A NOTE ON THE STAINING REACTIONS OF LEUCOCYTES.

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IN view of the increased attention which is now being given to the differentiation of leucocytes in connection with the subject of phagocytosis, it may not be inappropriate to direct attention to a few points in connection with the somewhat intricate staining reactions of these cells. Of fundamental importance in this respect is Ehrlich's classification of the aniline dyes into "acid" and "basic" dyes, according as the actual colouring principle occupies the position of acid or base in the "salt," which constitutes the aniline dye of commerce. Further, we may adopt from Ehrlich the terms "oxyphile" and "basophile," to denote the elements of the cell which respectively attract acid or basic dyes. Elements with each of these opposite chemical affinities are found in leucocytes.¹ Thus the nucleus of the leucocyte, like the nucleus of every other cell with which we are familiar, is invariably basophile, while the granules which are found in the leucocytes of the normal circulating blood of mammalia are, as will be shown, invariably oxyphile. The complete histological differentiation of a leucocyte depends upon staining it in such a manner that all the oxyphile and basophile elements are brought out in relief.

The following method of staining was planned with the direct purpose of satisfying these theoretical requirements. It probably does not differ, except in matters of detail, from other methods of staining which are actually in use, but it may nevertheless present a certain interest as an attempt to disentangle the principles which underlie those methods.

Cover-glass specimens of blood are prepared in the ordinary way, and are fixed either by dry heat (Ehrlich's method) or by chemical reagents (osmic acid, corrosive sublimate, etc.).

STAINING OF OXYPHILOUS ELEMENTS.

For this purpose the cover glass is floated from a few seconds to a full minute on a watery (say 1 per cent.) solution of an acid aniline dye (we have invariably employed eosin). If no staining has taken place within the minute it is advisable to interfere to hasten the process. We can readily accomplish this by introducing a trace of acid into our staining fluid, to compete with the acid staining principle for the base with which the latter is associated in the dye. By this means we favour the transfer of the acid staining-principle to the basic (oxyphile) elements of the cell. The addition of the minutest trace of acetic acid to the eosin solution results in the formation of what we may call a superacid dye and in the immediate staining of the specimen. In any case the specimen of blood usually becomes overstained, and requires to be decolorised in order to bring about an isolated staining of its oxyphilous elements. Such a decolorisation is instantly obtained by dipping the cover glass into a very dilute solution of sodium carbonate. It is well to cease decolorising before the stain has been entirely removed from the red blood corpuscles. At this stage the oxyphilous granules still retain the eosin stain, and stand out clearly in the unstained protoplasm of the leucocytes.

STAINING OF THE BASOPHILOUS ELEMENTS.

The basophilous elements in a specimen can be stained with any basic aniline dye, or with carmine or hæmatoxylin after they have been converted into basic dyes by combination with alum. We have employed Loeffler's² methylene

blue in most of our experiments because its action is extraordinarily rapid, and we were thus able to minimise the risk of dissolving out any of the elements of the cell.

This dye acts on the specimen of blood as follows: First, it stains all the basophile elements—nuclei—basophile granules, and bacteria when present; next, it proceeds to overstain—staining cell protoplasm and red blood corpuscles; and lastly, it dissolves out acid dyes from their combination with oxyphilous elements, or dissolves out the oxyphile granules themselves. For our purposes the action of the dye must be stopped as soon as it has sufficiently stained the basophilous elements. This is a matter of seconds or fractions of a second when the specimen has been prepared for the reception of the basic dye by the process of extracting the excess of eosin in weak alkali. By attention to the above details and by controlling the various stages of the process by examination of the specimen under the microscope, a very beautiful differential staining of the basophile and oxyphile elements of the leucocyte can be obtained.

RE-EXAMINATION OF EHRLICH'S CLASSIFICATION OF GRANULES UPON SPECIMENS PREPARED AS ABOVE.

Ehrlich, to whom almost all our knowledge of this subject is due, describes three main classes of granules in the protoplasm of leucocytes: (a) Large oxyphilous—generally called "eosinophilous"—granules, found in the leucocytes of the bone marrow and of the circulating blood; (b) minute neutrophilous granules, found in the ordinary polynuclear leucocytes of the circulating blood; (c) large basophilous granules, not normally present in the circulating blood of mammals. The first and the last of these classes of granules present no difficulties. The granules are large and are readily demonstrated by any method of staining which brings out oxyphile and basophile elements. We desire, however, to draw attention to the fact that the minute granules described by Ehrlich as neutrophilous are in reality not neutrophilous but oxyphilous, for we find that these granules are brought out with great distinctness when specimens of blood are stained as above in watery eosin. The description of these granules as neutrophilous appears to us to have been due to defects in Ehrlich's methods of staining. Thus the fact that these granules are generally not brought out on staining with glycerine-eosin, while the coarser granules are stained by this reagent, appears to us to depend not on any difference of staining reactions, but rather on a solution of the finer granules in the glycerine. The fact that these fine granules are stained by Ehrlich-Biondi's triple stain—a "neutral" aniline dye dissolved in excess of an acid dye—is probably due to the fact that they are well preserved in an acid medium. There is nothing to show that the fine granulations take up the neutral and not the acid stain from the Ehrlich-Biondi triple stain.

It appears to us that the classification of a granulation as neutrophilous or oxyphilous has a certain importance in view of inferences that may be drawn from a granulation as to the derivation of one form of leucocyte from another. A knowledge of the fact that all the granules found in normal circulating leucocytes are oxyphilous may also, it appears to us, serve as a corrective to premature inferences as to the non-phagocytic nature of eosinophilous cells. In view of the fact that the gonococcus can, as in the specimens before us, be found in large numbers in the interior of polynuclear cells containing abundant fine eosinophilous granules, the non-phagocytic nature of eosinophilous cells evidently does not deserve to be accepted as an article of faith.

is very close. The addition of a competing base—in photography the "accelerator"—leads to more rapid staining, while the addition of acid—in photography the "restrainer"—delays the staining process or decolorizes. In the case of acid dyes the processes are, of course, exactly reversed.

A REUTER'S telegram from Rome states that the two representatives of the Italian Government at the coming International Sanitary Conference at Dresden will be the Marquis Curtopassi, Italian Minister at Bucharest; and Dr. Pagliani, Director of the Italian Board of Health.

A SOCIETY for the Study of Children's Diseases has recently been founded at Amsterdam. The new Society owes its existence chiefly to the efforts of Dr. Voûte. The meetings will be held twice a year.

¹ It may be noted that the nuclei of the cancer-parasites figured by Ruffer and Walker (*Jour. of Path.*, Vol. 1, No. 2, Plates xiv-xvi), take on an acid instead of a basic dye. The absence of a basophile nucleus is also incidentally noted by Laveran in the case of the parasitic protozoa of malaria.

² As in the case of the acid dye the transfer of the stain to the tissue was made more rapid by the addition of a competing acid (formation of a superacid dye), so here the action of a basic stain is made more rapid by the addition of a competing base (formation of a superbasic dye). In the case of a basic dye the analogy with a photographic developing solution