THE OXYDASE OF MYELOID TISSUE

and

THE USE OF THE OXYDASE REACTION IN THE DIFFERENTIATION OF ACUTE LEUKAEMIAS.

by

JOHN SHAW DUNN, M.A., M.B., Ch.B.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

PRELIMINARY.

The oxidising property of leucocytes was pointed out by Vitali (1887)[25], when he showed that pus added to tincture of guaiacum produced guaiac-blue, without the addition of hydrogen peroxide. He found that the reaction did not take place if the pus were previously boiled, thus showing that the oxygenating substance was thermo-labile. Brandenburg (1900)[1], in a further investigation of this subject, was able, by extracting pus with chloroform water and precipitating with alcohol, to obtain a powder which possessed the oxidising property in a marked degree. He considered it to be of the nature of a ferment, and to have the constitution of a nucleo-albumin. It could be obtained readily from organs which contained abundant granular leucocytes, such as bone marrow, but not from purely lymphocytic organs, such as lymphatic glands or thymus, nor from normal liver. Erich Meyer (1904)[14] pointed out that the blood of myelogenous leukaemia, even when much diluted, had the power of spontaneously converting guaiacum to guaiac-blue in virtue of its high leucocytic content. Similarly, blood with a leucocytosis of over 20,000 per cubic millimetre was found to react positively. Meyer predicted that this property, which seemed to belong only to the granular leucocytes, might come to be used as a criterion in the differential diagnosis of leukaemias.

The/

The foregoing researches were carried out only with leucocytes in bulk, but the adaptation by Winkler (1907)[26] of the "indophenol-synthesis" test rendered possible the microscopic study of the oxydase reaction in individual cells. Winkler observed that fixation in alcohol or formalin did not destroy the ferment. His investigations led him to believe that all forms of leucocytes possessed it in greater or in less degree. On the other hand, Schulze (1909)[21] has shown by means of the same test that lymphocytes never give a reaction, whereas polymorpho-nuclear leucocytes and their corresponding myelocytes always give it, and even the non-granular precursors of the myelocytes may show it to some extent. Schulze applied this test to sections of organs from a case of acute leukaemia in which the leucocytes were almost exclusively of the large mononuclear, non-granular type. He found that a considerable proportion of these gave the oxydase reaction, thus confirming the view he had previously formed, on histological grounds, that they were myeloblasts and that the case was one of acute myeloid leukaemia. These results were confirmed later by Peters (1909)[19] in two similar cases, so that Meyer's prediction as to the diagnostic value of the reaction has been verified.

The work of Winkler and Schulze opens up an interesting and/

and important field. It affords to histology a new means of studying the relationships of the various forms of leucocytes and of their distribution in the tissues under normal and pathological conditions. Further it renders evident in these cells a very pronounced chemical property which is no doubt related to certain of their functions and thus brings physiology into closer touch with anatomical facts.

The first part of present work deals with the general application of the oxydase reaction and with the facts obtainable by means of it, in the tissues, especially the blood and blood forming organs in normal adults and embryos and also under pathological conditions. Some experimental work is included referring to the nature and chemical behaviour of the substance which produces the reaction. Some observations on the application of the ozydase reaction in the differention of the acute leukaemias being necessarily accompanied by fairly extensive accounts of the anatomical changes in these conditions, have been dealt with separately in a second part.

PART 1.

THE OXYDASE OF MYELOID TISSUE.

THE "INDOPHENOL-SYNTHESIS" TEST for OXYDASE.

This test depends on the production by active oxygen of an insoluble dye from a combination of two soluble substances. The two reagents generally used are alpha-naphthol and dimethyl-para-phenylendiamin. These, when brought together in aqueous solution in presence of an alkali, become slowly oxidised even in air with the production of a blue dye, indophenol-blue, which, being insoluble in water, precipitates. The addition to the mixture of an oxidising agent greatly accelerates the dye formation, and the oxidising ferment of leucocytes brings it about with extraordinary rapidity. In applying the test, equal parts of 1 per cent aqueous solutions of the two reagents are mixed and the film or section to be tested is immersed in the mixture for one to five minutes or longer as may be required. If the preparation is then washed in water, and examined in water or glycerin, the granular leucocytes are seen stained a dark opaque blue, from precipitation of indophenol in their protoplasm, while the nuclei remain unstained. Red corpuscles lymphocytes, and tissue cells generally show no reaction.

In practice it is found most convenient to use a saturated aqueous solution of alpha-naphthol which

is/

is rather less than 1 per cent. The dimethyl-para-phenylendiamin obtained commercially is frequently partially oxydised and only partly soluble. It is also very deliquescent so that accurate wieghing is difficult. However, the solution need not be made of accurate concentration as a little more or less makes no appreciable difference in its efficiency. The solutions I use are generally about $\frac{1}{5}$ to $\frac{1}{2}$ per cent, and the results with different samples are in perfect accord. Freshly prepared solutions act more slowly than those a few days old, but after a fortnight or three weeks these solutions begin to be less efficient and should be rejected. The addition of 1 per cent Na O H to the appha-naphthol solution as suggested by Schultze (21) is unnecessary and in dealing with films is actually disadvantageous as it may cause the film to leave the cover-glass. The alkalinity which is necessary for the occurrence of the reaction is provided by the phenylendiamin which is a base.

As the indophenol is very rapidly soluble in alcohol and in xylol the preparations cannot be mounted in canada balsam. Specimens mounted in water or glycerin fade very rapidly, and the fading is not prevented by alkalinising the medium. An unfixed film of normal blood exposed to the reagents for two minutes shows deep blue staining of the leucocytes if examined at once, but/

but if it be kept mounted in glycerin for four hours practically all trace of the colour disappears. If the film is fixed by alcohol or osmic acid (1 per cent) previous to staining the colour may persist for twenty-four hours, and if a portion of a film is left unfixed previous to staining, it will be found that though the film seems to stain evenly there is a a relatively more rapid decolorisation of the leucocytes in the unfixed area. Accordingly the fading is not explained merely by slow solution in the medium, it is most probably due to destruction of the dye by continued action of the oxidising, or some other ferment. The effect of pus cells on commercial indophenol was tested by adding equal quantities of the dye to equal quantities of pus. boiled and unboiled. After incubation overnight the sediments were obtained by centrifugalising; the quantity of indophenol recovered from the tube containing the unboiled pus was much diminished; apparently some of it had been destroyed.

The fading of the colour in films does not occur so long as these are kept dry, but begins as soon as they are mounted. Preparations which have faded can be restained again and again, showing that the oxydase remains active.

Winkler (1908 [27]) recommended the use of a mixture of colophonium and benzene as a mounting medium, but specimens/

specimens mounted in this last only a few days. I found (1910) that by mounting in undiluted water-glass one could preserve stained films and sections unchanged for many weeks. This medium does not dissolve the indophenol, and appears to prevent the action of the ferment from destroying it. Later observations have shown that it is more convenient to dilute the commercial water-glass with an equal amount of water to render it less viscid and avoid tearing of sections. The preparations are equally lasting but it appears that even in water-glass some fading occurs after a month or two if the number of cells in the preparation which produce indophenol is small. In films or sections containing abundant myeloid tissue, e.g. from leukaemia, no appreciable alteration is observed after two years.

VARIATIONS of THE "INDOPHENOL-SYNTHESIS" TEST.

The combination of reagents used in this work is only one of a series, and it has been selected on account of the rapidity of its action, but other combinations give a similar result with varying degrees of rapidity.
Thus the dimethyl-para-phenylendiamin may be replaced by para-phenylendiamin, or the alpha-napthol may be substituted by various phenols. The results of examination of a number of such combinations are given below; the tests were carried out with normal blood films:-

Reagents	Reaction.
Dimethyl-para-phenylendiamin ÷ Ortho-chlorphenol.	Slight in fifteen minutes, and almost confined to eosinophils
" ÷ Meta-chlorphenol.	Well marked in fifteen minutes.
" ÷ Para-chlorphenol	Slight in fifteen minutes in eosinophils.
" ÷ O-cresol " ÷ M-cresol	) Well marked in five minutes.) Disappearance of dye very) rapid.
" ÷ Resorcin	Very slight in thirty minutes.
" ÷ Pyrocatechin	Fairly well marked in thirty minutes.

The use of para-phenlylendiamin instead of dimethyl-para-phenylendiamin with alpha-naphthol gives a reagent slower in action, but sufficiently rapid to be useful; the same may be said of meta-chlorphenol and ortho- and meta-cresol, with dimethyl-para-phenylendiamin.

The attempt was made to synthetise indamines from combinations of meta-toluene-diamin with para-phenylendiamin and of dimethy-para-phenylendiamin with dimethylanilin by means of the leucocytic oxydase, but only the very faintest indications of dye formation were obtained in the cells after some hours' exposure.

Schulze (22) has recently recommended a mixture of the sodium salt of Beta-Naphthol, Microcidin, with Dimethylparaphenylendiamin-Hydrochloride. This is not so rapid in action as the more commonly used mixture but gives sharper pictures. I have not used it to any great extent.

THE REACTION IN NORMAL TISSUES.

Normal blood is most conveniently studied in cover-slip films. These may be treated without fixation but in that case the red corpuscles become lysed, and the dye formation tends to spread outside the leucocytes. A brief fixation, e.g., 1 per cent osmic acid for five seconds. or alcohol for five minutes, does not interfere with the reaction to any extent, and gives much cleaner and more satisfactory results.

The film is placed face downwards on the surface of a fairly freshly prepared mixture of the two reagents in a hollow glass block and covered. The addition of alkali is unnecessary, and may cause the film to leave the cover slip. An exposure of two minutes is sufficient for films of ordinary thickness; if a longer time is allowed, the nuclei of the leucocytes become obscured. Thick films stain rather slowly and unevenly. When the staining is completed the film must be washed in cold running water, to remove excess of reagents; it may than be lightly counterstained with dilute basic fuchsin and mounted in water-glass; it is somewhat difficult to exclude tiny air bells. The darkly stained leucocytes are easily seen on low power examination. With oil-immersion lens the blue staining

is/

is seen to occur in the form of an opaque deposit on the granules (Fig. 1). The isolated granules of broken-up leucocytes, particularly of eosinophils, stain heavily.

The eosinophil cells show the reaction earliest and most markedly. The neutrophils also have a heavy deposit, varying slightly in degree in individual cells. The large hyaline leucocytes react less markedly but still very distinctly, and the dye settles out in the form of granules in the protoplasm. The basophil granular leucocytes are more feebly stained than are any of those mentioned. The lymphocytes, like the red corpuscles and the blood platelets, show not a trace of blue staining even if the film is exposed to the reagents for an hour, by which time all the leucocytes which contain oxydase are completely obscured by the dense collections of indophenol accumulated round them.

Bone marrow examined in films shows a well-marked reaction in all granular cells, but the loose granules of leucocytes broken up in the process of making the films lie in abundance between and upon all the elements present and stain deeply, thus interfering with the critical examination of certain elements. So far as one has been able to observe, the megakaryocytes do not give the reaction. The fat globules present in the marrow take/

take on a violet colour, as the indophenol is soluble in fat.

For the examination of solid tissues, sections cut by the freezing process have generally been considered essential. However, I have found paraffin sections to give good results. The tissues have usually been infiltrated in paraffin melting at 40° C., on the top of the oven, and finally embedded in hard paraffin melting at 52°C. after only half an hour at the higher temperature. This process appears to damage the oxydase only very slightly if at all.

The bone marrow is the only normal organ which shows any considerable reaction. In sections of it the granular polymorphs and myelocytes of the various kinds react positively. The myelocytes show a slightly less degree of staining than polymorphonuclears.

The nucleated red corpuscles and the small lymphocytes which form no inconsiderable proportion of normal marrow are unstained.

In most of the other organs a close scrutiny of sections with the oil-immersion lens, controlled by suitable counter-staining shows that the only cells which give the reaction are the granular leucocytes in the blood vessels. The lymphocytes in the spleen lymphatic glands and other structures never show any staining/

staining. No reaction has been observed with these reagents in the large mononuclear spleen-cells which have been shown by Loele (1911)[12] to contain "phenolophile" substance and to give a brown stain with alkaline alpha-naphthol. This reaction develops very slowly however (24 hours).

The parotid and lacrymal gland-epithelia are exceptions to the general rule. These cells, as was discovered by Schulze (1910)[23] also possess an indophenol oxydase and give a positive reaction. Their oxydase is evidently more labile than that of myeloid cells as it is not demonstrable in sections cut by the paraffin process.

These observations on the organs have been confined to tissues fixed by formalin, and are in no way contradictory of the work of von Gierke (1911)[4], who has recently demonstrated, by means of the Indophenol synthesis test, the presence of oxidising ferments of an extremely labile character in muscle-fibres and gland-epithelia. His results are obtainable only in fresh, unfixed material. It is probable that the oxydase investigated chemically by Vernon (1911)[24], in parenchymatous organs is of the same character.

In the blood of children from the time of birth the same cells give the reaction as in adult blood, and the mature/

mature forms give it in equally marked degree. The large forms of true lymphocyte observed in the blood of childhood, like the ordinary small lymphocytes give no reaction.

THE REACTION IN FOETAL TISSUES.

The oxydase test proved itself peculiarly suitable for demonstrating the distribution of myeloid tissue in the organs of the foetus at different ages. The information obtained by its use was supplemented by subsequent examination of the same films or sections after staining with Leishman's or some other stain suitable for the demonstration of leucocytic granules and other protoplasmic characters. It was frequently necessary to make rough sketches of certain microscopic fields indicating the behaviour of special cells with regard to the reaction, so as to compare the results obtained by ordinary tinctorial methods

The smallest foetus examined was approximately at the tenth week. The whole foetus was fixed in 5 per cent aqueous formalin and embedded in paraffin after clearing. Sections were cut 5 μ in thickness. The only cells stainable by the indophenol synthesis were fairly large mononuclear cells occurring singly or in groups in the connective tissue surrounding the umbilical vein in the liver. (Fig. 2.)

Leishman's stain showed that practically all of these contained coarse eosinophil granules. They were accompanied by large non-granular mononuclear cells with basophile protoplasm which gave no reaction.

In/

In a foetus of 3½ months the organs were examined by means of sections cut by the freezing process. The quantity of myeloid tissue was fairly large. The bulk of it occurred in the connective tissue surrounding the portal vein and its branches in the liver (Fig.3) The thymus was composed chiefly of unstained lymphoid nodules, but in the intervening connective-tissue septa there were fairly numerous myeloid cells (Fig.4). The spleen was very small, and contained only a few cells giving the reaction; these showed no definite arrangement. The blood and the bone marrow of this foetus were examined in films. No neutrophil cells were found in specimens stained by Jenner's fluid. The leucocytes present were for the most part either coarsely granular eosinophil cells or non-granular mononuclears with basophil protoplasm. The eosinophils all gave a strong oxydase reaction, and it was not difficult to determine that a certain proportion of the non-granular cells also reacted, but less strongly. These cells were without doubt the non-granular mother cells of the myelocytes, and it was evident that in them the appearance of the oxydase had preceded recognisable granular formation. A certain number of these cells showed no trace of a reaction even after prolonged exposure to the reagents, but it was not found possible to obtain any very accurate estimate of the relative proportions of positively and negatively reacting cells.

