OBSERVATIONS ON LEUCOCYTHÆMIA.

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(PLATE IX.)

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I INTEND, in the present paper, chiefly to give an account of the histological changes in the blood in leucocythæmia, and to discuss shortly some of the opinions held with regard to their interpretation. In English literature comparatively meagre accounts of the disease have been given during recent years, and our information with regard to it has remained nearly the same for a considerable length of time. This is due mainly to the fact that with the former means of examination the limit of knowledge was practically attained, but also, in part, to the circumstance that as cases of the disease are far from frequent any one observer has not had sufficient opportunity for carefully observing and comparing the facts in different cases. By the employment of greatly improved methods, however, chiefly on the part of continental observers, some new observations of importance have been made, sufficient to induce the hope that ere long the nature of the disease will be definitely ascertained, though its cause may remain unknown for some time longer. The facts here brought forward will be partly gathered from papers by other workers, though I shall not attempt to give a historical *résumé* of the whole subject, and partly the results of my own observations. It is evident that the true explanation of the changes found in leucocythæmia will depend to a great extent on the theory which is ultimately proved to be the correct one regarding the origin of the white and red blood-corpuscles and their relation to one another (if any). I have, however, avoided entering at length into a discussion of this subject, and have merely indicated the points which seem best supported by evidence.

In addition to examining the blood in its fresh unaltered condition, and in various preserving fluids, I have also employed methods of making permanent preparations. Ehrlich's method of making films of blood on cover-glasses, which are dried and afterwards fixed by heating for some time at a temperature of about 120° C., or by passing several

times quickly through a Bunsen flame, is of great service on account of its rapidity and the ease with which staining reagents can afterwards be used. I have also devised a method¹ of fixing the films before any drying occurs, by dropping the cover-glasses with the films downwards into a saturated solution of corrosive sublimate, the films on the cover-glasses being afterwards treated as ordinary sections, stained in various ways, etc.; and a method of dropping a small quantity of the fresh blood into Flemming's strong solution, which hardens it and fixes it at the same time, so that the drops can be embedded in paraffin and sections made. For subsequent staining I find safranin or Ehrlich's acid hæmatoxylin most suitable, along with the usual contrast stains. These two methods are of use especially in connection with the study of the structure of the nuclei of the cells and in relation to the question regarding their multiplication by division. We can treat the cells of the bone-marrow, etc., by exactly the same methods, and we are thus afforded a variety of ways of comparing the cells of the blood with those in the bone-marrow, and of judging of their identity.

To ensure clearness I may here repeat the chief characters of the leucocytes in normal blood. Some of these are uninucleated, others are multinucleated, or, to speak more correctly, have a multipartite nucleus, whilst there are also a few intermediate forms between these two classes. The smallest uninucleated leucocytes (about 7 μ in diameter) are sometimes called "lymphocytes" to distinguish them from the larger forms, which are simply spoken of as "uninucleated leucocytes." The multinucleated leucocytes are the most numerous variety, and I have found that their number is approximately twice that of the uninucleated corpuscles. More precise proportions are given by Hayem, Einhorn,² and Müller and Rieder, but their results coincide pretty nearly with what I have stated. Some of the leucocytes of normal blood contain coarse granules, as was first observed by Schultze,³ and these were afterwards discovered by Ehrlich⁴ to stain deeply with eosin. These cells, which are accordingly called "eosinophile" leucocytes, number about 2 to 4 per cent. of the leucocytes in normal blood. In normal blood it is rare to find even the largest leucocytes exceeding 10 μ in diameter, *i.e.* when measured in the unaltered spherical condition.

It will also be convenient to mention the chief facts described by Ehrlich and his pupils regarding the various "granules" found in leucocytes and other cells. Ehrlich found these granules to be of five varieties, which he designated α -, β -, γ -, δ -, ϵ -granules. The α -granules, oxyphile or eosinophile, already mentioned, also stain deeply with watery

¹ *Journ. of Anat. and Phys.*, 1891, p. 258, and 1892, p. 393.

² Einhorn, "Ueber das Verhalten der Lymphocyten zu den weissen Blutkörperchen." Inaug. Dis., Berlin, 1884.

³ Schultze, *Archiv f. mikr. Anat.*, Band i. p. 1.

⁴ Ehrlich, "Ueber die specifischen Granulationen des Blutes," *Archiv f. Anat. u. Phys.*, 1879; *Phys. Abtheil.*, pp. 166, 571.

solutions of the various acid aniline stains—aurantia, tropæolin, etc. The β -granules, according to Ehrlich's¹ original description, are closely allied to the above. He considered them to be a previous stage of the α -granules, and probably to differ from them chemically in containing more water. If a mixture of eosin and indulin in watery solution be used as a stain the α -granules are stained red, the β -granules black. The β -granules are called by Schwarze² and v. Limbeck³ "amphophile," and are said by them to stain both in acid and alkaline aniline colours. The γ - or "mast-cell granules"⁴ are coarse granules which stain with basic aniline stains, and are so called because they are similar to the granules found in the variety of connective tissue corpuscles known as mast-cells. Cells containing such granules are said by some to occur in the blood only in pathological conditions, but I have seen a few in perfectly normal blood. The δ - or basophile granules are fine granules closely related to the preceding, and stain readily with basic aniline dyes. The ϵ - or neutrophile granules are revealed by staining with a mixture of an acid and an alkaline stain, *e.g.* acid fuchsin and methyl-green. They are exceedingly minute, and occur in the ordinary multinucleated leucocytes of the blood. Ehrlich⁵ has found that the protoplasm of the leucocytes gradually acquires neutrophile granules as they pass from the uninucleated to the multinucleated condition. Of these five varieties the α -, γ -, and ϵ -granules are of most importance in relation to the subject of leucocythæmia.

