficulty lies in getting a sufficiently thin film, because as a rule there is too much fluid in the mixture to allow of a single layer of corpuscles being obtained. After trying many methods I find the following one to be the simplest and best. A large drop of blood is taken up on a glass slide, and a drop of stain, not larger than the drop of blood, is quickly placed close beside it with the end of a glass rod. Then, with the other end of the rod the two drops are thoroughly mixed together, and small quantities of the mixture (say, of the size of a pin's head) are transplanted on to several other slides (just previously wiped clean from dust), where each is covered with a coverslip gently let down upon it. The smaller the droplet of mixture transplanted the thinner the resulting film, and the advantage of the use of the glass rod in this manner is that we can transplant droplets as small as we please and consequently obtain films of any tenuity we may wish for. Specimens for a considerable class may thus be made from a single drop of blood. Aqueous solutions of many stains may be employed and will not generally dissolve the red corpuscles if the amount of solution used is not in excess of the amount of blood.

One of the most useful stains is an old polychrome filtered saturated aqueous solution of methylene blue in 0·5 per cent. salt solution. Large areas, with separate corpuscles in single layer, yet not too much crushed, are obtained, just as in the best dried films. The leucocytes and blood plates are stained almost at once, although the background is almost colourless. A little later the red corpuscles take a blue tinge, and the chromolinin granules and centrosomes of a certain percentage of them⁵ (which are not easily disclosed in dry stained films) begin to show up—the granules coloured blue and the centrosomes deep ruby red. The centrosomes of leucocytes are also coloured red; and it is important to note that the blood plates show similar minute red points, which, if they also are centrosomes, as is possible, will establish the long-contested cellular nature of these bodies. Trypanosomes can be well stained by several reagents. I am beginning to use this method generally in place of the ordinary unstained wet films.

2. *Agar method*.—Ordinary nutrient agar is melted and mixed with filtered saturated solutions of various stains (e.g., polychrome methylene blue) and poured on sloped glass slides, so as to obtain very thin films of the stained agar on the glass. The agar sets on the glass almost at once but does not dry until some time later. While its surface is still moist a coverslip charged with a droplet of blood is dropped upon it. The blood spreads out beautifully, the red corpuscles being generally disposed in a single layer of perfect discs. In a few minutes the elements take up the stain by adsorption from the stained agar, centrosomes and chromolinin granulation becoming apparent later. The picture is similar to that of Method I. but perhaps a little inferior, as the staining is not so vivid and the background is not so clear. Deetjen, so far as he can be understood, seems to have used this method but only partially in his study of blood plates⁶; but on the whole I do not think it is as good, even for this purpose, as Method I. The agar should be as deeply stained as possible, so as to stain the cells quickly; but the film of it on the glass should be as thin as possible, so as to allow sufficient light through it.

3. *Drained drop method*.—Thin liquid films are usually obtained by pressure between two surfaces of glass, but the following method gives a very thin liquid layer on a single surface. A coverslip is charged with a fairly large droplet of blood slightly spread out upon it and is then inverted on a shallow cell made with vaseline on a glass slide. This gives

¹ The two short articles which follow are records of work done at the Johnston Laboratories, University of Liverpool.

² Ross, Moore, and Walker: Transactions of the Pathological Society of London, vol. lviii., part 1, 1907.

³ Nissle: Archiv für Hygiene, München, Band lxi.

⁴ Soulié: Bulletin Médicale de l'Algérie, 1890, p. 230.

⁵ Ross, Moore, and Walker: Transactions of the Pathological Society of London, vol. lviii., part 1, 1907.

⁶ Deetjen: Archiv für Pathologische Anatomie und Physiologie, Band clxiv., p. 239, 1901.

a usual hanging-drop preparation; but the coverslip is now pressed down evenly and gently on the yielding vaseline until the lowest surface of the hanging drop touches the upper surface of the glass slide below it. There is thus formed a column of blood touching both the slide and the coverslip. Round this column on the under surface of the coverslip a clear zone of extreme tenuity and about a milligramme in breadth will be found. So shallow is the blood here that leucocytes and clusters of blood plates cannot get past it, while the red corpuscles can only squeeze through gradually. The cells are therefore spread out in this zone as perfectly as in any ordinary liquid or dry film, but only by adhesion to the glass, while, as the cell is air-tight, drying does not occur for hours. Moreover, the zone acts as a kind of filter for the leucocytes, which will be seen in hundreds adhering living to the glass and still kept moist with serum. For staining, the blood may be mixed beforehand with a solution of some stain, or, perhaps better, with a few small particles of the undissolved stain. Exquisite pictures of all the elements can thus be obtained without any flattening by pressure.

Specimens made by these methods are, of course, not permanent, as the elements soon break up. But by mixing the solution of stain used for Method I. with glycerine or Farrant's preservative the preparations will last in fair condition (enough, for example, to show the chromolitin granulation of the red corpuscles) for some months. For good permanent preparations, however, more complex methods must apparently be used, such as that of Walker, described in the paper already referred to.⁷

A CASE OF CEREBRO-SPINAL MENINGITIS; ISOLATION OF THE SPECIFIC ORGANISM; PREPARATION OF A VACCINE; RECOVERY.