In a foetus at 7½ months the organs were examined in frozen sections. Abundant myeloid tissue was seen in liver, spleen and thymus. In the liver it was distributed in a zone round the periphery of the portal tracts, close to the liver cells. (Fig. 8). In the spleen it occurred chiefly in the neighbourhood of the small vessels: definite lymph nodes could not yet be distinguished (Fig. 5). In the thymus the myeloid cells were very abundant in the connective-tissue septa and around the blood vessels, but a good many were scattered also amongst the unstained lymphoid cells (Figs. 6 & 7). The Hassall's corpuscles showed a certain degree of blue staining. The cervical axillary and inguinal lymphatic glands were examined. Their cortical areas showed only unstained lymphoid tissue, and the medullary areas contained large numbers of oxydase-containing cells. In the films of blood and marrow from this foetus all the granular leucocytes and many non-granular leucocytes with lobed nuclei gave a well-marked reaction.

In the organs of a foetus at full term the appearances were very similar to those just described. The sections in this case were cut by the paraffin process. In the liver the zonal arrangement of the myeloid cells was very well marked (Fig. 10). In the thymus the lymphoid tissue was now relatively much more abundant, but there was still a considerable amount of myeloid tissue in the fibrous septa (Fig. 11). In the spleen the Malpighian bodies stood out unstained/

unstained while the pulp contained numerous myeloid cells ranged most abundantly just at the periphery of these.(Fig. 9) It appeared as if the lymph nodes had developed in relation to the vessel walls, and had by their growth pushed outwards the perivascular ring of myeloid tissue seen at the earlier age. Only a few myeloid cells could be found, occurring singly, in the connective tissue of the renal pelvis and round the renal vessels. The kidneys of several foetuses of different age were examined, but in none was there any special development of myeloid tissue in this situation, though lymphatic glands in the neighbourhood contained a good deal. This negative finding is of some interest in relation to the occurrence of bone marrow in the hilum of the kidney under pathological conditions (McKenzie, Browning and Dunn, 1909.)[13]

For the sake of comparison with the foetal organ, the thymus of a child of 18 months was examined. The reaction obtained indicated the persistence of strands of myeloid tissue in the fibrous septa, but the large masses of lymphoid tissue were quite unstained (Fig. 12).

In general, it was found that the leucocytes in foetal blood and organs gave the reaction with a degree of intensity corresponding with the stage of development of the cells. The non-granular cells showed it only in a limited/

limited degree or not at all: the granular cells always reacted markedly but rather less intensely than the polymorpho-nuclear leucocytes of the adult.

THE REACTION IN PATHOLOGICAL CONDITIONS.

Blood-films from a variety of diseased conditions were subjected to examination by the oxydase test: in particular from wasting diseases, such aa carcinoma, diabetes and renal disease, and from severe anaemias. In no case was there any evidence of alteration in the oxydatic activity of the mature leucocytes. In conditions associated with leucocytosis there was no more than the normal slight variation in activity between individual cells.

The information obtainable in diseased organs referred chiefly to conditions associated with local accumulations of leucocytes. In acute suppurative inflammation the disposition of exuded leucocytes is accurately sketched out. It is also noted here that the structureless fibrinous exudate, present, from example on the serous surface of an inflamed appendix shows a diffuse bluing, quite apart from and additional to the heavier deposit occurring on the leucoctyes.

In lymphatic glands which drain an acutely inflamed area the lymph-sinuses are seen to be dilated and crowded with polymorpho-nuclear leucocytes, many of which are undergoing phagocyctic absorption. The large mono-nuclear phagocytes/

phagocytes give no reaction but the included leucocytes continue to give a reaction until they are completely absorbed and digested. The process corresponds exactly with what is observed in films of exudates experimentally produced in the peritoneal cavity of the rabbit.

In certain conditions the oxydase reaction is serviceable in revealing the presence and extent of a leucocytic exudate which might escape observation by ordinary staining.

(a.) For example :-

A. In the mucous membrane of a vermiform appendix removed during the quiescent period following an attack of appendicitis there may be little evidence of a definite inflammatory lesion, but it is shown by the oxydase reaction that abundant granular leucocytes are present in the small cellular areas of the mucosa between the lymphfollicles : in some cases they are in great excess and suggest a definitely inflammatory condition - (c.f. Loele[10]) B. In the kidney of a case of acute nephritis ordinary staining showed only slight catarrh of the convoluted tubules and the glomeruli were very slightly more cellular than normal. On application of the oxydase test it was seen that these glomeruli were the seat of a fairly well-marked leucocytic infiltration indicating a lesion/

lesion more in accordance with the severe clinical symptoms than could be readily appreciated from ordinary histological examination. This has also been commented on by von Gierke (1911)[4]. C. In glands from cases of lymphadenoma the extent of infiltration of the diseased glands by eosinophile leucocytes can be readily estimated as these cells give a marked reaction.

As might be expected the tissues in myeloid leukaemia present striking pictures when examined by this method as the myeloid infiltrations in the organs give a pronounced reaction (Fig 13). In examination of these cases paraffin sections give much more definite pictures than do those cut by the freezing process. In the latter the blue staining occurs somewhat diffusely probably on account of some diffusion of the oxydase through the tissues after death. It is also noteworthy that staining occurs very slowly unless large quantities of the oxydase reagents are used. This is due not to insufficiency of the reagents for the numerous cells, but to lack of sufficient dissolved oxygen in the reagents to supply the necessary molecules for formation of the indophenal. The reaction in relation to leukaemia is more fully discussed in the second part.

CHRONIC INFLAMMATIONS.

A number of observations were made in conditions of subacute and chronic inflammation to determine the behaviour of the cells concerned.

In frozen sections of the breast-tissue in chronic mastitis, in which abundant tissue mast-cells were present, it was determined that these cells give no reaction (1910). This is in agreement with the observations of Kreibich (1910)[8].

Plasma-cells were obtained in great numbers in some granulation-tissues and in certain inflamed fibrous tumours of the gums. Theywere examined in fresh films and in frozen sections and were found to give no reaction.

A similarly negative reaction was obtained in the case of the peculiar large foamy cells observed in granulation-tissues and in the walls of chronic abscesses. In one case of a simple chronic abscess of the mamma it was found that the foam-cells that were supported by vascular tissue gave no reaction but in those that lay free in the abscess the nuclei gave bluing with the indophenol reagent. This result has not been again obtained though films and sections of two similar cases have been examined: its meaning is not yet understood.

The/

The negative reaction in tissue mast-cells, plasma-cells and foam-cells is at any rate suggestive that their origin and nature differ from those of the granular blood leucocytes

THE OCCURRENCE OF THE OXYDASE IN DEAD TISSUES.

While in living tissues the presence of this stable oxydase can be referred practically only to myeloid tissue derivatives it appears that in dead tissues in the body a substance with similar properties, if not the same substance is frequently diffusely distributed.

Meyer (1904).[14] observed that tubercular caseous material possessed the property of bluing guaiac. This appears to bealways the case quite apart from secondary pyo-genic infection and the presence of leucocytes. Also I have found that in a considerable number of caseating tubercular lesions, without pyo-genic infection the caseous material shows a diffuse blue staining when exposed to the indophenol reagents. The bluing is confined to the dead structureless material and is sharply bounded by a line running round parallel to the edge of the living granulation-tissue and about a millemetre from it. Tubercle Bacilli themselves do not give the reaction so that they are probably not responsible for this phenomenon.

In three different syphilitic gummata, free from secondary infection the same thing was observed in the caseous material. In simple infarctions at an early stage when there is peripheral leucocytic exudation these leucocytes show their usual reaction but the dead tissue also shows it diffusely. In old infarctions which have become/

become caseous and are surrounded by a thick fibrous capsule the reaction still persists, in the caseous material only and shows a distribution such as was described in tubercular caseous lesions. On the other hand in completely healed and vascularised cicatrices of old inflammatory lesions where there must at one time have been extensive leucocytic exudates, e.e. in obliterated appendices, the fibrous scar-tissue shows no reaction. Accordingly it seems probable that the oxydase persists in dead structures, as it must do for some months at any rate in old infarcts, on account of the want of access of the blood stream to remove it.

The same diffuse reaction may be observed in necrotic areas in tumours but no reaction has been observed in tumour cells of any kind, including those of two cases of diffuse myeloma, a primary sarcoma of bone marrow.

THE RELATION OF THE OXYDASE TO THE STRUCTURE OF THE LEUCOCYTES.

It is a matter of some theoretical interest to determine if possible what constituent of the leucocytes con--tains the oxidising substance. Winkler [26] observed that the nuclei of the cells took no part in the reaction. Schulze (1910)[22] is of opinion that the granules carry the oxydase and this is certainly suggested by the way in which isolated granules of broken up eosinophile leucocytes react. On the other hand, Fursenko (1911)[2] considers it possible that the granules merely take up the dye which is formed in the protoplasm and Pappenheim (1911)[18] also suggests that the staining of the granules is of the nature of lipoid staining and similar to vital staining.

In observations in dry films it is apparent that the dye settles out as a precipitate which certainly at first forms on the granules but very soon appears throughout the protoplasm obscuring all detail and ultimately it is laid down on th surface of the cell and even on the margins of adjoining cells: accordingly whatever is the actual seat of the oxydase the oxidising action can extend considerably beyond it and the location of the dye is independent of any special staining affinity of the tissue/

tissue elements.

The large granules of the amphophile leucocytes of the rabbit are very well suited for observation and these cells may be readily obtained in quantity in peritoneal exudates. If the exudate be mixed directly, while fresh, with a quantity of the indophenol reagents and observed under the oil-immersion lens the develop-ment of the reaction can be readily followed, as under these conditions of "vital" staining it occurs somewhat slowly. It is seen that the granules alone are stained and the intervening protoplasm remains perfectly clear. Naegeli has made similar observations on living eosinophile corpuscles in the sputum of bronchial asthma and expresses the opinion that the granules alone carry the oxydase (1912)[16]. In further investigation of this point a quantity of red-marrow from the ribs of a horse was subjected to tryptic digestion in the way adopted by Petry (1911)[20]. to obtain the free eosinophile granules. Films made from the centri-fugalised deposit after 24 hours digestion contained large numbers of these enormous granules lying free and isolated. They still retained their eosinophilic tendency and they gave a well marked oxydase reaction, so that in this case at any rate the granules are definitely proved to be the carriers of the oxydase.

Similar conclusions are to be arrived at from the work/

work of Kreibich (1910)[9] who observed staining of the leucocytic granules by adrenalin and other phenols, the stain being formed as a result of oxidation of these substances. Loele (1911)[10] & [11] also obtained similar results in the way of granule-staining with alpha-napthol in alkaline solution and with alpha-naphthol combined with minute quantities of aniline dyes, the staining being dependent on an oxidising process.

While in the granular leucocytes the bulk of the evidence favours the view that the oxydase is carried by the granules the non-granular cells which react positively, namely the large hyaline leucocytes of the blood and the non-granular myeloblasts of foetal marrow or of acute leukaemia, present apparent exceptions. But even in the case of these cells it is possible that the oxydase is confined to specialised elements of the protoplasm. Both of these classes of cells show granules in their protoplasm in dark ground illumination(Naegeli 1912[17]) von Jagie 1911[5].)and as will be shown later the development of the oxydase reaction in myeloblasts is accompanied by a modification of the protoplasmic structure though it precedes the formation of stainable granules. Further

the/

the inhibitory effect which is exercised by albumin on the oxydase (vide infra) would lead one to expect that this substance would be carried by some special constituent and not by the general body of protoplasm.

ON EXTRACTION OF THE OXYDASE FROM LEUCOCYTES.

Brandenburg (1900)[1]. and Meyer 1904)[14] used chloroform water to extract the oxydase, but in using this one found it difficult to obtain a clear solution and also the great bulk of the oxydase remained in the bodies of the leucocytes. Better results were obtained by the following method : a quantity of thick pus was obtained from a case of empyema (pneumococcus alone present) and this was mixed with an equal quantity of chloroform. The mixture, which became tough and ropy, was then shaken up with an equal quantity of water and set in an oven at 50°C for a fortnight, being shaken up daily. At first it remained uniformly turbid but gradually a clear layer began to separate above and by the end of a fortnight the clear layer was equal to the amount of water added. By this time the pus cells were quite disintegrated and the oxidising effect of the pale debris on guaiac was much diminished while the clear fluid above had a strong oxidising effect. The whole was finally shaken up again and then centrifugalised and the clear fluid was collected and distributed in test-tubes, 10.cc. in each, and these were heated for an hour in a water bath kept at 75.° C.. This brought about coagulation of a certain amount of albumin and drove off the chloroform, and at the same time the effect of the extract on guaiac, when cooled, was greatly enhanced. After clearing by centrifugalisation this extract could be sealed

up in sterile tubes and sterilised at 57°C. and seemed to retain its properties very permanently. Extracts thus obtained varied in strength to some extent but of any of them .025 c.c. was sufficient to produce deep blue coloration of 1 c.c. of ten per cent suspension on Tinct. Guaiaci in one minute at room temperature: this is a degree of activity approaching that of undiluted pus but not quite equal to it.

The extracts thus obtained are quite clear and have a slightly alkaline reaction. On heating to 85°C. or on boiling they become slightly turbid and lose their oxydising power. They give positive results on testing for albumin by the Xanthoproteic reaction, by Millons reagent and by Adamkiewicz's method and the Buiret gives a pink colour indicating presence of proteose or peptone. After dialysis of ten cubic centimetres for 36 hours the oxidising activity is only slightly diminished; this result was obtained on four occasions. Putrefactive and pyogenic organisms grow readily in the extracts. No appreciable digestive effect has been obtained with them on coagulated albumins after several days incubation.

The activity of extracts is most conveniently estimated by means of guaias. It was found best to employ a ten per cent suspension of Tincture of Guaiac in water. This forms a white emulsion which will remain unchanged for about an/

hour under ordinary circumstances but later begins to turn blue from oxidation, in the superficial layers. The addition of .01 c.c. of pus to 1 c.c. of this white emulsion produces a uniform deep blue colour in about half a minute at room temperature and the relative activity of an extract may be roughly gauged by the degree of bluing which .01 c.c. produces in a given time. The results obtained are only comparative and no satisfactory method of standardisation has yet been devised. The rapidity and completeness of the oxidation of the guaiac are greatly increased at higher temperatures up to 80°C.

It may be shortly noted here that extracts which are very active in oxidising guaiac have absolutely no effect on certain other chromogenic substances which have been used in testing for blood by a catalytic reaction. Guaiac used in this way with H_2O_2 will detect blood in a dilution of 1 in 20,000, aloin with H_2O_2 in 40,000, Benzidin with H_2O_2 in 200,000, alkaline Phenol Phthalin with H_2O_2 1 in 1,000,000 (1 in 80,000,000. Kastle 1909)[6] Of these four, guaiac is the only one with which the oxydase solution gives any direct oxidation, even with heating, so that in respect of these reactions the action of the oxydase does not go on parallel lines with that of the active oxygen produced when Hydrogen Peroxide is broken up.

THE EFFECTS OF HEAT AND OF CERTAIN REAGENTS ON THE OXYDASE.