During the last four years I have had opportunities of examining the blood in seven cases of leucocythæmia, in most of these on several occasions. It will be convenient for further reference if I simply name the chief facts in these cases, giving the average number of the corpuscles.

I am indebted to Professor Greenfield for allowing me to examine four of these cases and to make use of the notes:—

CASE 1. M. R., 35, F.—Red corpuscles about 3,000,000. Leucocytes about 160,000. Enormous enlargement of spleen, lymphatic glands not enlarged.

CASE 2. D. K., 23, M.—Red corpuscles about 3,750,000. Leucocytes about 750,000. Spleen greatly enlarged, lymphatic glands not enlarged.

CASE 3. C. M'L., 33, F.—Red corpuscles about 2,800,000. Leucocytes from 250,000 downwards (*vide infra*). Enormous enlargement of spleen, lymphatic glands not enlarged.

CASE 4. G. R., 10, M.—Red corpuscles (before death) 721,250. Leucocytes 283,000. Great enlargement of spleen, general enlargement of lymphatic glands, also general leucocytic infiltration of the tissues and great enlargement of kidneys.

¹ Ehrlich, "Ueber die specifischen Granulationen des Blutes," *Archiv f. Anat. u. Phys.*, 1879; *Phys. Abtheil.*, pp. 166, 571.

² Schwarze, "Ueber eosinophilen Zellen." Inaug. Dis., 1880.

³ v. Limbeck, "Grundriss einer klinischen Pathologie des Blutes," 1892, p. 132.

⁴ Westphal, "Ueber Mastzellen." Inaug. Dis., 1880.

⁵ Ehrlich, "Beiträge zur Physiologie u. Pathologie der verschiedenen Formen der Leucocyten," *Zeitsch. f. klin. Med.*, Band i., 1880, p. 553.

CASE 5. A. H., 22, F.—Red corpuscles (only one examination) 947,500. Leucocytes 687,500. Enormous enlargement of spleen, no enlargement of lymphatic glands, but tumour-like mass in mediastinum (*vide infra*).

CASE 6. A. G., 32, M.—Red corpuscles about 1,800,000. Leucocytes about 160,000. Moderate enlargement of spleen, no enlargement of lymphatic glands.

CASE 7. T. M'G., 3, M.—Red corpuscles 1,890,000. Leucocytes 196,000. Considerable enlargement of spleen, lymphatic glands just palpable.

So far as the condition of the blood is concerned these cases naturally fall into two classes. In the first class, which includes the first three cases, the blood showed a great variety of colourless cells, some of which are not present in normal blood; the spleen was enormously enlarged, whilst the lymphatic glands were unaffected. These cases are examples of the type of leucocythæmia, formerly called *splenic*, but now generally designated by recent German writers as *spleno-medullary*, and I shall endeavour to show that there is strong evidence that in such cases the bone-marrow is the primary seat of the disease. In the other four cases the characters of the leucocytes were widely different, these being nearly all of the small uninucleated variety. When this character of the blood is associated with enlargements of the lymphatic glands the term "*lymphatic*" leucocythæmia is often applied, but we may have the same changes in the blood without any enlargement of the lymphatic glands. I shall first describe in detail the characters in the blood of the first type, of which I find that my description agrees closely with that given by Ehrlich,¹ Spilling,² Müller,³ Wertheim,⁴ and others.

In this form of leucocythæmia the *eosinophile* cells are generally increased in number, a fact first observed by Ehrlich shortly after his description of the eosinophile granules. Ehrlich at first affirmed that these cells were always increased, but this is not the case, as in the "*lymphatic*" form they may not be increased in number, and this is, I believe, the general rule. In Cases 4–7 eosinophile cells were exceedingly rare, and Wertheim records a case in which the same condition was found. Still, in the spleno-medullary form the large number of cells in the blood bearing eosinophile granules is a striking feature. These cells, of whose staining reactions a minute account is given by Schwarze, are normally found in greatest number in the bone-marrow, but they also occur in small numbers in the spleen and in the blood, as already mentioned. Ehrlich, Schwarze, and others, believe that the bone-marrow is the special seat of their formation, and this is made practically certain by the recent observations of

¹ Ehrlich, *Deutsche med. Woch.*, 1883, p. 670; and "Ueber die Bedeutung der neutrophilen Körnung," *Charité-Annalen*, Band xii.

² Spilling, "Ueber Blutuntersuchungen bei Leukämie." *Inaug. Dis.*, Berlin, 1880.

³ Müller, "Zur Leukämie-Frage," *Deutsches Archiv f. klin. Med.*, Band xlviii., 1891, p. 47.

⁴ Wertheim, "Zur Frage des Blutbildung bei Leukämie," *Zeitsch. f. Heilkunde*, 1891, p. 281.