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THE patient, a male child, aged six months, was admitted to the City Hospital, Fazakerley, Liverpool, on Feb. 27th, 1907, certified to be suffering from cerebro-spinal fever. The diagnosis having been verified a vaccine treatment was decided upon, with results which seem sufficiently noteworthy to justify a report upon the clinical manifestations and course of treatment.

History of onset.—The child is stated to have been taken suddenly ill a fortnight before admission to hospital with vomiting and convulsions. Retraction of the head and neck was observed four days subsequently and squint within a week. Emaciation had been sufficiently marked to attract the attention of the mother within ten days of onset.

Condition on admission.—The general nutrition was fair, but loose folds of skin about the arms and thighs pointed to some recent wasting. The skin was pale and dry, presenting no rash. Beyond a few scattered rales no physical signs of disease were observed in the lungs; respiration was normal in character and frequency. The pulse was of fair tension, regular, and of normal frequency. Vomiting, a marked feature of the initial illness, had ceased; nourishment was taken readily and without difficulty. The general appearance and attitude of the child pointed to a profound disturbance of the nervous system. He lay on his side with his elbows and knees flexed, screamed shrilly at intervals, whilst his expression bore evidence of recurrent attacks of pain. The motor symptoms were definite and characteristic. The head was slightly extended on the spine and attempts to flex it were firmly resisted. The cervical and dorsal spines were fixed rigidly in orthotonos. The erector muscles of the spine were contracted and prominent with disappearance of the spinous processes between them. There was no trismus. The upper extremities were flexed at the elbows and the knees were drawn up upon the abdomen. These rigidities were tonic and not the result of an involuntary effort to resist movement.

⁷ Loc. cit.

No clonic spasms or convulsions were observed. Double internal strabismus was marked and constant. Deglutition was unaffected. The age of the child precluded an accurate record of sensory impressions. Hyperæsthesia of the joints and skin was very marked. A light touch on the skin of the abdomen was sufficient to produce a scream of pain. No areas of anæsthesia were observed. There was no intolerance of ordinary daylight but the electric light called forth an immediate expression of pain. Similarly, although ordinary sounds produced little effect, loud noises, such as a clapping of the hands, caused obvious distress. No marked changes in the superficial or deep reflexes were observed. Kernig's sign was present but Babinski's sign was absent. The knee-jerks were slightly exaggerated. From the time of admission to the commencement of the vaccine treatment on March 11th the condition of the child became progressively worse. The general nutrition failed rapidly until no subcutaneous fat could be detected on pinching the skin. Spasm of the muscles of deglutition was induced by each attempt to swallow and the extreme retraction of the head and neck appeared to offer a physical obstruction to the passage of food. The contraction of the erector muscles of the spine produced a complete tetanoid opisthotonos. The elbows, hips, knees, and ankles became rigidly flexed, and the eyes fixed and staring. The pupils remained equal and active throughout. No paralyses or areas of anæsthesia were observed. Variations in the quality and timing of the pulse occurred, but retardation was never a marked feature. From March 9th to 11th vomiting recurred repeatedly and rectal feeding was resorted to. During this period the presence of the diplococcus intracellularis meningitidis had been detected in the cerebro-spinal fluid repeatedly, but lumbar puncture produced no alleviation of the irritative phenomena.

The first vaccine inoculation was made on March 11th. With the exception of a remission in the temperature of the body no marked change was observed in the clinical manifestations during the succeeding 48 hours. On the 14th, however, a definite decrease in the degree of extension of the head and neck was noted, the spine could be straightened with the exercise of moderate force, and lateral movements of the head were made voluntarily. On the evening of the 14th an exacerbation of the pyrexia occurred, not accompanied by an aggravation of the irritative phenomena. A second inoculation was made on the 16th followed by a period of apyrexia lasting 24 hours. On the 17th the eyeballs were observed to be fixed less rigidly, some lateral movements occurred voluntarily. The improved position of the head and neck at this period made feeding by the mouth again possible. Vomiting ceased entirely. Hyperæsthesia and Kernig's sign persisted. During the period from the 17th to the 21st the temperature was raised and irregular; a third inoculation was made on the latter date, followed, as were the previous inoculations, by a period of apyrexia persisting on this occasion six days. The squint was now occasional only, the position of the head and neck approaching the normal, and the flexion of the joints of the extremities was easily overcome. A moderate degree of pyrexia from the 27th to the 29th was met by a further dose of vaccine on the latter day. The temperature subsequently remained normal until the end of treatment. Kernig's sign was first noted as absent on April 2nd. From this date to the conclusion of treatment a rapid and progressive improvement in the condition of the child was maintained. The irritative phenomena and hyperæsthesia disappeared. The appetite and state of nutrition became normal. No affection of the special senses persists.

Our investigations into the efficacy of vaccine treatment in this disease have been restricted by the freedom of the city of Liverpool from cerebro-spinal fever in epidemic form, but there are, we think, some encouraging features in the history of this case and its treatment. The patient came under treatment at a time when his condition justified a most unfavourable prognosis. The extreme and progressive retraction of the head and neck, accompanied by other advanced nervous phenomena, the return of the vomiting (a most grave symptom), and the rapid emaciation pointed to the probability of a fatal termination. The apyrexia following the first injection of vaccine recurred after subsequent injections. So rapid and complete a recovery with no definite relapses or involvement of the special senses could, we think, scarcely have been expected with the customary methods of treatment at our disposal.