The constituent of the leucocytes which confers on them this remarkable oxidising property has considerable powers of resistance to deleterious influences, and would appear to be a comparatively stable body. As has been already mentioned, its effects may still be observed in blood films which have been preserved for eighteen months, and I have obtained a satisfactory reaction in paraffin sections kept unmounted for even longer. However, there is no doubt that it does become imparied with age, though slowly, for in the films mentioned the reaction was not so rapid nor so strongly marked as in fresh blood films. It can also be determined by the examination of old films that the cells which give the reaction most strongly in fresh blood, namely, the polymorpho-nuclears, particularly the eosinophils, retain the oxidising property longest, and may still continue to give a good reaction when the mononuclears have lost that power. It seems also to be the case that though exposure of the leucocytes to various external influences does not produce total destruction of the oxydase, nor even a degree of deterioration which can be estimated by the active alpha-naphthol-dimethy-para-phenylendiamin reagent, still, some change is produced, though slight, and this will naturally fall most heavily on the cells which possess the slightest degree of oxydase activity./

activity. Accordingly, when such cells as the non-granular predecessors of the granular myelocytes are being examined, it is advisable that the tissues should be as fresh as possible and should be used unfixed, and that every precaution should be taken to avoid exposing them to any influence which may be prejudicial.

In order to estimate the resistance of the oxydase to heat, the leucocyte cream was obtained from a few cubic centimetres of fresh human blood, and sealed up in a number of capillary tubes. These were exposed to various temperatures for varying lengths of time, and then films were made from their contents and examined in the usual way. The leucocytes from a tube exposed at 57° C. for four days gave a reaction which seemed as active as in normal blood. The same was true of those exposed to 70° C. and to 80° C. for an hour, but those exposed to 85° C. for five minutes gave absolutely no reaction even on prolonged staining. Identical results were obtained with pus cells from a glandular abscess due to staphylococcus aureus.

Experiments were made with a number of substances which act as poisons on ferments generally. In each case a small quantity of leucocyte cream was added to excess of the poison in a small test tube; this was stoppered and set in the incubator at 37° C. for an hour. The leucocytes were then sedimented and washed in physiological saline solution to free them from the reagent used. Where acids had been employed/

employed, the mixture was neutralised previous to washing. The washed sediment was finally converted into films and examined for oxydase. In the case of the following substances no damage appreciable by the ordinary test was observed:-

Sulphurous acid (B.P.)	
Sodium thio-sulphate	20 per cent. solution
Formaldehyde	40 " "
Osmic acid	1 " "
Toluylendiamin	1 " "
Phenylhydrazin	Saturated solution
Quinine tartrate	" "
Phloridzin	1 per cent. solution
Resorcin	2 " " .
Hydrocyanic acid	2 " "

Exposure to 2 per cent. hydrocyanic acid in excess for days did not destroy the reaction. Sufficient proof of the resistance of the substance to destruction by, or solution in, alcohol, chloroform, and xylol is obtained when one remembers that pieces of tissue which have been dehydrated and cleared in these reagents will, after embedding in paraffin, show the indophenol reaction readily and markedly. Schulze (1909)[21] has pointed out that the oxydase still persists in tissues which have been preserved in dilute formalin for years, but that it is destroyed by the long-continued action of alcohol.

The following substances were found to destroy the oxydase in an hour when tested as described above:-

$\frac{N}{10}$ Oxalic acid.

$\frac{N}{10}$ Hydrochloric acid.

$\frac{N}{1}$ Acetic acid.

Corrosice sublimate, saturated solution.

Phenol, 5 per cent.

Picric acid, saturated solution.

$\frac{N}{10}$ Oxalic acid was found to destroy it in films in ten minutes.

EFFECT OF FILTRATION OF THE EXTRACT.

The following is an account of one of three experiments illustrating the effect of passage of the extract through a Berkefeld filter.

A new Berkefeld cylinder was sterilised by boiling and 300 c.c. of sterile distilled water were drawn through it by suction, to dryness. 100 cubic centimetres of an extract were then passed through and the filtrate was taken off as it came through in ten tubes of 5 c.c. each, until 50 c.c. had passed: the remaining 50 c.c. were received in two portions of 25 c.c. each. The activity of the different portions towards guaiac was then estimated as follows:- Doses of ·01, ·025, ·05, ·075 and ·1 c.c. of each of the twelve samples were set out in small test-tubes and similar doses of the same extract unfiltered were placed in test-tubes as a control. One cubic centimetre of the guaiac emulsion was then added to each tube and the results were read at the end of five minutes. They are given in tabular form:-

Doses.

	·01 c.c.	·025 c.c.	·05 c.c.	.075 c.c.	·1 c.c.
First Portion	Very faint green	Very faint green	Very faint green	Very faint green	Very fain green
2nd.	Ditto	Ditto	Ditto	Ditto	Ditto
3rd.	Ditto	Ditto	Ditto	Ditto	Ditto
4th.	Faint green	Faint green	Faint green	Pale green	Greenish Blue
5th.	Pale green	Greenish Blue	Turquoise	Turquoise	Blue
6th.	Pale blue	Blue	Blue	Blue	Deep blue
7th.	Pale blue	Blue	Deep blue	Deep blue	Deep blue
8th.	Blue	Deep blue	Deep blue	Deep blue	Deep blue
9th.	Deep Blue	Ditto	Ditto	Ditto	Ditto
10th.	Ditto	Ditto	Ditto	Ditto	Ditto
11th.	Ditto	Ditto	Ditto	Ditto	Ditto
12th.	Ditto	Ditto	Ditto	Ditto	Ditto
Unfiltered	Ditto	Ditto	Ditto	Ditto	Ditto

These results show that the first 15 c.c. of the extract which passed had greatly diminished activity and that the portions which followed showed increasing activity apparently reaching that of the unfiltered extract. However, although the end result in five minutes was the same in the last five series the reaction really appeared more quickly with the unfiltered extract and with graduated rapidity in the remaining tubes. It was apparent that the filter was at first almost impermeable to the oxydase, not on account of the/

the size of its molecule, but for some other reason. The filter could be gradually accommodated to the passage of the oxydase, just as was found by Muir and Browning (1909)[15] to be the case with haemalytic complement.

The surface of the filter was afterwards washed carefully with a stream of distilled water and five cubic centimetres of distilled water passed through in the reverse direction. This fluid contained abundant oxydase and corresponded in strength with the seventh portion passed through, but this gives no idea, of course, of its relation to the amount lost in filtration.

On shaking up the half of a Berkefeld filter, ground to fine powder, with ten c.c. of the same extract, and centrifugalising it was found that the oxydase was almost completely removed from the clear supernatant fluid, but it was present in more concentrated form in the sediment of the powdered filter, to which it had apparently adhered: in this particular the oxydase behaves differently from haemolytic complement as described by Muir and Browning. No further experiments have yet been made in this direction.

EXPERIMENTS TO OBTAIN AN ANTI-OXYDASE.

The following series of experiments was made in the endeavour to obtain an anti-substance to the oxydase which would destroy it or inhibit its action.

A quantity of pus as free as possible from red-blood corpuscles was obtained from an abscess. The organism present, in pure culture, was Staphylococcus Aureus and on testing with the Indophenol reagent it possessed no oxydising property. This pus was washed four times in physiological saline to free the leucocytes from the liquor puris. It was then sterilised by exposure to a temperature of 57° C. for an hour in three successive days. The pus was brought down completely by centrifugalisation for an hour and its amount measured. A known quantity was then diluted with an equal amount of sterile saline solution to render it more fluid and the equivalents of 1½ and 2 c.c. of undiluted pus were injected into a rabbit at intervals of ten days. The material that was injected gave an active oxidation of guaiac and in films of it exposed to the Indophenol reagents the leucocytes stained deeply and readily.

The blood serum was obtained from the animal ten days after/

after the second injection and heated at 57° C. for an hour. It is now referred to as anti-leucocytic serum. It was tested to determine :-

1. Whether it possessed a specific immune body for human leucocytes and not for human red-corpuscles or blood-serum.

2. Whether it produced inhibitory effects on the oxydase of human leucocytes.

1. Whether it possessed a specific immune body for human leucocytes and not for human red corpuscles or blood serum.

This was investigated by estimations of the deviation of haemolytic complement. In testing the effect of the anti-serum with leucocytes use was made of (a) fresh leucocyte cream from human blood obtained by centri-fugalisation and (b) Pus from an empyema containing pneumococci only (these organisms had no oxidising effect). Four experiments were made with (b) using . lcc of a 5 per cent suspension as the dose of leucocytes. One experiment was made with (a) using equal doses as nearly as possible similar to those of (b). The results with (a) were rather less marked than those with (b) but were still conclusive and as the absence of organisms renders this experiment/

experiment less open to criticism it alone will be recorded.

EXPERIMENT.

No. of tubes	1.	2.	3.	4.	5.
5 per cent suspension of human leucocyte cream ...	.1	.1	.1	.1	.1
Anti-leucocytic serum ...	.05	.05	.05	.05	.05
Guinea-pig complement ...	.005	.01	.02	.03	.1

The tubes were set in the incubator at 37°C. for 1½ hours and shaken at intervals. They were then removed and centrifugalised and the clear supernatant fluids were transferred to other tubes to be tested for complement. For this purpose Ox corpuscles sensitised by rabbit v Ox I.B. were used. .5 c.c. of 5 per cent suspension were added to each tube and the whole incubated for an hour with shaking at intervals.

RESULTING LYSIS.	Nil	Nil	Nil	Nil	Very slight

In a set of tubes used at the same time in which a dose of anti-leucocytic serum was .1 instead of .05cc.

Result	Nil	Nil	Nil	Nil	Nil

The dose of complement for .5 cc of ox corpuscles was .005 after similar incubation. Controls set for the purpose at the same time showed that a full dose of complement was not deviated by the dose of leucocytes or by the dose of anti-leucocytic serum alone.

The combination of.05cc of the Anti-leucocytic serum with .1 cc of 5 per cent suspension of leucocytes thus deviated nearly twenty doses of complement. and the deviation was more marked with an increased dose, .1cc of the anti-serum.

The Anti-Leucocytic serum was tested in exactly the same way against human red corpuscles using .5 cc of a five per cent suspension of the latter as the dose. This combination did not produce deviation of a full dose of complement. The haemolytic dose of the Anti-Leucocytic serum for human red corpuscles was found to be .2 cc. for .5 cc. of a 5 per cent suspension of these in presence of excess of complement.

With human serum in doses of .05, .005, .0005, .00005, and .000005 cc. and .05 cc. of the Anti-Leucocytic serum a full dose of complement was not deviated. These results were obtained on several occasions. Accordingly the Anti-serum possessed marked anti-leucocytic properties and had very little anti-substance to human red corpuscles or blood-serum.

2. Whether the Anti-Leucocytic serum produced inhibitory effects/

effects on the oxydase of human leucocytes.

In the course of the above experiments examination was made on each occasion of the leucocytes which had been exposed to the effect of the anti-leucocytic serum and complement and other experiments were made using larger doses of the Anti-Serum (up to .2 cc.). Films were made from the leucocytes in the deposit of each tube and these were tested for oxydase by the indophenol reagents. In no case was there any apparent diminution of the oxydase even where the largest doses of Anti-serum and complement had been employed.

The Anti-Leucocytic serum was also tested against a clear extract containing abundant active oxydase: .2 cc. of thisextract produced deep-blue coloration of 1 cc of 10 per cent guaiac emulsion in a minute at room temperature. Doses of .2 cc. of the extract in .4 cc of physiological saline solution were set out in twelve tubes. To six of these were added varying doses of the Anti-leucocytic serum and .075 cc. of giunea-pig serum for complement: to the other six tubes similar doses of normal rabbit-serum and .075 cc. of complement.

EXPERIMENT.

	1	2	3	4	5	6	Control 7
Oxydase Extract	.2 cc	.2 cc	.2cc	.2cc	.2cc	.2cc	.2cc
Anti-Leucocytic serum.	.02	.035	.075	.1	.125	.2	alone
Complement	.075	.075	.075	.075	.075	.075	
The tubes were incubated at 37°C for an hour and shaken at intervals.							
1 cc of 10 per cent guaiac emulsion added: result in five minutes.	pale greenish Blue		varying to			very pale green	Deep Blue.

This would appear to indicate inhibition but it was found that in the other six tubes with doses of normal rabbit serum in place of anti-leucocytic serum, the degree of inhibition of the oxydase was identical in the corresponding tubes. Further when all the tubes were heated for five minutes at 75°C to coagulate the albumins present, and fresh doses of guaiac were added to each, the new guaiac was rapidly and equally blued in all, thus showing that the oxydase had not been destroyed.

Accordingly no anti-oxydase properties can be attributed to this serum which judging by complement deviation tests is markedly anti-leucocytic.

Another rabbit had been previously inoculated in a similar way with doses of sterilised pus and the serum from it/

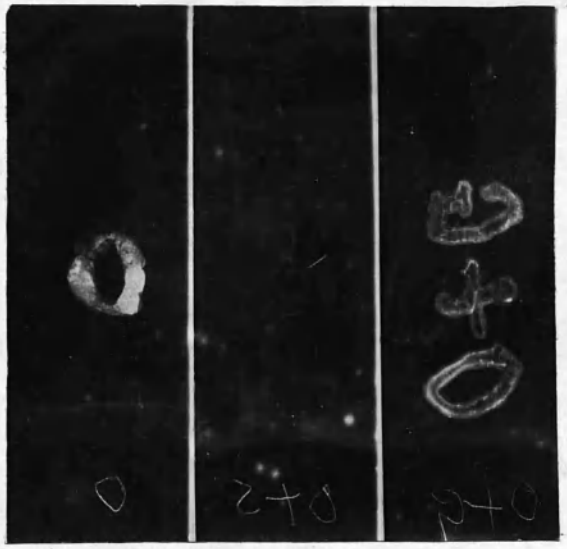
it appeared to be strongly anti-leucocytic but was also slightly"anti-serum" so that it could not be absolutely relied upon. However, it gave no evidence of possessing anti-oxydase properties when tested as above.

The markedly inhibitory effect exercised by normal sera on the action of the oxydase on guaiac introduces some difficulty in the just interpretation of such experiments with extracts and anti-sera, and it is found that in this particular the indophenal reaction is of no more assistance. The addition of normal serum to oxydase extract is even more efficient in inhibiting indophenal formation by the latter than in preventing oxidation of guaiac.

This is most easily demonstrated as follows:-

A thin film of oxydase extract diluted with an equal amount of water is spread on a slide. A similar film is made from a mixture of equal parts of exydase extract and normal human serum. the films are dried and immersed in a fresh mixture of alpha-naphthol and dimethyl-para-phenylen-diamin. The film with the extract alone stains blue in a few minutes: no bluing of the film of oxydase plus serum occurs even after 24 hours. A print of two such films is here shown along with a third in which the oxydase extract was diluted with an equal amount of 15 per cent gelatin. The latter has no inhibitory effect. These films had been exposed to the action of the indophenol reagents for 24 hours: staining showed in the O and O - G films almost immediately but no shadow at all has developed on the O - S

film. Of course this inhibitory effect is only recognisable with reference to these colour-tests, and may not represent any actual important relationship of the oxydase itself.



On the other hand it seems definitely to indicate that cell-granules which carry the oxydase in an extremely active state are not likely to be of albuminous constitution, but may be albuminoid, as is stated by Petry who analysed quantities of the eosinophile granules of horse leucocytes (1911)[20].

The anti-oxydase which was obtained to laccase a vegetable ferment, by Gessard[3] was found to retard the action of the ferment in bluing lac-juice but the ferment was uninfluenced by normal sera.