Müller,¹ who found these cells undergoing mitotic division in the bone-marrow but not in the blood or in the spleen. I have also seen mitotic figures in these cells in the marrow, two of which are shown in Fig. 1. In the bone-marrow of the rabbit, for example, these cells may readily be shown by making films by either of the methods described above, and afterwards staining with hæmatoxylin and eosin or with Ehrlich-Biondi fluid. By the latter stain the granules are coloured a deep reddish purple; when stained with eosin they appear of a crimson-red colour, more brilliant than that of the red corpuscles, which also take the stain deeply. The eosinophile cells in the marrow present a considerable variety in the character of their nuclei, but most have a single round or oval nucleus which may show an indentation at the side. In their general characters they resemble the ordinary marrow-cells which contain no eosinophile granules. They also vary much in size, some of them being very much larger than any of the leucocytes present in normal blood. Now, in this form of leucocythæmia, we find many cells in the blood which not only have eosinophile granules, but also resemble the eosinophile cells in the marrow as regards their size and the character of their nuclei. In some of the smallest the nuclei resemble those of the ordinary multinucleated leucocytes of the blood, but these are much rarer than those which contain a single round or oval nucleus (Fig. 1). Some of these cells reach 12 μ or 13 μ in diameter. By an extensive series of observations Müller and Rieder² show that the increase in *number* of the eosinophile cells in the blood is not peculiar to leucocythæmia (spleno-medullary), and that they are sometimes even more numerous in other conditions. They consider that a much more important point is that, in this disease, there are eosinophile cells in the blood much larger than those normally present, and in fact resembling those normally found in the bone-marrow. This I can confirm, and accordingly many of the eosinophile cells found in this form of leucocythæmia are to be looked upon as really abnormal elements. If we compare a film of leucocythæmic blood with one of bone-marrow, fixed and stained in the same way, the similarity in the characters of the eosinophile cells in the two specimens is very striking.

Another important feature is the presence in the blood of *large uninucleated corpuscles*, which contain no eosinophile granules. These corpuscles sometimes reach nearly 16 μ in diameter, and as they occur in large numbers they constitute a very striking feature in the microscopic field. The occurrence of these cells was noted by many of the earlier observers. They generally possess a single large nucleus, which is sometimes indented at one side, or may be even horse-shoe shaped,

¹ Müller, "Ueber Mitose an eosinophilen Zellen," *Archiv f. exper. Path. u. Pharmak.*, Band xxix., p. 221.

² Müller u. Rieder, "Ueber Vorkommen und klinische Bedeutung der eosinophilen Zellen," *Deutsches Archiv f. klin. Med.*, Band xlviii., p. 96.

but it never shows the complicated multipartite character seen in many of the ordinary leucocytes. In sections of blood fixed in Flemming's solution the nucleus is seen to be poor in chromatin, staining much less deeply than the nuclei of the multinucleated leucocytes; it has a distinct membrane, and shows in its interior little masses of chromatin, with a few fine threads, suggesting the presence of a very open intranuclear network. The surrounding protoplasm is well coloured with safranin if the specimen is not much decolorised, whilst the protoplasm of the multinucleated leucocytes is quite uncoloured. In dried film preparations the nuclei of these cells are more diffusely stained, but in some of them a nuclear network may be made out with fair distinctness, as Müller describes (*vide* Figs. 2 and 3). In Cases 2 and 3 I made careful examinations of the blood on the warm stage on several occasions, and always failed to detect any evidence of amœboid movement in these corpuscles, whereas the multinucleated leucocytes in their neighbourhood showed very active movements. These cells, therefore, differ in many particulars from the multinucleated leucocytes of the blood, and are not present in normal conditions. They are sometimes present in leucocythæmia in very large numbers; in one case I found that they formed more than 50 per cent. of all the colourless cells present in the blood. I was at an early period struck by the similarity of these corpuscles to the so-called "marrow-cells" which, in fact, they resemble in all the above-mentioned particulars, and after making many comparisons by various methods I believe that they are identical. A similar view has been taken by many others. Spilling¹ describes an extensive study of the staining reactions of the cells of the blood, with a great many aniline colours, in a case of leucocythæmia, and finds the distinctive feature to be the presence of large uninucleated corpuscles (evidently identical with the cells in question) whose protoplasm contains neutrophile granules, whereas the uninucleated leucocytes of normal blood contain no such granules. He also found that in this respect they resembled the cells of the bone-marrow, and concluded that in this disease the latter passed into the blood. Ehrlich¹ confirmed these observations, and came to the same conclusion, stating that in leucocythæmia alone do we find large uninucleated corpuscles containing neutrophile granules. Müller¹ also describes the cells, and assigns to them the same origin. He considers it more correct to call both them and the large eosinophile cells, which are not normally present in the blood, "marrow-cells," applying the term "blood-cells" to the colourless corpuscles of normal blood. These cells are also described by Gollasch² (who calls them "myelocytes"), v. Limbeck,³ and Hayem.⁴ The last-mentioned calls them "leucocytes hypertrophics," but does not speak with regard to

¹ Spilling, Ehrlich, and Müller, *loc. cit.*

² Gollasch, "Friedlaender-Eberth Mikroskop-Technik," p. 161.

³ v. Limbeck, *op. cit.*, p. 160.

⁴ Hayem, "Du Sang," p. 352.

their origin. They certainly constitute one of the most important features of the blood in this form of leucocythæmia.

The ordinary multinucleated leucocytes of the blood are also increased in this form of leucocythæmia, but possess all their usual characters; whilst the small uninucleated forms, or lymphocytes, show no increase. There may also be present in the blood many corpuscles with "mast-cell" granules. These are about the same size as the eosinophile granules, but are readily distinguished from them by their staining reactions. The origin and significance of these cells are not known.