With regard to the actual nature or constitution

of/

of the oxydase very little is known. In the extracts which have been described it probably forms only a fraction of the matter present in solution and it would be difficult to imagine any criterion by which the purity of a sample of it could be established. We know that it is a product of cellular metabolism, that it can be destroyed by heat, that it is active in probable infinitesimal doses, and is apparently inexhaustible. These facts have led most observers to label it a ferment and no very definite objection can be made to its inclusion under that term. Its relatively resistent character finds analogy among the vegetable ferments in laccase which is not destroyed by a temperature of 70°C.

CONCLUSIONS.

1. The synthesis of indophenol from alpha-naphthol and dimethyl-para-phenylendiamin, in presence of oxygen, is produced with great rapidity by -

(a) The polymorpho-nuclear neutrophil leucocytes

(b) The eosinophil leucocytes,

and also fairly rapidly by -

(c) The large hyaline leucocytes in normal blood

(d) The myelocytes in marrow and in leukaemic blood.

It is produced less readily by -

(e) Basophil granular leucocytes,

and by -

(f) A certain proportion of myeloblasts in foetal blood, and marrow.

It is not produced by lymphocytes nor red blood corpuscles nor by normal tissue elements, with the exception of parotid and lachrymal gland epithelium.

2. This property of myeloid cells may be made use of as a means of recognising them as such even before they have developed their characteristic granulations, but the absence of the reaction from a non-granular, lymphocyte-like cell does not necessarily indicate that that cell is not myeloid, for a certain proportion of quite definite myeloblasts give no reaction: these are no doubt the most embryonic examples, in which this functional characteristic is as yet undeveloped. A positive oxydase reaction in a

cell/

cell of this kind is a definite proof of its myeloid nature.

3. The constant and well-marked occurrence of this property in the large hyaline leucocytes in normal blood is confirmatory of Ehrlich's view that these originate in the bone marrow.

4. This property of myeloid cells renders the "indophenol-synthesis" test of great value in demonstrating the distribution of myeloid tissue in normal foetal organs and of granular leucocytes in the organs in myeloid leuk--aemia. It may also be employed to reveal the presence and extent of a leucocytic exudate in certain pathological conditions, where the exudate is difficult to recognise by ordinary staining methods.

5. The synthesis of indophenol by myeloid cells may be produced from other combinations of reagents similar to those mentioned, but less readily.

6. The oxidising substance in the myeloid cells exhibits a considerable degree of resistance to the influence of heat and of certain poisons and solvents. It is therefore possible to observe its effects, at any rate in the majority of the myeloid cells, in tissues which have been passed through the paraffin process. In the investigation of/

of the more embryonic forms of myeloid cells it is desirable that such observations should be controlled by examination of unfixed films or of unfixed tissues cut by the freezing process.

7. The oxydase is held by the granules, and probably by the granules only, in the granular cells, and even in the non-granular cells which contain it, it is probably attached to some specialised particles in the protoplasm, and not distributed diffusely.

8. The oxydase is of the nature of a ferment, though a very stable and resistent body. Its capacity for bluing guaiac and for synthesising indophenol is remarkably diminished by the presence of albumins. This fact may be interpreted as throwing some light on the constitution of cell-granules, in which the oxydase exists in a very active state towards the indophenol reagents.

9. The oxydase is very firmly attached to the substance of the leucocytes and is only obtainable in fairly strong aqueous solution when the cells have been fully disintegrated.

10. No evidence has yet been obtained indicating the possibility of procuring an anti-oxydase to this substance.

REFERENCES TO PART I.

1. Brandenburg. München med. Wchnschr. 1900 S. 183.
2. Fursenko. Zentralbl. f. allg. Path. XXII. 1911. No. 2. p97.
3. Gessard. Quoted by Kastle, U.S.A. Hygienic Laboratory Bulletin, Washington, 1910. No. 59, "The Oxydases", p.66.
4. v. Gierke. München med. Wchneschr. 1911. No. 44 p. 2315.
5. v. Jagic. Ibid. 1910. P.500.
6. Kastle. U.S.A. Hygienic Laboratory Bulletin, Washington 1909 . No. 51 Chemical Tests for Blood. P.30.
7. Kastle. Ibid No. 59, "The Oxydases", P.126.
8. Kreibich. Wiener klin. Wchnschr. 1910. No. 41, p.1443.
9. Kreibich. München med. Wchnschr. 1910. No. 22 P.1204.
10. Loele. Zentralbl. f. allg. Path. XXII. 1911. P.433.
11. Loele. München, med. Wchnschr, 1910. No. 46. P.2414.
12. Loele. Frankf. Zeit. f. Path. Nd. IX. H.3. P.436.
13. McKenzie, Browning and Dunn. Trans. Path. Soc. of Gt. Britain and Ireland, July, 1909.
14. Meyer, Erich. München med. Wchnschr, 1904. S.1587.
15. Muir and Browning. Jour. of Path and Bact. Vol. XIII. 1909. P.232.
16. Naegeli. Blutkrankheiten u Blutdiagnostik, Leipzig. 1912. p.95.
17. Naegeli. Ibid. P.181.
18. Pappenheim. Fol. Haematol XI. 1911. P.47.
19. Peters München med. Wchnschr. 1909. S.1478.

20. Petry. Biochem. Zeit. XXXVIII. H. 1 & 2, P.92.

21. Schulze. München med. Wchnschr. 1909. S.167.

22. Schulze. Ibid. 1910. No. 42. p.2171.

23. Schulze. Beitr. z. Anat, u.z. allg. Path. Jena. Bd. 45 P. 127.

24. Vernon. Jour. of Physiol. XLII. P.402.

25. Vitali. Chem. Centr.-Bl., Leipzig. 1887. S.1528.

26. Winkler. Fol. Haematol, 1907. Bd. IV. S.323.

27. Winkler. Ibid. 1908. Bd. v. S. 17.

LIST OF ILLUSTRATIONS TO PART I.

Microphotographs of blood films and sections of tissues stained by the indophenol-synthesis test for oxydase.

Fig. 1. - Film of normal blood. (1) Polymorpho-nuclear leucocyte deeply stained. (2) Large hyaline leucocyte showing a less degree of staining. (3) Lymphocyte unstained. (x 500.)

Fig. 2. - Portal vein in liver of ten-weeks' foetus. The myeloid cells in the wall of the vessel are indicated as dark dots. (x 50.)

Fig. 3. - Small branch of portal vein in liver of foetus at 3½ months, with myeloid cells in its wall. (x 50.)

Fig. 4. - Thymus of foetus at 3½ months. (x 100.)

Fig. 5. - Spleen of foetus at 7½ months, showing perivascular arrangement of myeloid tissue. (x 20.)

Fig. 6. - Thymus of foetus at 7½ months, showing arrangement of myeloid cells in connective-tissue septa and around vessels. (x 20.)

Fig. 7. - Thymus of foetus at 7½ months, showing arrangement of myeloid cells round two vessels. A Hassall's corpuscle is visible near the periphery. (x 100.)

Fig. 8. - Liver of foetus at 7½ months, showing darkly-stained zone of myeloid tissue at periphery of main portal tract. (x 20.)

Fig. 9 - Spleen of full-term foetus. The lymph-nodes have now/

now developed and appear as irregular unstained areas. The myeloid cells in the pulp are somewhat more numerous at the margins of these. (x 20.)

Fig. 10. - Liver of full-term foetus. There is a well-marked zone of myeloid tissue at the periphery of the main portal tract. (x 20.)

Fig. 11. - Thymus of full-term foetus, showing myeloid tissue arranged mainly in connective-tissue septa and around vessels. (x 20.)

Fig. 12. - Thymus of child of 18 months. Persistent myeloid tissue in connective-tissue septa. (x 100.)

Fig. 13. - Blood film from a case of myeloid leukaemia. There is a well-marked reaction both in polymorphonuclear and in mononuclear cells. (x 1000.)

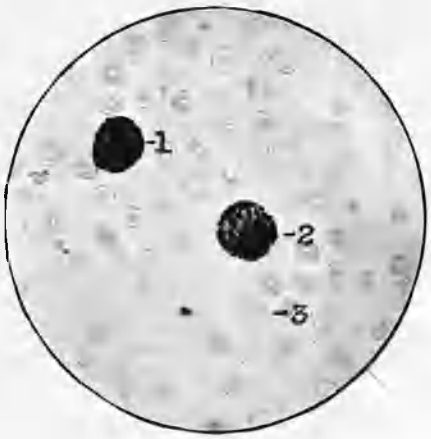


Fig. 1

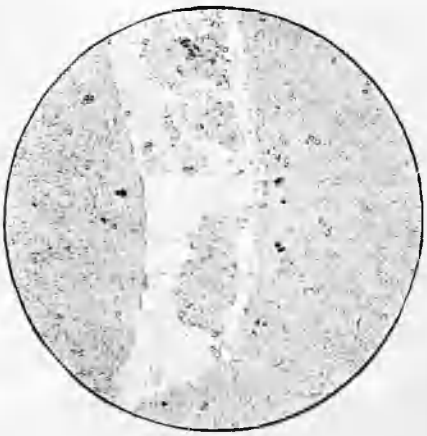


Fig 2

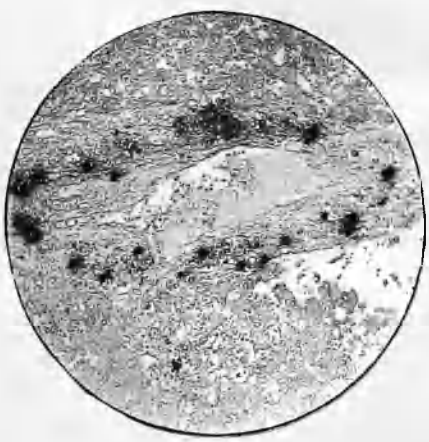


Fig 3

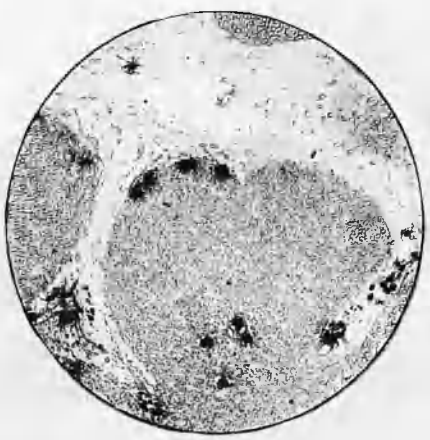


Fig 4

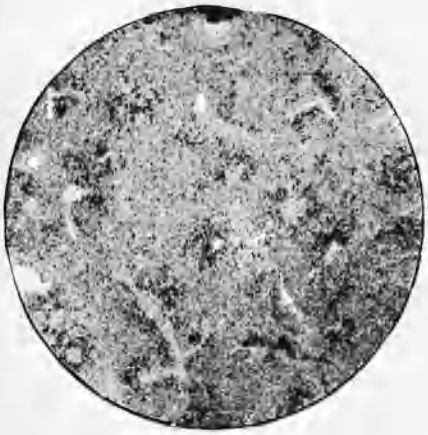


Fig 5

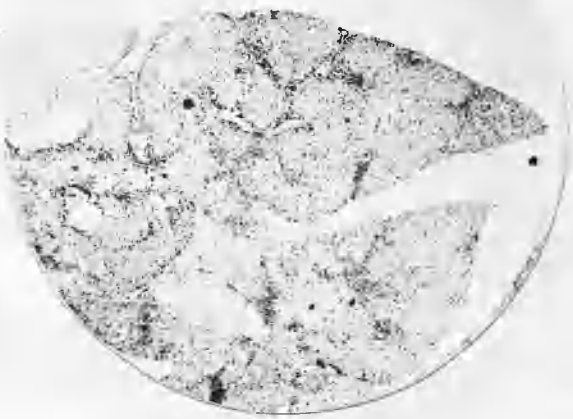


Fig 6

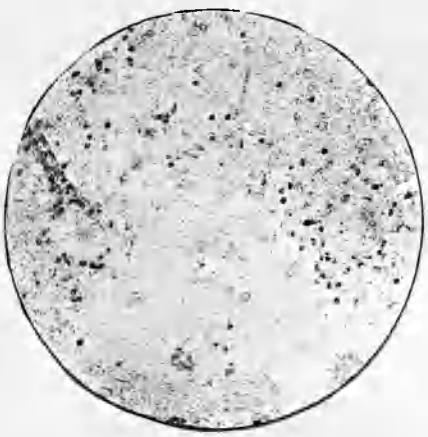


Fig 7

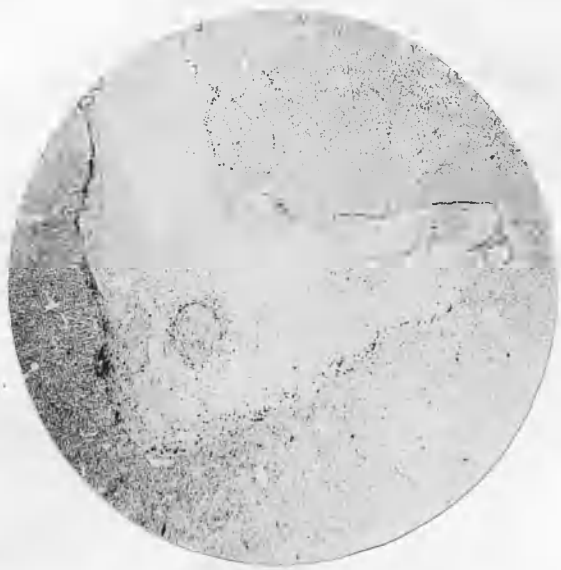


Fig 8

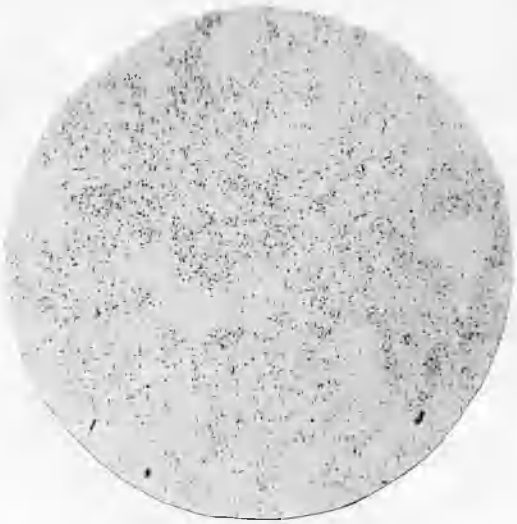


Fig 9

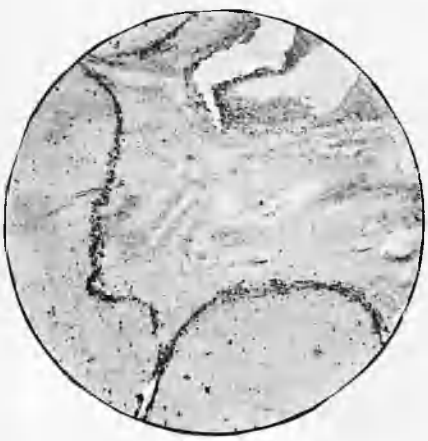


Fig 10

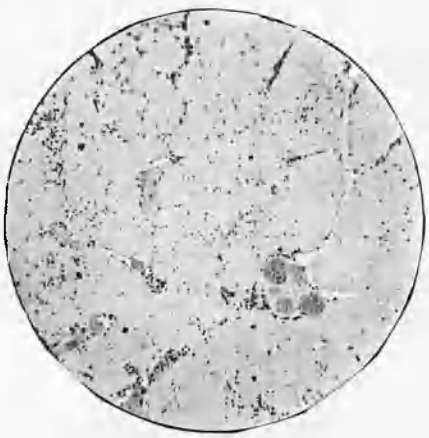


Fig 11

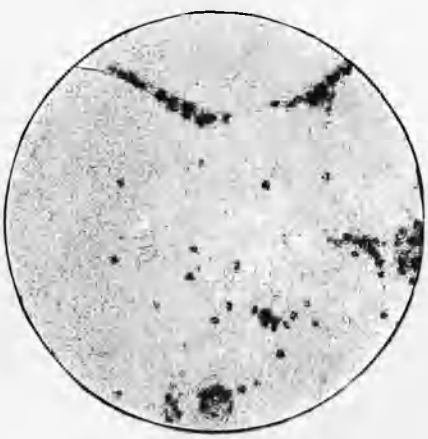


Fig 12

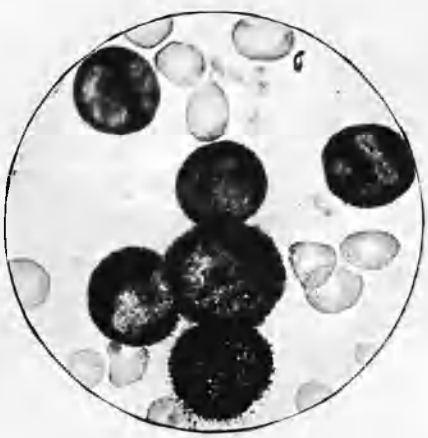


Fig 13

PART II.

THE USE OF THE OXYDASE REACTION. IN THE DIFFERENTIATION OF ACUTE LEUKAEMIAS.

Until a few years ago all cases of acute leukaemia were classified as lymphatic. It was recognised that in such cases the lymphatic glands and the spleen might show little evidence of a leukaemic change, while the bone-marrow might be extensively altered, but the fact that the leucocytes in the blood and in the organs were non-granular was proof, according to Ehrlich's view (1905)[4], of their lymphocytic nature, and the disease was regarded as essentially lymphatic.

An advance from this standpoint was first made possible after embryological research, had shown that the bone-marrow of the foetus is in its earlier stages, composed of non-granular cells, from which the granular myelocytes take origin later. This fact was found to explain the nature of certain puzzling cases of leukaemia with a "mixed" blood-picture; myelocytes and lymphocytes being both present together in excess: these "lymphocytes" were really the non-granular precursors of the myelocytes and their presence merely indicated a more than usually embryonic type of blood-formation, and not the co-existence of two different forms of leukaemia. (Lit. see Browning 1905[1]).

An/

An important contribution to this subject was then made by W.H. Schulze (1906)[18]. He described a case of acute leukaemia in which the leucocytes in the blood numbered 560,000 per cubic millemetre, and were almost exclusively large mononuclear cells with basophile, non-granular protoplasm: clinically an acute lymphatic leukaemia. By thorough histological examination of the organs after death Schulze was able to show that the lymphatic germ-centres in the spleen, lymph-glands and lymphatic tissues generally had in this case remained quite inactive and that a remarkable development of large non-granular cells had taken place in the bone-marrow, in the pulp of the spleen, and in the medullary parts of the lymph-glands. He concluded that these large cells were in reality non-granular marrow-cells and that the case was one of acute myeloid leukaemia, although only a very small proportion of granular leucocytes was present in the blood and blood-forming organs. Other cases of acute myeloid cases were subsequently described by various observers, though only those confirmed by anatomical proof were accepted as absolutely free from doubt. In some of these cases, even cases of very acute course, the blood-picture showed fairly large proportions of granular myelocytes and transitional stages in the development of these from myeloblasts, e.g. cases of Meyer and Heineke 1907[12], Fabian, Naegeli and Schatiloff 1907[5]. Only a few exhibited the extremely/

extremely lymphoid character of that describer by Schulze, e.g. case of Pappenheim and Hirschfeld (1908)[15]. With regard to the possibility of differentiating such cases as the latter clinically by examination of the blood there was always some dissension. The proportion of myelocytes present being small, might have been of the nature of the "irritation-myelocytosis" which may occur in lymphatic leukaemias. Reliance was placed by some observers on the finer histological characters of the embryonic leucocytes to distinguish myeloblasts, the non-granular marrow-cells, from lymphoblasts, the corresponding mother-cell of the lymphocytes.

Naegeli (1900)[13] who first named and described the myeloblasts stated that the nuclei of these cells possess 3 or 4 nucleoli: those of lymphocytes never more than two. This is certainly true in normal embryological blood-formation but it has been suggested that under pathological conditions this criterion may not be reliable (Butterfield (1908)[2]).

In general myeloblasts are larger cells and have a greater body of protoplasm than lymphocytes, but large forms of lymphocyte occur and small forms of myeloblast with narrow protoplasm are known (Naegeli 1900[13]), and may even form a large proportion of the cells in the blood in leukaemia (Isaac and Cobliner 1912)[8].

'Azure/

'Azure granules' of a certain type are peculiar to lymphocytes but they do not occur in all lymphocytes. Schridde's fuchsinophile granulation was at first supposed to be characteristic of lymphocytes, but it has later been shown that myeloblasts also contain granules stainable by the Altmann - Schridde method (Butterfield, Heineke and Meyer, 1909)[3].

The chromatin of myeloblast nuclei, as in myelocytes, is arranged in a fine regular network, while in lymphocyte nuclei it is in the form of coarser and more irregular bands, (Naegeli 1900)[13]. Whether this invariably holds good under extreme pathological conditions is not yet determined.

In view of the divergence of opinion on this subject it was suggested by Schulze (1909)[19] that the chemical behaviour of the cells might afford a surer means of differentiation, and, in fact, he found that in the case already referred to and in another similar case, the majority of the cells forming the leukaemic infiltrations in the organs, though chiefly non-granular, gave the indophenol-oxydase reaction, thus proving their myeloid nature. Confirmatory evidence of the value of this reaction has been obtained in a number of cases. Peters (1909)[17] obtained a positive oxydase reaction in "almost all" the large cells, in a case where the leucocytes numbered 57,000 per cubic millemetre and 96 per/

per cent were myeloblasts. Marchand (1911)[10] obtained a similar result in a case with 250,000 leucocytes per cubic millemetre, almost all large non-granular cells. In the case described by Isaac and Cobliner (1912)[8], where the blood contained 33,000 leucocytes per cm.m., with 8 per cent of myeloblasts and 81 per cent of cells resembling small lymphocytes, all of these cells were said to give the oxydase reaction in blood-films. The blood-formation in the organs was of the myeloid type as described by Schulze and showed a greater proportion of large myeloblasts than would have been expected from the blood-picture. In sections of the organs only the large cells gave a positive oxydase reaction.

A case described by Kahn (1912)[9] is noteworthy. In this case the blood showed 200,000 leucocytes per cm.m. and almost all were large non-granular cells. Of these "about half" gave a positive oxydase reaction of varying degrees of intensity. The myeloid nature of the case was confirmed by post-mortem examination. In discussing the case Kahn states that the absence of the oxydase from a large proportion of the myeloblasts is due to the "toxic effect of the leukaemic poison". He gives it as his opinion also that "if in an acute leukaemia even only a proportion of the leucocytes, which by ordinary staining methods are non-granular, give the oxydase reaction, this proves the occurrence of transitions between non-granular and/

and granular cells and the diagnosis of myeloid leukaemia may be made with certainty.

Thomas (1911)[21] has also observed absence of oxydase reaction in the large non-granular cells in the organs in a case of sub-acute myeloid leukaemia, in which the blood-picture was of the 'mixed' type.

It is noteworthy that since Schulze in 1906 first accurately differentiated acute myeloid leukaemia of the extreme lymphoid type, most, at any rate, of the acute large-celled leukaemias reported have also been found to be myeloid. It has even been suggested that all acute leukaemias are of myeloid nature (Scott 1907[20], Kahn 1912[9], Isaac and Cobliner 1912[8]). However, in a case of acute large-celled leukaemia described by Graetz (Case 3, 1910[7]) the large cells appeared to be derived from enlarged lymph-follicles and they gave no oxydase reaction. Graetz considered this case to be lymphatic. In another case of acute leukaemia described by Marchand (1911)[11], the cells in the blood were chiefly small lymphocytes, and they were found to take origin from lymph-follicles. Here also the oxydase-reaction was negative.

The total number of acute leukaemias reported in which the oxydase test has been applied is not yet very large and as these cases are not of very common occurrence opportunity has been taken to apply the test in some recently observed/

observed, and to control the results as far as possible by histological examination of the blood-forming organs.

CASE I.

R. McC. Male, aged 59 years, was admitted to the Glasgow Western Infirmary on October 27th, 1911, suffering from pains in the back, weakness and loss of weight of about three months' duration.

When examined on admission he was somewhat wasted and his skin showed a yellow pallor. There was evidence of enlargement of both the liver and the spleen. A haemorrhage was visible in the left retina.

The blood estimation gave:-

Red Blood Corpuscles - 2,030,000 per cm.m.
Haemoglobin - 53 per cent.
Leucocytes - ?

The red blood-corpuscles were well-coloured, and there was little polychromatophilia. Poikilocytosis was present. Megaloblasts and myelocytes were present. No differential count was made of the leucocytes.

On 3rd. November the blood count gave:-

Red Blood Corpuscles - 2,930,000 per cm.m.
Haemoglobin - 50 per cent.
Leucocytes - 13,000 per cm.m.

On 4th. November ·4 gramme salvarsan was injected intravenously.

8th. November. The patient's condition was worse.

Red blood Corpuscles - 1,460,000 per cm.m.
Haemoglobin - 40 per cent.
Leucocytes - 56,000 per cm.m.

10th. November. Symptoms progressively worse.

Temperature 103.2° Pulse 104. Resp. 32.

13th. November.

Red Blood Corpuscles	-	1,283,000 per cm.m.
Haemoglobin	-	32 per cent.
Leucocytes	-	203,000 per cm.m.

He died on 16th November, 1911.

THE EXAMINATION OF THE BLODD.

Films of blood obtained on three different dates, 8th, 15th, and 16th. November, were subjected to careful examination. Differential counts of 1000 cells each were made on films stained by Jenner's Eosin - Methylene-Blue and by Ehrlich's Triacid Stain, and use was also made of Pappenheim's Methyl-green Pyronin. This latter, a mixture of two basic dyes, gives the nuclei a bluish-green colour and stains basophile protoplasm red in a degree corresponding to the degree of basophilia. It is very useful in the recognition of myeloblasts of varying degrees of ripeness but as it does not stain eosinophile or neutrophile granules it is hardly suitable for differential counts. The results of the differential counts on the dates mentioned were as follows:-

8th. November Leucocytes, 56,000 per cm.m.

	Jenner	Triacid
Neutrophile Polymorphonuclears	5.4 per cent	6 per cent
Neutrophile Myelocytes	3.2 "	7.6 "
Eosinophiles	.2 "	.2 "
Basophiles		
Myeloblasts	89.6 "	82.8 "
Small Lymphocytes	1.6 "	3.4 "
	100.0 "	100.0 "

Nine/

Nine megaloblasts and twelve normoblasts were seen, while counting 2000 leucocytes.

15th. November Leucocytes 203000 per cm.m.

	Jenner	Triacid
Neutrophile Polymorphonuclears	3.1 per cent	4.9 per cent
Neutrophile Myelocytes	5.2 "	11.9 "
Eosinophiles		.4 "
Basophiles	1.4 "	.2 "
Myeloblasts	89.4 "	80.9 "
Small Lymphocytes	.9 "	1.7 "
	100.0 "	100.0 "

Five Megaloblasts and four normoblasts seen while counting 2000 leucocytes.

16th November.

	Jenner	Triacid
Neutrophile Polymorphonuclears	1.8 per cent	2.5 per cent
Neutrophile Myelocytes	3.8 "	8.3 "
Eosinophiles		
Basophiles		
Myeloblasts	93.0 "	87.3 "
Small Lymphocytes	1.4 "	1.8 "
	100.0 "	100.0 "

One megaloblast and four normoblasts seen while counting 1000 leucocytes.

It might be noted here that among the megaloblasts are included a few very embryonic forms, the protoplasm of which is markedly basophile and entirely lacking in haemoglobin. These cells are easily recognised by the character of the nucleus which is similar to that of the ordinary megaloblast, (c.f. Scott 1907[20], Pappenheim and Hirschfield 1908[15], and others).

The polymorpho-nuclear leucocytes seen in the films are for the most part of normal aspect, but in some the granules are more faintly stained than normal and in others the granules are diminished in number.

Under the heading of neutrophile myelocytes are included all mononuclear and transitional cells which contain neutrophile granules and while many of these have the usual characters of neutrophile myelocytes with well-developed granules, a considerable proportion of younger cells are seen in which part of the protoplasm is still basophile and the neutrophile granules few in number. It will be noted in a review of the various counts that the percentage of myelocytes is always greater in the count of a film stained with Triacid than in the corresponding film stained with Jenner, and this increase of myelocytes is balanced by an decrease of myeloblasts/

myeloblasts. This may be considered to be almost a rule in comparative estimations of the kind and depends on the presence of cells with granules which give the neutrophile tint with the specific neutrophile stain but which have too slight an acidophile tendency to pick up the Eosin out of the Eosin-Methylene-blue mixture.

The eosinophile and basophile leucocytes are present only in very small numbers as is usually the case in acute myeloid leukaemia. The cells counted as small lymphocytes are mononuclear cells with basophile protoplasm, and are seldom much, if at all, larger than red blood corpuscles.

All the remaining cells amounting to from 80 to 93 per cent of the total leucocytes in the various estimations, have been classed together as myeloblasts, the non-granular precursors of the myelocytes. As it is important to indicate to what extent these cells possess oxydase properties it will be necessary to describe them in some detail. Their diameter varies from 10 to 25 microns. Their nuclei, which in films stained by Jenner, are always paler than the protoplasm, are round, oval, indented or even sometimes lobed. With triacid staining the nuclei are pale-green in colour and closely surrounded by the protoplasm which is of varying shades of pale brown and shows no neutrophile granulation./

granulation. In films stained by Jenner the protoplasm appears reticular and more basophile than the nucleus but there is a certain lack of uniformity in individuals which renders necessary the recognition of three types. First. A small proportion, 3 per cent of the total leucocytes, are characterised by the density and uniformity of the basophile protoplasmic reticulum which stains deep crimson with Pappenheim's stain and deep blue with Jenner. In the nuclei of these cells 3 or 4 nucleoli are generally somewhat indistinctly visible. Second. The largest group, amounting to 68 per cent of the total leucocytes, have basophile reticular protoplasm, but this shows, generally over a small area only, an appearance of widening of the reticular meshwork sometimes accompanied by a very slight acidophile staining of the clearer matrix in the meshes, though no granules arevisible by any staining method. The area of protoplasm so affected often lies against an indentation of the nucleus.