Still another element abnormally present in the blood in this disease is the *nucleated red corpuscle*. As is now well known, though these cells are normally present only in the bone-marrow in the adult, they may occur in the blood of the adult in a variety of anæmic conditions, but this is the only disease in which they are found where there may be comparatively little anæmia present, and my experience has shown that they occur in larger numbers than in any anæmic condition. For example, in Case 2, in which the red corpuscles numbered nearly 4,000,000 per c.mm., they were so numerous that one or two could be seen in every field of the microscope. Many of them are of the "normoblast" type, *i.e.* they are about the size of an ordinary red corpuscle, and possess a single round nucleus, which may show a reticular structure, or may be almost homogeneous, especially if it is of small size. Some may be of larger size, and the nucleus is sometimes fragmented, or even of a rosette form, or it may be in the form of small round fragments (Fig. 2). The significance of the nucleated red corpuscles is discussed below.

In the cases which I have examined, the numbers of the corpuscles in the blood varied during the time of observation, but the abnormal elements were never absent. In one case a very striking fact was observed. In the course of four months the number of the leucocytes diminished from about 250,000 per c.mm. to a normal number, but the same abnormal elements—large uninucleated corpuscles, nucleated red corpuscles, etc.—were still present. The spleen also retained its great size, and the general condition of the patient was little improved.

I examined the blood of Cases 2 and 3 on the warm stage on various occasions, always with the same results. The large uninucleated corpuscles ("marrow-cells") were always found to be motionless, though various temperatures were employed. The smaller coarsely granular corpuscles showed amœboid movement, the larger showed slower and less extensive movements, whilst those of the largest size were motionless. The smaller finely granular corpuscles, which one could recognise as the typical multinucleated leucocytes, showed movements quite as active as in the normal condition. Hayem, Löwit, and Müller have obtained somewhat similar results, and the last mentioned finds that the large eosinophile cells resemble the similar cells of the normal marrow in being devoid of amœboid movement. I have not come across any evidence that

cells which normally possess amœboid movements lose them in leucocythæmia, as some writers have stated.

We come now to consider what is the significance of the cells described, when they occur in the blood in leucocythæmia. The presence of these three abnormal elements—large uninucleated corpuscles (with neutrophile granules), numerous eosinophile cells of various sizes and characters, and nucleated red corpuscles which are not accounted for by the degree of the anæmia—can, I believe, only be satisfactorily explained by a pathological condition of the bone-marrow. It is very difficult to conceive of any process either in the blood or in the spleen which could produce the changes which we find. The condition might well be explained as an extension of the bounds of the bone-marrow, so that its cells appear in the blood. Now, in the normal bone-marrow, a considerable number of marrow-cells are in process of indirect division, as Müller¹ describes. Cornil² has made a similar observation, and finds that the mitotic figures become more numerous when the marrow is mechanically irritated, and I have seen these cells in all stages of mitosis, (*vide* Fig. 4). The eosinophile marrow-cells also divide in the same way, as mentioned above, and produce the eosinophile cells of the blood. Most probably the marrow-cells produce, by their division, smaller uninucleated corpuscles, which enter the blood and become multinucleated leucocytes. An excessive proliferation of marrow-cells of both varieties, causing their appearance in the blood, and producing an excessive number of ordinary multinucleated and eosinophile leucocytes, might accordingly explain the characters of the colourless cells found in the blood in this form of leucocythæmia. The appearance of the nucleated red corpuscles in the blood might be explained either by an increased proliferation on their part, or by their being simply carried into the blood-stream owing to the disturbance of the normal cellular arrangements in the marrow by the proliferating marrow-cells. The latter is, I think, the more probable explanation of the large number present, though I have seen one or two undergoing indirect division in the blood. I do not intend at present to enter into a consideration of the structure of the marrow, and the relations of the various cells to the circulating blood, but I may mention that in the normal marrow the nucleated red corpuscles may be seen lying chiefly at the periphery of the vascular channels, and it is evident that any structural change in the marrow would readily cause them to enter the blood-stream in their immature condition. And, further, I think it probable that when the red corpuscles appear in the blood in the nucleated condition they do not readily lose their nucleus, as many of the nuclei show signs of fragmentation and splitting up into irregular forms—a condition which I have not seen in the nucleated red corpuscles of healthy marrow (though it is

¹ Müller, *loc. cit.*

² Cornil, "Sur la multiplication des cellules de la moelle des os," *Arch. de Phys.*, 1887, p. 47.

common in certain pathological conditions, *e.g.* in pernicious anæmia). It is also evident that if this proliferation of marrow-cells occurs, it may seriously interfere with the process of formation of red corpuscles, and the occurrence of anæmia may thus be explained. A somewhat similar explanation of the anæmia is suggested by Stephen Mackenzie in his Lettsomian Lectures for 1891.¹