The third group consists of cells similar to the second but with protoplasm which contains more or less numerous basophile granules. These granules are scattered throughout the protoplasm and they vary in size, the smallest being the size of neutrophile granules, the largest 3 to 4 times that size. With Jenner's stain they have a violet tint, with Pappenheim's stain they appear/

appear red or sometimes brown. These cells amount to about 22 per cent of the total leucocytes.

The separation of these three types is not, of course, intended to indicate any difference in nature of the cells but merely for convenience of description. They merge closely into one another and no doubt represent different stages of maturity of the one class of cell, as is indeed indicated by the oxydase reaction.

In films stained by this method for a short time, five minutes, it is found that the majority of the leucocytes show blue staining, some very densely, others very faintly, while all intermediate degrees of staining occur. A certain proportion remain entirely unstained. In films treated for longer periods the depth of blue-staining increases and the number of unstained cells is reduced to a minimum, but never quite abolished (Fig. 1). As a result of several estimations it is found that about 10 per cent of the leucocytes show no reaction. It is difficult to give an absolutely accurate figure as the leucocytes often lie in groups and those which give a strong reaction produce a certain amount of precipitation of indophenol on other cells lying beside them. By counterstaining with Pappenheim's stain it is seen that the cells which give no reaction are either small lymphocytes or myeloblasts/

myeloblasts with deeply basophile, protoplasm. These are believed to include the first type of myeloblast. Of the cells which give a reaction a large number showed only a faint diffuse blue staining of one area of protoplasm and often opposite to an indentation of the nucleus. These cells represent roughly the second and third types of myeloblast. The granular myelocytes and polymorphs are stained intensely blue, just as they are in films from chronic myeloid leukaemia.

The following is an account of the post-mortem examination, which was performed 24 hours after death, by Professor Muir.

External Appearances. The body is that of a powerfully-built man, slightly emaciated. The skin has a lemon-tint.

Thorax. The pericardial sac is normal. The heart is somewhat enlarged, especially the left side. The fat on the surface is moderate in amount. The arterial valves are competent. The mitral orifice measures 1·5 inches in diameter: the tricuspid 1·8 inches. The valve-segments are healthy. The left ventricle is slightly hypertrophied and dilated: the right ventricle is also slightly hypertrophied. The myocardium shows extreme patchy fatty degeneration.

Both pleurae are obliterated by fibrous adhesions. There are some fibrous nodules, the remains of old tubercular lesions, in the upper lobe of the right lung. The lungs/

lungs are oedematous. There is a patch of early pneumonic consolidation in the upper part of the lower lobe of the left lung.

Abdomen. An inguinal hernia is present on each side and there is some chronic peritonitis around the inguinal rings. There is no ascites.

The Liver is much enlarged and weighs 5 lbs. 14 oz. There are a few fibrous adhesions over its upper surface. The organ is of fairly firm consistence and on section is of a somewhat pale brownish-red colour. The portal tracts are somewhat more distinct than normal. A very intense reaction for free iron is given on testing with Hydrochloric Acid and Potassium Ferrocyanide.

The spleen is enlarged and weighs 1 lb. 3 oz. It is fairly firm in consistence and of rather pale reddish colour. The malpighian bodies are visible, but not prominent. A well-marked reaction for free iron is given,

The right kidney is somewhat enlarged, weighing 6 oz. The capsule strips fairly readily. The surface generally is of pale yellowish colour and there are numerous slightly prominent areas measuring $\frac{1}{8}$ to $\frac{5}{8}$ inch. in diameter, of whitish colour. On section these areas present ill-defined margins. They suggest foci of leucocytic infiltration. The colour of the kidney otherwise suggests the presence of fatty degeneration. The left kidney is similar in appearance, the whitish areas being less numerous. The

Stomach/

Stomach and Intestines present no change of note.

Bone-marrow. The bone-marrow in the right femur is of greyish-pink colour in the upper two thirds of the bone, the lower third being fatty. This grey marrow is moderately firm in consistence and has a somewhat translucent appearance. The rib-marrow is of pale-red colour.

Lymphatic glands. The deep cervical lymphatic glands are all somewhat enlarged, the largest measuring 1¼ inch long by ¾ inch in diameter. On section they are all of a pale dull greenish colour, which fades rapidly on exposure to the air. The axillary glands are of the size of small beans, of firm consistence and of greenish colour.

The mesenteric, retroperitoneal and inguinal glands are not apparently enlarged, but are of firm consistence. On section they are of pale greyish-pink colour.

The Tonsils are slightly enlarged and on section are of greenish colour.

HISTOLOGICAL EXAMINATION.

Portions of the various organs were fixed in 5 per cent formalin and in Corrosive Sublimate solution and some portions were fixed in Formol-Müller (1 in 10) for examination by the Altmann-Schridde method. The stains employed were Haemalum and Eosin, Triacid, Methyl-green-pyronin, Leishman's method for sections, and the Altmann-Schridde method. The Oxydase test was applied to sections of tissues fixed in formalin/

formalin and cut both by freezing and by the paraffin method.

The femur-marrow in sections appeared as a solid cellular tissue from which the fat had almost entirely disappeared. The cells composing it were of fairly uniform large size with abundant protoplasm. The great majority of them had basophile protoplasm and showed no granules, and they corresponded with the myeloblasts seen in the blood. Some of them showed mitotic figures. Eosinophile cells and neutrophiles were scattered irregularly throughout, but nowhere numerous. Nucleated red corpuscles were also seen throughout the sections. Megacaryocytes were few in number. The appearances in films of femur and rib-marrow confirmed the great predominence of myeloblasts and the scantiness of neutrophiles and eosinophiles. In the films stained by Jenner or Leishman numerous myeloblasts with metachromatically stained violet granules were seen as in the blood. The sections of marrow treated with the oxydase reagent showed blue-staining of almost all the cells. A few scattered cells stained more intensely than others and some small groups showed very little staining. (Fig. 2).

In the Spleen the lymph-follicles appeared small and were composed of small lymphocytes which contrasted sharply with the large round cells filling the pulp. The pulp was increased/

increased in amount so that the lymph-follicles appeared widely separated. These large cells in the pulp corresponded with those in the bone-marrow. A rather larger proportion of granular cells was present here, and nucleated red cells were also seen. A considerable amount of iron-pigment was present in the phagocytes. In sections stained by means of the oxydase reaction the pulp appeared almost uniformly blue and the unstained lymph-nodes stood out in sharp contrast. (Fig. 3).

In the Liver the portal tracts showed fairly extensive infiltration by cells similar to those in the marrow. Some groups of these cells could also be observed in dilated capillaries in the liver lobules. The liver-cells contained iron. The leukaemic infiltrations presented a striking picture in sections stained by the Oxydase method. (Fig. 4).

The pale nodules in the kidneys proved to be areas of massive myeloid infiltration. The great majority of the cells were myeloblasts, but granular myelocytes and nucleated red corpuscles were also seen. In sections stained by the oxydase method these nodules and the cells infiltrating the kidney generally stood out in sharp contrast to the unstained tubules. (Fig. 5).

The Cervical and other lymphatic glands, exhibited extreme myeloid infiltration of the medulla. In the larger cervical glands only a few small lymph-nodes remained, easily recognisable, by the small lymphocytes forming them, among the/

the masses of larger marrow-cells. The peripheral and medullary sinuses were filled with marrow-cells. In the smaller glands the amount of residual lymphatic tissue was greater. Its amount could be estimated with great accuracy in sections stained for Oxydase, where it appeared in the form of pale areas surrounded by the blue-stained myeloid tissue. (Figs. 6 and 7).

In sections of the bone-marrow and other organs stained by the Altmann-Schridde method no granules resembling those found in lymphocytes were observed in any of the large mononuclear cells.

There is no suggestion of development of genuine tumour-growth in relation to any of the infiltrations in the organs.

Summary.

The onset and course of this case is not that of the most acute forms of leukaemia, and though serious symptoms existed only for four months it is possible that leukaemic changes in the organs had been present with sub-leukaemic blood-picture for some time previously. However, the rise in the number of leucocytes in the circulation occurred with great rapidity, from normal limits to over 200,000 per cubic millemetre in ten days time, and the leucocytes present show very embryonic characters as nearly 90 per cent are, in Ehrlich's sense, non-granular. Examination of/

of the blood by ordinary staining methods certainly suggests that these non-granular cells are myeloblasts, and that the myelocytes are developing from them. A certain proportion of typical myeloblasts such as Naegeli described, and such as may be observed in the marrow or the liver of a five-months foetus, are present, and an unbroken chain of transitional forms may be observed between these and the granular myelocytes. The oxydase reaction is positive in the great majority of these cells and is positive in greatly varying degree in different examples, a fact which confirms the idea that transitional forms of many varying degrees of maturity are represented.

The histological examination of the organs shows that the type of blood-formation is myeloid, as described by Schulze, and the myeloid nature of the deposits is again fully confirmed by the positive oxydase reaction.

CASE II.

T.H. male, aged 13 years, was admitted to the Glasgow Western Infirmary, on December 1st, 1911, under the care of Dr. Barclay Ness. He was suffering from progressive general weakness of three months' duration, and had had severe headaches, ulceration of and bleeding from the gums, and also haematemesis. Ten days before admission purple spots suddenly appeared on the skin. The patient had tuberculosis of the left knee, with sinus-formation, of four years' standing.

On admission he was much wasted and his skin was very pale. A fairly profuse purpuric eruption was present. The gums were ulcerated and bled readily. The temperature was 99°, Pulse 116 per min., Respirations 24 per minute.

On physical examination the liver was found to be enlarged and palpable. The spleen also was enlarged and could be felt under the costal margin. There was no palpable enlargement of lymphatic glands. The urine contained albumin but no blood. Blood was present in the faeces.

The patient went rapidly down-hill. The temperature rose to 101°, the pulse rate to 136 and the respiration-rate to 32 per minute. He died on 3rd. December, two days after admission.

The Blood was examined on December 2nd.

The/

The Red Corpuscles numbered 2,200,000 per cm.m. The haemoglobin amounted to 40 per cent. Colour Index (·9) The leucocytes numbered 81,000 per cm.m.

Films were stained by Jenners Stain, Triacid, Methyl-green pyronin, Haemalum and Eosin, and by the Oxydase method. On examination of a Jenner-stained film the red corpuscles showed only slight poikilocytosis and variation in size. There was little polychromatophilia or granular degeneration. The red corpuscles stained fairly well with eosin. Four megaloblasts and two normoblasts were seen during a count of 1000 leucocytes and a few early megaloblasts without haemoglobin were observed throughout the films. The most striking feature was the presence of large numbers of large non-granular cells with basophile protoplasm which seemed at first to be the only form of leucocyte present.

A differential count of 1000 leucocytes on a film stained with Jenner gave the following figures:-

	per cent.
Neutrophile Polymorphonuclears	·6
Neutrophile Myelocytes	5.5
Eosinohpile Polymorphs.	·3
Eosinohpile Myelocytes	·2
Basophile (myelocyte)	·1
Large non-granular mononuclears	88·3
Smaller non-granular mononuclears	5.0
	100·

A similar count on a film stained with Triacid gave:-

	per cent.
Neutrophile Polymorphonuclears	2·0
Neutrophile Myelocytes	3·5
Eosinophile polymorphonuclears	·1
Eosinophile myelocytes	·4
Basophile polymorphonuclears	·2
Large non-granular mononuclears	88·5
Small non-granular mononuclears	5·3
	100·

The neutrophile polymorphs seen were of fairly normal appearance; in some the granules stained rather faintly. In some of the neutrophile myelocytes the protoplasm was faintly basophile, indicating immaturity. The large non-granular mononuclear cells, which constituted such a large proportion of the total, showed considerable variation in size, ranging from 12 to 30 microns in diameter. The nucleus in these cells was, with Jenner's Stain, paler than the protoplasm, and regularly showed from four to six definite nucleoli (Fig. 9). The nucleus occupied from 4/5 to 7/8 of the cell body, and was generally round or oval, but occasionally indented or even lobed. Mitotic figures were observed in a few in the course of many examinations. The protoplasm exhibited an intensely basophile reticulum without visible granulation, and surrounded/

surrounded the nucleus closely. It was somewhat labile and easily crushed. Vacuoles were present in some cells but Auer's bodies were never observed in specimens stained with Giemsa or Leishman's stain. In a very few examples meta-chromatic violet (pre-neutrophile) granules were seen. While in the great majority the protoplasmic reticulum was close and homogeneous, a certain percentage, amounting to 10 per cent. of the total leucocytes, exhibited a slight degree of loosening and transparency of it, so that the protoplasm appeared pale though still darker than the nucleus. This was regarded as an evidence of ripening of these cells.

Of the cells counted as small mononuclears the majority were simply small examples of the large ones already described but it was noticeable that the nucleoli were less easily seen in these cells. It is possible that some of them were ordinary small lymphocytes, but this could not be accurately estimated. (Fig. 8).

With triacid staining the protoplasm of the non-granular cells appeared brown and the nuclei bluish-green. With Pyronin-methyl-green the nuclei appeared green: nucleoli crimson, and protoplasmic reticulum crimson. With this stain also the cells with the looser reticulum appeared distinctly paler than the others.

From the histological features of the large cells in this case it was considered certain that they were myelo-blasts/

blasts, and that though only 6 per cent. of granular cells were present, the case was one of myeloid leukaemia. The oxydase test was then applied and careful estimations made extending over 4000 leucocytes in different films. The result of this investigation gave:-

		per cent.
Positive Reaction	Well-marked	12
	Faint	2
Negative Reaction		86

(See Fig. 10).

The reaction really showed great variations in the degree of intensity in the various cells, being most pronounced in the polymorphonuclears and in the myelocytes. In the 2 per cent which gave a faint reaction this showed as a slight diffuse bluing usually of one area of protoplasm and generally of the broadest part of the protoplasm, lying opposite an indentation of the nucleus. None of those cells counted as small mononuclears showed the faintest trace of a reaction.

As the total proportion of cells which gave a positive reaction (14%) exceeded the total proportion of granular cells (6 per cent) by 8 per cent, it was obvious that this 8 per cent. must be made up by some of the large non-granular cells, and it no doubt represents those non-granular cells in which the protoplasmic reticulum appeared looser/

looser. As those paler cells are distinguished from the great majority of the non-granular cells only by this protoplasmic character it seems certain that they represent transitional stages in the ripening of the latter towards the granular forms. In other words, the majority of the non-granular cells though they give a negative oxydase reaction, are embryonic myeloid cells, or myeloblasts.

Report of the post-mortem examination (performed 40 hours after death).

The body is that of an emaciated boy. The skin generally is pale and there is a fairly extensive petechial rash. There is some swelling and haemorrhage visible along the gums. An open sinus is present above the left knee, communicating with the interior of the joint.

Thorax. The pericardial cavity contains an ounce of blood-stained fluid. Both layers of pericardium exhibit numerous minute haemorrhages. The valves of the heart are normal: the cavities are slightly dilated: the muscle exhibits diffuse fatty change.

The pleurae are free from adhesions: each pleural sac contains 4 oz. of blood-stained fluid, and petechiae are present in both layers of pleura. The lungs are well-aerated and free from oedema: they exhibit no gross lesion.

Abdomen. The peritoneal cavity contains 2 ounces of blood-stained fluid, and minute haemorrhages are present here/

here and there throughout the membrane.