The important question therefore arises, What evidence have we that an increased proliferation of colourless cells actually occurs in this disease? The presence in the blood of cells in process of indirect division has now been noted by a considerable number of observers, and there can now be no doubt of their occurrence. Flemming,² I believe, was the first to observe them. He found them, however, to be very few in number, there being only one in process of division in several thousands of colourless cells. He considered that the number of mitotic figures in the blood was insufficient to explain the progress of the disease, and concluded that the chief seat of proliferation was in the bone-marrow or spleen, or that the colourless corpuscles also divided by direct division. Some of the cells in process of division which he figures are of considerable size—larger than the ordinary multinucleated leucocytes of the blood. Their occurrence has since been observed by Spronck,³ Biondi,⁴ Müller,⁵ Wertheim,⁶ and others. Wertheim examined the blood in thirteen cases of spleno-medullary or medullary leucocythæmia, and found mitotic figures in all, generally in considerable numbers. Writing in the beginning of last year I said that I had failed to find them in the cases which I had examined. Since that time I have succeeded in finding them in one well-marked case of this variety of leucocythæmia (Case 2). The figures were in various stages, and were nearly all in cells of such a size as to leave no doubt that the cells in process of division were the large abnormal elements—"marrow-cells" (*vide* Fig. 3). A few occurred in cells about the size of an ordinary leucocyte, but these were much rarer. Müller⁷ distinguishes, in leucocythæmic blood, mitotic "marrow-cells" from mitotic "blood-cells," the former being of larger size and the figures poorer in chromatin. The latter point of difference I did not find well marked. The proportion of cells showing mitosis was relatively small, but I scarcely ever failed to find a few after careful seeking. They were seen both in sections of the drops of blood, and also in dried preparations, but in the latter the figures were less perfectly preserved. On one occasion the spleen was punctured, and in the blood

¹ *Vide* Abstract in *Brit. Med. Journ.*, 1891, p. 57.

² Flemming, *Archiv f. mik. Anat.*, Band xx., p. 57.

³ Spronck, *Ned. Tijdschr. v. Genesk.*, 1889, No. 20. Ref. *Fortschr. d. Med.*, 1889, p. 740.

⁴ Biondi, *Archivio per le Scienze Mediche*, 1889, p. 291. Ref. *Centralb. f. allgem. Path.*, 1890, p. 172.

⁵ Müller, "Zur Frage der Blutbildung," p. 68, from *Sitz. d. Kais. Akad. d. Wis. in Wien*, Band xvi., Abt. iii., June 1889.

⁶ Wertheim, *loc. cit.*

⁷ Müller, "Zur Leukämie-Frage," *loc. cit.*

from the puncture there appeared to be rather more mitotic figures than in the blood taken from the lobe of the ear. Observations, however, on normal animals have shown me that the blood taken in this way from the spleen does not differ much from the blood in the general circulation, and the presence of more cells in mitosis would probably indicate that the cells in the blood had a better opportunity for division in passing through the spleen pulp. In Case 3 I did not find any mitotic figures, though I searched carefully on several occasions, but it must be noted that during the time of observation the number of colourless cells in the blood was diminishing.

There still remains the further question, viz., Is the multiplication of the cells in the blood the essential feature of the disease, or do the mitotic figures seen in the blood simply represent an overflow, as it were, from the bone-marrow? The latter supposition is *a priori* the more probable, seeing that we must believe that the proliferation of cells began in the marrow, and might even be in progress for some time before it caused important changes in the blood. Wertheim records an interesting case of medullary leukæmia running an acute course, which has an important bearing on this question. During the life of the patient he found a considerable number of mitotic figures in the cells of the blood, but at the post-mortem examination, which took place shortly after death, an extraordinary number was found in the bone-marrow, and a smaller number in the spleen, though in that organ also they were more numerous than in the blood. In this case the enlargement of the spleen was not great, which fact may possibly be accounted for by the comparatively short duration of the disease. In another case also, in which there was great enlargement of the spleen, he found mitotic figures in great abundance in the bone-marrow—more numerous than in the blood or in the spleen. I have not yet had an opportunity of examining the bone-marrow in such a case immediately after death, as none of the above patients died when under observation. I may mention, however, that in sections of a leucocythæmic spleen, I have found a great number of eosinophile cells in the network of the pulp. The presence of these is readily explained on the theory that the disease had started in the marrow, and that these cells had been carried by the blood to the spleen, where they had accumulated, and probably undergone further proliferation.

With regard to the other four cases of leucocythæmia, a markedly different condition was found in the blood. The essential change may be described as an enormous increase in the number of the uninucleated leucocytes, such as are normally present in the blood. These cells have a very narrow rim of protoplasm round the nucleus, which is comparatively rich in chromatin, and stains deeply. There was little or no increase of multinucleated leucocytes; in fact, in some cases, these appeared to be absolutely, as well as relatively, diminished in number. Eosinophile cells were exceedingly scanty, many fields of the microscope

often being searched without a single example being seen. Nucleated red corpuscles were generally absent, though in one case (Case 5) a considerable number were seen; but in this instance the anæmia was intense, the number of the red corpuscles being 947,500 per c.mm. So far, I have not observed any nucleated red corpuscles in the blood in this variety of leucocythæmia, until the number of the red corpuscles has fallen below 1,500,000. The large uninucleated corpuscles (marrow-cells) were also generally absent, though in Case 4 some were present in the blood, especially towards the end of the disease. The statement of Müller and Rieder¹ that "marrow-cells" are always present in this form of leucocythæmia must also be regarded as incorrect. For example, I have numerous specimens of leucocythæmic blood taken from a patient shortly before death, in which all the leucocytes present, with the exception of a few multinucleated forms, were of the small uninucleated variety, not exceeding 8μ in diameter. These corpuscles are generally perfectly spherical, but in Case 6 many were seen to be of irregular form when the blood was examined in the fresh condition, and stained preparations—both dried films and sections of blood fixed in Flemming's solution—showed the nuclei also to be of irregular shape, conforming to the outline of the cell (*vide* Fig. 5). These corpuscles, when examined on the warm stage, showed no amœboid movements. I have seen no other description of a similar condition in the leucocytes. Cells with "mast-cell granules" are never numerous in this form of leucocythæmia, and generally appear to be altogether absent. The differences in the characters of the blood in the two types of leucocythæmia come out with great distinctness, if films of the blood are stained with the Ehrlich-Biondi stain of methyl-green, orange, and acid fuchsin. In the last described variety, nearly all the cells are small uninucleated corpuscles, which show a single nucleus stained a green colour, surrounded by a thin rim of protoplasm, which is practically uncoloured. In the former, a great variety of cells is seen—large uninucleated corpuscles with pale green nuclei, some with fine neutrophile granules in the protoplasm, which are stained a violet tint, others with coarse eosinophile granules stained a deep purple, numerous multinucleated leucocytes, whose protoplasm also gives the neutrophile reaction, and nucleated red corpuscles with bright green nuclei and yellow perinuclear substance. Wertheim² also describes a case of this form of leucocythæmia characterised by an increase of the uninucleated leucocytes alone, without any increase of eosinophiles, etc., and with no nucleated red corpuscles in the blood; and Obrastzow,³ describes two cases of acute leucocythæmia of this variety, in which, after repeated examinations, he failed to find any nucleated red corpuscles. Hayem⁴ also failed to find them in a

¹ Müller and Rieder, *loc. cit.*

² Wertheim, *loc. cit.*

³ Obrastzow, "Zwei Fälle von acuter Leukämie," *Deutsche med. Wochenschrift*, No. 50, 1890. Ref. *Centralb. f. allgem. Pathol.*, 1891.

⁴ Hayem, *op. cit.*, p. 857.

case of the same kind, and says that the other form of leucocythæmia (which, however, he calls "splenic") is the only disease in which, with comparatively little anæmia, nucleated red corpuscles in considerable number may be considered as practically constant elements of the blood. Detailed descriptions of this form of leucocythæmia are not nearly so numerous as of the other, as it appears to be the less common variety. Wertheim failed to find any mitotic figures in the blood in the above-mentioned case, and Obrastzow in his two cases only found one dyaster after a prolonged search. So far, I have not found any mitotic figures in the blood in cases of this disease, but in only one case did I make sections of the blood fixed in Flemming's solution, which method is much more suitable for showing them than the dry method, especially if the cells be of small size. That these uninucleated leucocytes also multiply by mitosis in the lymphatic glands, etc., has been shown by Flemming, Bizzozero, Gulland,¹ and many others, and it is difficult to understand why mitotic figures should not be as numerous in the blood in this form of leucocythæmia as in that first described.

In one of the above cases (Case 5) the mediastinum was occupied by a large tumour-like mass, which showed a dense fibrous stroma enclosing spaces which were filled with small uninucleated leucocytes. Whether this had formed in the remains of the thymus I cannot say. A similar condition is mentioned by Coats.² In another case, the fibrous tissue throughout the body showed an infiltration with the same cells, most marked in the liver, kidneys, and lungs. The lymphatic glands were also greatly enlarged, the enlargement being due to an accumulation of the same cells, with little or no increase of the supporting structures, whilst in the mediastinum there was a large firm mass whose structure was the same as in the first case. The enlargement of the spleen was found to be due to an enormous distension of the reticulum of the pulp, with the same small uninucleated corpuscles, whilst the Malpighian bodies were small and indistinct. In the bone-marrow it was found that the proper elements of the marrow—nucleated red corpuscles, marrow-cells, etc.—had to a large extent disappeared, their place being taken by the small uninucleated leucocytes. Only a few nucleated red corpuscles were seen, and those were of large size with fragmental nuclei. A practically similar condition of the spleen, bone-marrow, etc., along with an increase of the same cells in the blood, is described by Neumann, and he applies the term "lymphoid" to the change in the bone-marrow, in which he considered that the disease originated. It seemed to me, from an examination of the case just described, that the changes present could only be satisfactorily explained by a great multiplication of the uninucleated leucocytes in all the situations mentioned. Unfortunately the tissues were not prepared at the time (two years ago) with a view to showing mitotic figures, and though a

¹ Gulland, "Reports of the Roy. Coll. of Phys. Lab. Edin., 1891, p. 156.

² Coats, "Manual of Pathology," p. 60.

careful search of the sections which I have, shows appearances very suggestive of mitoses imperfectly preserved, I cannot speak with absolute certainty. Bizzozero,¹ however, has made important observations bearing on this point. In three cases of leucocythæmia, in which there was a general infiltration of the connective tissues with leucocytes, along with enlargement of the lymphatic glands and spleen, he found mitotic figures amongst the leucocytes in a great variety of situations. They were specially numerous in the lymphatic glands and in the spleen, but were also present in the infiltrations in the liver and the kidneys. He therefore concludes that such pathological changes are not due simply to an emigration of leucocytes from the vessels, and accumulation in the tissues, but to an actual proliferation of the leucocytes after they have reached the tissues. Mitotic figures were also observed in the lymphatic glands in Wertheim's case (though they were not so numerous as Bizzozero describes), and also in Obrastzow's two cases mentioned above.