The Liver weighs 1¾ lbs. and does not appear enlarged. On section the liver substance shows evidence of diffuse fatty degeneration. The portal tracts do not appear enlarged. On treatment with Hydrochloric Acid and Potassium Ferrocyanide a very faint green colour is given indicating presence of free iron.

The Spleen, which weighs 12 oz. is much enlarged and its edges are rounded and the capsule tense. The organ crackles when handled, on account of the presence of bubbles of gas from a gas-forming organism. On section the pulp is of dark red colour and somewhat diffluent. The lymph-nodes are quite obscured. Iron reaction almost nil.

The Kidneys are pale and yellowish in colour from fatty degeneration: iron reaction negative.

The mucous membrane of the Stomach shows numerous petechial haemorrhages.

The Intestinal mucous membranes and the Peyers patches show no departure from normal.

The mesenteric and retro-peritoneal lymph-glands are not enlarged but are slightly firmer than normal.

The tonsils are slightly swollen and superficially ulcerated. The cervical lymphatic glands are very slightly/

slightly enlarged and are firm, oedematous and of dark-red colour.

The axillary and inguinal lymph-glands are not enlarged, but are slightly firmer than normal.

Bone-marrow.

The rib-marrow is pale-reddish in colour and very fluid and translucent. It contains little fat. The marrow of the left femur is of pale greyish-pink colour throughout, with areas of congestion and some small haemorrhages.

The left-knee joint shows a fairly extensive tuberculosis affecting chiefly the synovial membrane and involving the bones only superficially.

HISTOLOGICAL EXAMINATION.

Sections of the femur-marrow showed total disappearance of fat-cells. The whole tissue was made up of almost uniform large cells with basophile protoplasm packed closely together and supported by a very light reticulum, hardly visible. Mitotic figures were present in considerable numbers. In sections stained by Leishman's method a very few eosinophile and neutrophile myelocytes could be observed. Red corpuscles, a few nucleated, were also scattered quite irregularly. Very few megakaryocytes could be seen. The appearances in dry films confirmed the above. In wet-fixed films (corrosive sublimate) the fine reticulum of chromatin in the nuclei of the large cells was well shown. Films and sections treated by the oxydase method showed a very few scattered cells with positive result and these were for the most part eosinophile or neutrophile myelocytes. The proportion of matured cells was indeed much smaller in the bone-marrow than in any of the organs showing leukaemic infiltrations.

Spleen. The structure of this organ was to some extent altered by the presence of small gas cavities, but many areas were sufficiently well-preserved to show the pathological changes, and the staining properties of the cells were little impaired. The lymph-follicles were few in number and widely/

widely separated: of many only the atrophied remains were visible and they were composed of small lymphocytes only. (Fig. 11). The pulp contained a large number of red blood corpuscles, and also a large proportion of the large basophile mononuclear cells. These were scattered diffusely and in groups and were accompanied by small numbers of eosinophile and neutrophile myelocytes. In one or two places they occurred in dense round nodes of more active growth resembling follicles and these sometimes occurred near the remains of atrophied lymph-follicles, but could be readily distinguished from them: they possessed no central vessel. They could be seen to exert pressure on the surrounding tissues by activity of growth. The oxydase reaction was positive in a considerable proportion of the cells throughout the pulp: mostly granular cells but also faintly in some of the non-granular.

In the Liver the portal tracts contained rather small but dense accumulations of the large mononuclears, with a few granular myelocytes and nucleated red cells. The margins of these infiltrations were irregular and some accumulations of similar cells could be observed in dilated capillaries in the lobules. Mitoses were frequently observed. (Fig. 12). The oxydase reaction in sections of liver showed a small but unfiromly distributed proportion of positively reacting cells throughout the infiltrations

The/

The proportion of these certainly exceeded the small proportion ofgranular cells present. (See Fig. 13).

In the kidney there was a scanty distribution of leukaemic cells in places in the connective-tissue.

In the cervical and axillary lymphatic glands the lymphatic nodes were composed of small lymphocytes only and were of small size. The large basophile mononuclears appeared in considerable numbers in the medullary sinuses and also in the peripheral sinus, and were also observed in the gland-tissue in the immediate neighbourhood of these sinuses, where they were undergoing proliferation and forming in some places small round collections of cells, similar to those in the spleen and quite apart from the lymph-nodes. Numerous red corpuscles were also present in the sinuses and were seen being taken up by phagocytes there. The changes in some mediastinal glands were similar to those described. Some glands from the left groin showed merely fibrous thickening of the medulla, the result of chronic inflammatory change,and no leukaemic infiltration was recognisable.

In several mesenteric glands the lymphoid-tissue was almost entirely destroyed, but persisted in the form of irregular areas composed of small lymphocytes only. In the medullae of these glands there were numerous neutrophile and eosinophile myelocytes, and also nucleated red corpuscles.

These/

These cells were in greater abundance here than in any other tissue examined. They were accompanied by large numbers of the large non-granular mononuclears. The oxydase reaction was present in a very considerable proportion of cells in the infiltrations here. (Fig. 14).

The tonsils, which showed slight superficial ulceration, exhibited a change, which was at variance with the findings recorded elsewhere. The lymph-nodes here were enlarged, and were composed of large cells hardly distinguishable by any feature from those forming the infiltrations elsewhere in the body. Similar cells were also present in considerable numbers around the crypts and penetrating into the epithelial layers.

There is nothing suggestive of genuine tumour-growth in any of the organs.

SUMMARY.

This case presents a typical picture of acute leukaemia. Its brief course is marked by haemorrhages into the skin and from the mucous membranes, by fever and by ulcerative gingyvitis. The lymphatic glands are not enlarged. The blood-picture shows a considerable increase of leucocytes of which the great majority are large non-granular cells. These present the typical characters of Naegelis myeloblasts, and transitional forms between them and/

and myelocytes are present, though scanty. The oxydase reaction conclusively proves the myeloid nature of a certain proportion of these, which differ from the others only by a slight alteration of the protoplasm indicative of ripening. The absence of the reaction from the majority of them is to be explained by their very embryonic character rather than by pathological suppression of a function already developed. They correspond accurately with the first type of myeloblast described in Case I, which also gave negative oxydase reaction. The type of the blood-formation in the marrow, in the pulp of the spleen and in the medullae of lymph-glands, and the atrophy or inactivity of the lymph-nodes are confirmatory of the view based on the blood-examination that the case is one of acute myeloid leukaemia.

This case differs from Case I, not only in being more acute and of more embryonic type, but also in showing much less abvanced changes in the organs. In the cervical glands for example, there is little enlargement, and the condition of leukaemic infiltration is at an early stage, which renders possible some observations on its mode of occurrence. It is seen that the lymphatic tissue remains indifferent and is composed of small lymphocytes only: the myeloblasts are most numerous in the sinuses and in the neighbourhood of these: one or two foci of more massive development of myeloblasts which are present are also in the /

the neighbourhood of the sinuses. (Similar foci were observed in Spleen and in mesenteric glands). These appearances so far as they go are in favour rather of the metastatic origin of the myeloid tissue than of the metaplastic development of it from cells previously present in the organs.

CASE III.

For the blood-films from this case I am indebted to Dr. Matthew J. Stewart of the Pathological Department of Leeds General Infirmary. Unfortunately, no post-mortem examination was made after death, so that the organs could not be examined histologically.

The patient was a girl of 11 years of age, and presented symptoms of acute leukaemia. Accurate details are not available.

The blood-examination showed:-

Red-Blood Corpuscles	-	1,160,000 per cm.m.
Haemoglobin	-	45 per cent.
Leucocytes	-	250,000 per cm.m.

Differential estimations of the Leucocytes gave the following results:-

Jenner's Stain	1000 Leucocytes per cent.
Neutrophile Polymorphonulcears	2·2
Neutrophile Myelocytes	·3
Large Non-Granular Mononuclears	93.5
Small Lymphocytes	4.0
	100

Triacid/

Triacid	1000 Leucocytes per cent.
Neutrophile Polymorphonulcears	1·7
Neutrophile Myelocytes	·6
Eosinophile Polymorphs	·6
Large non-granular mononuclears	92·8
Small Lymphocytes	4·3
	100

Oxydase Reaction	1000 Leucocytes
Positive Reaction	3·3
Negative Reaction	96·7

Thirty normoblasts and four megaloblasts were seen during a count of 2000 leucocytes. Some early megaloblasts without haemoglobin were observed throughout the films.

In this case it will be seen that the Jenner and Triacid counts give almost identical figures:- 97 per cent of non-granular and 3 per cent of granular cells, and that the oxydase reaction is negative in all but the granular cells. Accordingly this reaction gives no indication whatsoever of the occurrence of transition between the two series, and so/

so far as it goes the nature of the non-granular cells remains in doubt.

When the histological features of the leucocytes are considered, however, it is found that these cells present perfectly definite myeloid characters: they correspond in every detail with those described in Case II. The only differences recognisable between the blood-pictures in these two cases are the smaller percentage of granular cells here, 3 per cent in place of 6 per cent, and the less frequent occurrence of definite transition, forms such as cells with looser reticulum or with metachromatic violet granules. Absolute proof of the myeloid nature of the case is impossible in absence of histological examination of the blood-forming organs, but the characters of the cells seem too definitely those of Naegelis myeloblasts to render possible any other explanation. This case would therefore appear to represent a stage of embryonic blood-formation at which the value of Schulze's Oxydase Reaction as a test for myeloblasts breaks down. But when one remembers the large percentage of negatively-reacting cells present in Case II, which was proved to be myeloid by histological examination, the occurrence of such a stage as this, can be recognised as a probability.

CASE IV.

E.R. female, aged $4\frac{1}{3}$ years, was admitted to the Western Infirmary, Glasgow, under the care of Prof. Samson Gemmell on November 11th, 1911. She had been suffering from weakness, loss of colour and wasting for nine weeks. Swelling of the glands of the neck had commenced three weeks before admission and there had been deafness for ten days. A rash of purple spots appeared on the skin six weeks before admission, and this was still present. She had had no previous serious illness and was always a healthy child.

On admission, the patient was very anaemic and a purpuric rash was present on the skin. The temperature was $106 \cdot 8^{\circ}$. Pulse rate 140 per min. Respiration rate 40 per min.

A systolic murmur, apparently haemic was audible at the base of the heart. The spleen was not palpable. The Liver was enlarged and its lower edge was palpable below the costal margin. On examination of the blood the following figures were obtained:-

Red Corpuscles	-	1,643,000 per cm.m.
Haemoglobin	-	38 per cent,
Leucocytes	-	9,375 per cm.m.

The patient died on November 12th, 1911.

A/

A few blood-films had been taken after admission, and one of these, stained with Jenner's stain was available for examination.

The red corpuscles were fairly well-stained and showed little poikilocytosis or granular degeneration. One normoblast (polychromatophilic) and one megaloblast were seen while counting 1000 leucocytes.

A differential count of the leucocytes (1000 cells) gave the following result:-

	per cent.
Neutrophile Polymorphonuclears	1·0
Neutrophile Myelocytes	1·3
Eosinophile (Polymorph)	·1
Large Lymphocytes	75·2
Small Lymphocytes	19·3
'Reizungsformen'	3·1
	100.

There was, therefore, here a great relative and absolute diminution of polymorphonuclear cells and the lymphocytes were absolutely increased though the increase was not striking and did not at first suggest leukaemia.

The cells counted as large lymphocytes were fairly uniform in appearance and measured from 12 to 16 microns in diameter. Their nuclei stained more faintly than the protoplasm, and usually showed a pretty definite single nucleolus: no/

no cells containing a greater number were observed. In three cells the nucleus was divided into two, but no mitotic figures were seen. The nuclei generally were round or oval and showed no lobulation. The protoplasm exhibited a fine basophile reticulum, less densely basophile than that of typical early myeloblasts. In many of the cells a paler zone of protoplasm was seen immediately surrounding the nucleus. In one cell a few fairly large round azurophile granules, of the lymphocyte type, were observed in the protoplasm (cf. Pappenheim 1911)[15]. These cells corresponded with the description of large lymphocytes given by Naegeli (1912)[14]. The small lymphocytes were identical with those of normal blood. The cells described as 'Reizungsoormen' or irritation-forms were of large size, 18-20 microns in diameter, and had large nuclei with abundant chromatin in a rather coarse network of broad bands, and deeply basophile protoplasm of a somewhat clouded appearance. They were most probably very early megaloblasts.

A post-mortem examination was performed 32 hours after death: the following is the report:-

The body is that of a fairly well-nourished child, with a thick layer of fat. There is some evidence of commencing wasting. The skin is very pallid and a few scattered petechial haemorrhages are present on chest and abdomen.

Thorax. The pericardial cavity contains an ounce of clear/

clear fluid. Numerous minute haemorrhages are present on both layers of pericardium.

The heart, weighing 4 oz. has normal valves. Patchy fatty degeneration of the papillary muscles is seen in the left ventricle.

The right pleura contains 2 ounces of faintly turbid fluid: the left pleura a similar quantity.

The right lung exhibits several small areas of collapse, of dark purple colour. There are also a number of haemorrhagic areas visible on the pleural surface associated with small nodules of consolidation with central necrosis. The left lung shows a similar condition.

Abdomen. The peritoneum is normal.

The Liver is of large size, weighing 2 lb. 14 ounces. Its surface is of pale yellow colour. On section the lobules are distinctly marked off and outlined by pale grayish tissue in the portal tracts. The liver substance shows marked fatty degeneration. On application of Hydrochloric Acid and Potassium Ferrocyanide, a well-marked reaction for free iron is obtained.

The spleen which weighs 3½ ounces , is of firm consistence and of dark red colour. The lymph-nodes are obscured. A faint reaction for free iron is given. The kidneys are slightly enlarged and of pale yellow colour. Several whitish areas with indefinite margins occur throughout the cortex. A faint reaction for free iron is obtained/

obtained.

The Stomach, Duodenum and Pancreas show no gross lesion. The lymphoid tissue in the intestines is not more prominent than usual and no ulcers are present.

Lymphatic glands.

The submaxillary glands, which can be felt through the skin, are as large as walnuts, and are firm in consistence. On section they have a homogeneous appearance, and pale yellow colour. The other cervical glands are small and appear pink and translucent.

The mediastinal glands are slightly enlarged and firm. Around the head of the pancreas the lymphatic glands are distinctly enlarged and on section are of yellow colour. The mesenteric glands and glands along the common and external iliac arteries are similar.

The axillary and inguinal glands are very slightly enlarged and appear pink and translucent.

The tonsils are enlarged and are superficially ulcerated.

Bone-marrow.

The rib-marrow is more fluid than normal and is of rather pale colour.

The marrow in the right femur is of dark-red colour and fills the whole cavity of the bone.

HISTOLOGICAL EXAMINATION.

Blood-films taken post-mortem from the heart showed a considerable increase of small and medium-sized lymphocytes. Definite enumeration was impossible. Some films were stained by the oxydase method. A positive reaction was given only by the few granular cells present: in these the reaction was well marked: no faintly staining cells suggestive of transitional forms were observed.

Bone-marrow.