With regard to the starting-point of the disease in this form of leucocythæmia much greater difficulties present themselves than in the form first described. We cannot judge of this from the character of the cells as those small uninucleated leucocytes are normally present in the blood, in the blood-glands, and in fact, practically all through the body; and accordingly our only guide, and that an imperfect one, is from the organ which first shows enlargement. While it is true that the lymphatic glands are very often enlarged, and may probably in some cases be the starting-point of the disease, their enlargement is by no means invariable. In two of the above cases there was no enlargement of the lymphatic glands; in one of these the enlargement of the spleen was not considerable; whilst in another the lymphatic glands were just palpable in some situations. An interesting case is recorded by Leube and Fleischer,² in which, along with a great increase of the uninucleated leucocytes in the blood, there was neither enlargement of the spleen nor of the lymphatic glands, whilst the bone-marrow showed a "lymphoid" condition. They concluded that the disease had either originated in the blood itself, or in the bone-marrow. On the other hand, the enlargement of the glands is not confined to this form of leucocythæmia, but may also occur in the other; Neumann,³ for example, records a case, evidently of this nature, in which certain groups of glands were enlarged. It would appear to be implied by the description of some writers that, when the spleen is enlarged it produces an excess of leucocytes of the larger variety, which pass into the blood. But there may be enormous enlargement of the

¹ Bizzozero, "Ueber die Natur der secundären leukämischen Bildungen," *Virchow's Archiv*, Band xcix., p. 378.

² Leube u. Fleischer, "Ein Beitrag zur Lehre von der Leukämie," *Virchow's Archiv*, Band lxxxiii., p. 124.

³ Neumann, "Ueber myelogene Leukämie," *Berlin. klin. Woch.*, 1878, p. 118.

spleen, when only the small uninucleated leucocytes are in excess in the blood, and the tissue of the spleen is then seen to be crowded with corpuscles of the same variety, as Neumann has pointed out. If, therefore, the term "lymphatic" is employed, it ought only to be as indicating the character of the cells increased in the blood, etc., and not as implying that the disease has necessarily started in the lymphatic glands, or even that these are affected. By some writers the term is used in this way, *e.g.* Ziegler describes the lymphatic form of leukaemia as one in which the "lymphocytes" are increased. Others have employed the term "lymphæmia" apparently in the same sense, though probably "lymphocythæmia" would be generally considered to be a more correct one. Cases might be conveniently described as lymphæmia or lymphocythæmia where there is enlargement of the lymphatic glands, of the spleen, etc., as the case may be. It is apparent that if a proliferation of these cells starts in any gland or organ, and the cells, capable of further proliferation, pass into the blood, any organ in the body may be secondarily affected. And if these cells infiltrate the tissues, and pass on to the lymphatic glands, enlargement of these will result, though, as already stated, in some cases the lymphatic glands may be the starting-point of the disease.

So far as my experience goes, this "lymphatic" form of leucocythæmia is attended with a more rapidly advancing anæmia, and leads more quickly to a fatal termination than the other form. In one case (Case 4) the number of red corpuscles fell nearly two millions per c.mm. in less than two months, and registered 721,250 shortly before the death of the patient. The condition of the bone-marrow would seem to explain this, as there was almost an absence of the nucleated red corpuscles and of the cells which are believed to be their antecedents, the whole tissue being filled with small uninucleated leucocytes. I may also repeat that no nucleated red corpuscles were found in the blood till a few days before death, when one or two were seen after a prolonged search. In Cases 1-3, on the other hand, the red corpuscles showed comparatively little diminution during the time of observation, which in one case extended over four months. Wertheim says that in such cases (spleno-medullary or medullary) the formation of red corpuscles takes place to as great an extent as in health, but that the corpuscles formed are of poorer quality, and do not last so long. Of this, however, I believe the evidence is quite insufficient.

The character of the cells seen in the blood is also a distinctive feature of the form of leucocythæmia first described, but in this case the origin of the cells, so far as my experience has shown, can be traced to the bone-marrow, which I believe is in such cases to be looked upon as the starting-point of the disease. Although many cases have been described in which there could be seen, on naked-eye examination, gross lesions of the marrow,—puriform aspect, softening of the bones, etc., as

described by Waldeyer,¹ Ponfick,² Mosler,³ and others,—yet the marrow might conceivably contain a large number of cells in course of active division, and still present only a somewhat paler aspect than normal. Accordingly, a careful microscopic examination would always have to be made with a view to observing the exact characters of the cells, and especially the presence of mitotic figures, before one would be justified in saying whether it were normal or not. An important question, as already hinted, is whether the various kinds of cells in excess in the blood can be explained by the proliferation of one kind of cell, viz. the marrow-cell. If the eosinophile cells in the marrow are merely a variety of the ordinary marrow-cell, and not a distinct element, and if the marrow-cells also produce multinucleated leucocytes, such an explanation is probably the true one. I am strongly inclined to believe that it is so, but at present one cannot speak with absolute certainty. Biondi considers that the various forms of colourless cells in the blood in leucocythæmia are not distinct entities but derivatives of the same class of cell. There is therefore strong evidence for believing that the changes found in both forms of leucocythæmia are due to the indefinite proliferation of a certain kind of cell, in a manner analogous to what we find in the growth of certain tumours, *e.g.* sarcomata. Whether this proliferation is due to some peculiar form of irritation, and if so, what the nature of the irritant is, are questions which as yet cannot be answered. The frequency of malaria as an antecedent appears a well-established fact, but, as this connection is altogether wanting in the majority of cases (according to Gowers,⁴ it can be traced in about a quarter of the cases), the malarial poison probably only acts as a predisposing cause by its effect on the blood and blood-forming tissues. So also any direct proof that the disease is of bacterial origin is still wanting.

Whether the blood in leucocythæmia ever shows characters other than those described as the two types, I cannot, of course, judge from my comparatively limited experience. In some of the figures given by the earlier writers, the leucocytes in excess appear to be those of the multinucleated type, though some small uninucleated corpuscles are also represented. But so far as I can judge from the descriptions of those who have carefully studied the characters of the cells by modern methods, and so far as my own observations go, the multinucleated leucocytes are never in excess in this disease without the presence of abnormal elements, large uninucleated corpuscles (about $12\ \mu$), etc. Accordingly, the presence of these large cells—"neutrophile uninucleated corpuscles," "marrow-cells"—is considered by Ehrlich, Müller, v. Limbeck, and others, to be the most important factor in the

¹ Waldeyer, "Diffuse Hyperplasie des Knochenmarkes, Leukämie," *Virchow's Archiv*, Band lii., p. 305.

² Ponfick, "Über die Sympathetischen Erkrankung des Knochenmarkes," *Virchow's Archiv*, Band lvi., p. 534.

³ Mosler, *Berlin. klin. Woch.*, 1876.

⁴ Gowers, "Splenic Leucocythæmia" in "Reynold's System of Medicine."

diagnosis of leucocythæmia. There is a general agreement amongst observers that leucocythæmia is the only disease in which they are present in the blood, but further observations on this point are necessary. In cases where there is leucocytosis, on the other hand,—in suppurative conditions, acute fevers, etc.,—I have found that there is simply an increase of the leucocytes normally present in the blood, which increase is almost exclusively on the part of the multinucleated forms, so that instead of being about twice as numerous as the uninucleated corpuscles, they may in some cases be even twelve times as numerous. In a case of empyema, for example, they numbered 93 per cent. of all the cells found in the blood. On this point my results agree with those of Ehrlich and Einhorn. It is accordingly quite incorrect and unscientific to separate leucocythæmia from leucocytosis by the mere number of the leucocytes, for, apart from the real nature of the two conditions, the difference, so far as the colourless cells in the blood are concerned, is one also of quality and not of quantity only.

I believe that the changes found in leucocythæmia cannot be explained on any other theory than that of an excessive and indefinite proliferation of a certain kind of cell, in its nature resembling that seen in the growth of many tumours. Practically all recent observers are agreed that the spleen of the adult mammal does not perform the function of transforming white corpuscles into red, and also, in fact, that the leucocytes found in normal blood do not become red corpuscles. Nor do I believe, from the result of numerous observations, that those cells of the bone-marrow, which are similar in structure to the large uninucleated corpuscles of leucocythæmic blood (Figs. 2 and 3), produce the nucleated antecedents of the red corpuscles. Regarding this, however, there is greater difference of opinion, and one cannot satisfactorily discuss the question without giving a minute account of the characters of the various cells found in the marrow, and their relationships to one another. Even, however, on the supposition that these marrow-cells do form nucleated red corpuscles, the essence of the disease would rather be an excessive proliferation of the former, the failure to become transformed being a result of the rapid division. The theory of Löwit¹ that the cause of the disease is a failure of the uninucleated leucocytes to become multinucleated, with a consequent accumulation in the blood, is open to still greater objections. The presence of mitotic figures in the blood, the characters of the cells present, and especially in some cases the great *actual* increase of the multinucleated forms, are quite unexplained by such a theory.

These observations are necessarily somewhat incomplete, and many important questions are left unanswered; but as it is more than a year since I have had an opportunity of examining a case of leucocythæmia, I have thought it better to publish the facts which I have observed, in

¹ Löwit, "Ueber Neubildung u. Zerfall weisser Blutkörperchen," *Sitzb. d. Kais. Akad. d. Wis. zu Wien*, Band xcii., Abtheil. 3, p. 22.

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

the hope that they may serve as an aid to further research on this subject.

EXPLANATION OF PLATE IX.

FIG. 1.—

- a.* Eosinophile cells from the blood of a case of "spleno-medullary" leucocythæmia (Case 2), showing variety in the size and character of the cells.

Dried preparation—Hæmatoxylin-eosin.

- b.* Two eosinophile cells from the bone-marrow of a rabbit, showing stages of mitotic division.

Film preparation—Corrosive sublimate, Hæmatoxylin-eosin.

FIG. 2.—Other cells from the blood of the same case.

- a.* Large uninucleated corpuscles—"marrow-cells."
b. Ordinary multinucleated leucocyte.
c. Various forms of nucleated red corpuscles.

Dried preparation—Hæmatoxylin-eosin.

FIG. 3.—Cells from sections of leucocythæmic blood (same case), *vide* text.

- a, b, c.* As in Fig. 2.
d. Cells in various stages of indirect division.

Flemming's Solution—Safranin.

FIG. 4. Cells from the bone-marrow of a rabbit.

- a.* Ordinary marrow-cells.
b. Ordinary marrow-cells, undergoing mitotic division.
c. Nucleated red corpuscle (the hæmoglobin is not shown).

Film preparation—Corrosive sublimate, Hæmatoxylin.

FIG. 5.—Cells from sections of leucocythæmic blood ("lymphatic" form).

- a.* Small uninucleated leucocytes.
b. Multinucleated leucocyte.

Flemming's Solution—Safranin.

Reichert homogen. immersion $\frac{1}{8}$ th. Zeiss Oc. 4. In Fig. 5 the magnification was slightly higher.