Sections of the femur-marrow show almost total absence of fat-cells. The whole of the marrow is cellular and most of the cells are rather small, round cells with darkly staining nuclei. In sections stained by Haemolum and Eosin, or by Triacid, it is noticeable that unmixed accumulations of these small round cells occur in rounded masses around all the vessels (Fig. 15). The intervening tissue also contains many such cells, but is easily distinguished from these round-celled areas by the presence of abundant red blood corpuscles. With suitable staining (Leishman) a few neutrophile and eosinophile myelocytes, some nucleated red corpuscles, and a few large mononuclear leucocytes with basophile protoplasm are observed in the areas containing blood, but not among the perivascular lymphocyte accumulations. One or two degenerated megakaryocytes are observed.

In films the small round cells present the characters of/

of small and medium sized lymphocytes: their nuclei contain single definite nucleoli and in wet-fixed films (corrosive-sublimate) the nuclear chromatin is in the form of coarse bands. None of them show mitosis. The oxydase is given only by the granular leucocytes present.

Spleen. The lymph-follicles are slightly enlarged, and are fairly distinct, They are composed of small and medium-sized lymphocytes and have pale centres, which are not very cellular, containing only a few large lymphocytes. In the pulp a moderate proportion of small and medium lymphocytes.are present, but these do not form excessive infiltrations and the splenic structure is well-maintained. In addition the pulp contains a few eosinophile cells and a number of large basophile mononuclear cells (18-20 microns) in which occasional mitoses are seen. There is nothing to suggest that these are in any way connected with the lymphocytes, or that lymphocytes are produced from them. They generally lie in groups of 6 or 7 together. Possibly they may be myeloblasts and indicate adventitious myeloid formation, following destruction of the normal bone-marrow. Only the eosinophile cells give the oxydase reaction.

Kidneys. The pale areas in the cortex are composed of dense infiltrations of small and medium.lymphocytes, and a very few larger forms with more abundant basophile protoplasm.

The/

The Thymus is atrophic and shows no evidence of leukaemic infiltration.

The Liver in sections exhibits a remarkable degree of development of small and medium-sized lymphocytes in the portal tracts. These form infiltrations without definite margins and the cells extend out into the dilated liver-capillaries in large groups (Fig.16). The liver-tissue is in consequence much atrophied and the leukaemic infiltrations obviously constitute the greater part of the bulk of the organ. The cells are fairly uniform in appearance and correspond with those seen in the marrow. No mitoses are seen. Here and there occasional groups of eosinophile cells occur, and larger basophile mononuclear cells are scattered among the lymphocytes.

Only the eosinophiles give the oxydase reaction.

In the enlarged submaxillary lymphatic glands the structure of the organs is maintained. The follicles are distinct, and are increased in size and composed of small and medium-sized lymphocytes.

The medullary cords are also enlarged and composed of similar cells but there are present also many of the large basophile cells described in the spleen pulp.

The changes in the smaller glands from the neck are similar and in the periglandular fat, infiltrations of medium-sized lymphocytes are frequently seen. In none/

none of the cervical glands is there any very advanced leukaemic change and the same applies to the axillary and inguinal glands.

On the other hand the posterior mediastinal glands and the glands from near the pancreas and from near the external iliac arteries show very definite overgrowth of lymph-follicles, and medullary cords so that the whole gland-structure is in marked examples (pancreatic) almost entirely wiped out by homogeneous masses of medium-sized lymphocytes such as occur in marrow and in liver. There is no doubt from the appearances that these cells are developed from the lymph-follicles and are not the result of metaplasia or infiltration occurring from the direction of the gland medulla. (See Fig.17).

Schridde's fuchsinophile granules were observed in only a certain proportion of the small cells in the liver, marrow, and glands. They had the typical peri-nuclear arrangement of those occurring in lymphocytes.

SUMMARY.

This case is one of a fairly acute illness showing anaemia, purpura and fever. There is slight enlargement only of lymphatic glands: the liver is much enlarged. The examination of the blood reveals a condition which if not quantitatively still qualitatively is leukaemic. The cells present in greatest number have the characters of/

of small and large lymphocytes and granular cells are present in very small proportions. On post-mortem examination the main leukaemic changes are found in the bone-marrow, liver, and abdominal lymph-glands. The cells forming the infiltrations have the characters of lymphocytes and give no oxydase reaction. Though some of these cells in the blood and blood-forming organs are fairly large they never attain the dimensions of average myeloblasts and their nuclear characters always render them recognisable as lymphocytes. In the marrow they exhibit a certain tendency to follicle-formation and they appear to be growing into the marrow and destroying it from the direction of the blood-vessels. In the abdominal glands they are obviously derived from the pre-existing lymph-follicles, and do not occur as an infiltrative or metaplastic formation in the medulla. The case is, therefore, regarded as an acute lymphatic leukaemia or rather aleukaemia. There appear to be no grounds for supposing that the cells in any way correspond with the small myeloblasts described by Isaac and Cobliner 1912[8], in cases of acute myeloid leukaemia, or by Frank and Isaac (1912)[6] as a terminal development in chronic myeloid leukaemia. So far as the behaviour of the Oxydase reaction is concerned this case is only of interest in that it further confirms the absence/

absence of oxydase from cells of lymphatic origin.

CONCLUSIONS.

1. The occurrence of a positive indophenol-oxydase reaction in large non-granular cells in acute leukaemia is a certain proof of their myeloid nature and enables a diagnosis of acute myeloid leukaemia to be readily made from blood-examination.

2. The oxydase reaction is negative in the more embryonic forms of marrow cells. It is always negative in small myeloblasts and is probably always negative in the most typical stage of large myeloblasts with uniformly dense basophile reticular protoplasm. When it falls positive in these large non-granular cells it is associated with alterations in the protoplasm which are recognisable by ordinary staining methods and which indicate stages of ripening towards the granular myelocytes.

3. Cases of acute Myeloid Leukaemia may occur in which the type of blood-formation is so embryonic that the oxydase reaction is valueless for differential diagnosis, but even in such cases the histological characters of the large leucocytes may render a diagnosis possible.

4. Cells of lymphatic origin do not give the oxydase reaction.

REFERENCES FOR PART II.

1. Browning. Lancet. Aug. 19th, 1905.

2. Butterfield. Deutsch. Arch. 92, 1908.

3. Butterfield, Heineke and Meyer. Fol. Haem. Bd. VIII. 1909, p. 325.

4. Ehrlich. Diseases of the Blood. (Eng. Trans.) London 1905, p.544.

5. Fabian, Naegeli and Schatiloff. Virch, Arch. Bd. 190. H.3 p. 436. Case 10.

6. Frank and Isaac. Zeitsch, f. Klin. Med. Bd. 74. H.1&2, p.1.

7. Graetz. Beitr z. Anat, u.z. Allg. Path. Jena. Bd. XLIX. p. 338. Case 3.

8. Isaac and Cobliner. Fol. Haem. Bd. X. p.459

9. Kahn. Frankf. Zeit. f. Path. Bd. IX. p. 259.

10. Marchand. Münch. Med. Wschrft. 1911. p. 924.

11. Marchand. Ibid. 1911, p.1215.

12. Meyer and Heineke. Deutsch. Arch. f. Klin. Med. Bd.88, 1907 Case 17.

13. Naegeli. Deutsch. Arch. Bd. 92, 1908.

14. Naegeli. Blutkrankheiten and Blutdiagnostik. Leipzig. 1912. p. 173.

15. Pappenheim and Hirschfeld. Fol. Haem. Bd.V. p.347. Case I.

16. Pappenheim. Prolegomena. Fol. Haem. Bd. XI. H.1. p.1.

17. Peters. Münch. Med. Wschrft. 1909. p.1478.

18. Schulze. Beitr. z. Anat. u.z. allg. Path. Jena. Bd.XXXIX. p. 252.

19./

19. Schulze. Münch. Med. Wschrft. 1909. p.167.

20. Scott. Lancet. Nov.30th, 1907.

21. Thomas. Zeit. f. Klin. Med. Bd. 73. H.3,&,4, p.308.

ILLUSTRATIONS TO PART II.

Fig. 1. Case I. Blood-film: oxydase reaction. The polymorphonuclear leucocyte near the centre gives an intense reaction The other cells are stained with varying degrees of intensity: in two there is no reaction. x 500.

Fig. 2. Case I. Bone-marrow: oxydase reaction. The round clear areas are the diminished fat-cells. The pale areas are clumps of more embryonic myeloblasts. x 50.

Fig. 3. Case I. Spleen: oxydase reaction. Intense in almost all the cells in the pulp. Negative in the remains of the lymph-follicles. x 50.

Fig. 4. Case I. Liver: oxydase reaction. Intense in leukaemic infiltrations in portal tracts. x 50.

Fig. 5. Case I. Kidney: oxydase reaction. Indicates the distribution of myeloid cells in the connective-tissue. x 50.

Fig. 6. Case I. Large cervical lymphatic gland: oxydase reaction: pulp full of myeloid cells. Remains of lymph-follicles. appear unstained. x 50.

Fig. 7. Case I. Section of small cervical lymphatic gland: oxydase reaction. Pulp full of myeloid cells. Remains of lymph-follicles unstained. x 50.

(Sections 2 - 7 cut by paraffin process).

Fig. 8/

Fig. 8. Case II. Blood-film: Jenner's stain. Six typical myeloblasts are present characterised by the darkness of the protoplasm. x 500.

Fig. 9. Case II. Blood-film. Jenner's stain, to show nucleoli 3 - 4, in myeloblasts. x 500.

Fig. 10. Case II. Blood-film. Oxydase reaction. Positive in one polymorphonuclear and in a ripening myeloblast: negative in three other myeloblasts. x 500.

Fig. 11. Case II. Spleen: Invasion of pulp by large myeloblasts. (a) Atrophic remains of follicle with small lymphocytes. (Leishman) x 100.

Fig. 12. Case II. Liver: Portal-tract with leukaemic infiltration. Haemalum & Eosin. x 100.

Fig. 13. Case II. Liver: oxydase-reaction in fairly large proportion of cells forming a collection in a portal tract. Frozen section. x 100.

Fig. 14. Case II. Mesenteric gland: oxydase reaction in numerous cells in medulla (mostly granular cells). Frozen section. x 50.

Fig. 15. Case IV. Bone-marrow: To show invasion of marrow (dark with red corpuscles) by perivascular follicles of lymphocytes (paler). (Triacid) x 100.

Fig. 16/

Fig. 16. Case IV. Liver. Infiltrations of lymphocytes in two adjoining portal tracts. Intracapillary accumulations. Atrophy of liver cells. x 100.(Haemalum & Eosin).

Fig. 17. Case IV. Lymphatic gland from External Iliac artery showing overgrowth of lymph-follicles. x 100. (Haemalum & Eosin).

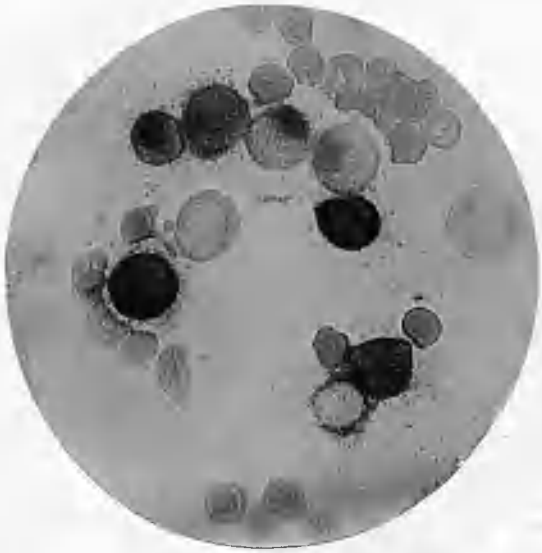


Fig 1

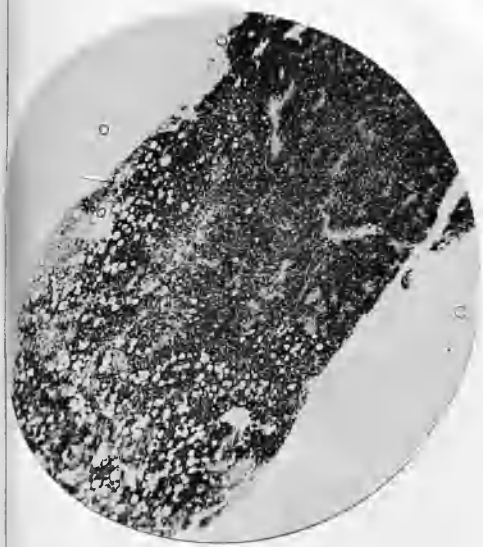


Fig 2

Fig 3

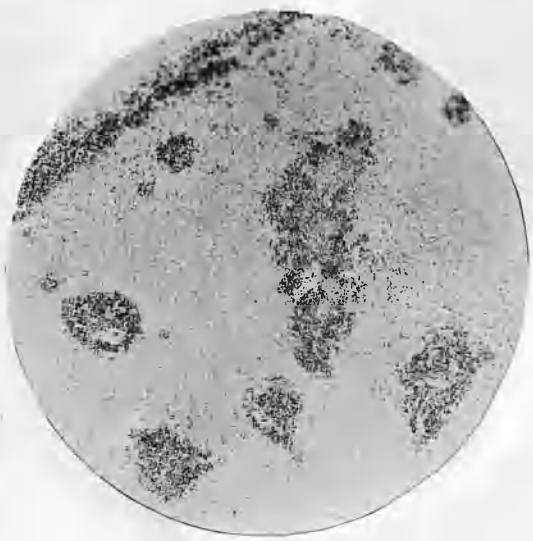


Fig 4

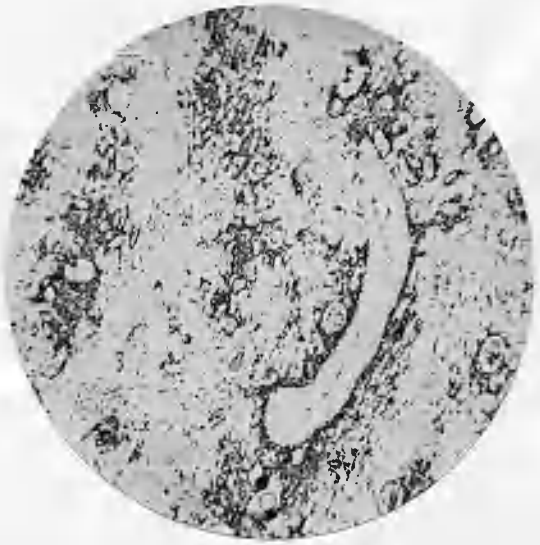


Fig 5

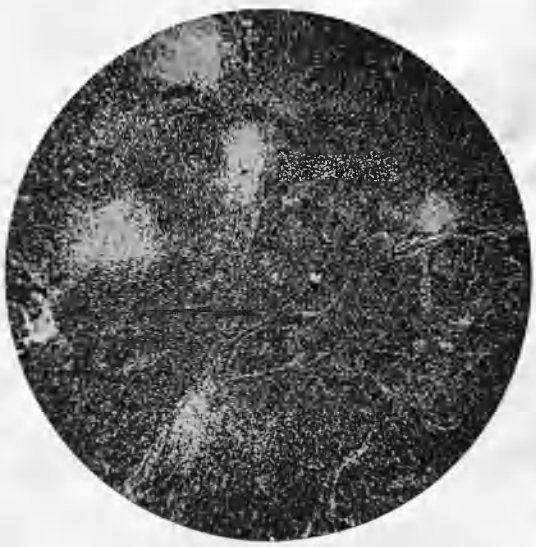


Fig 6

Fig 7

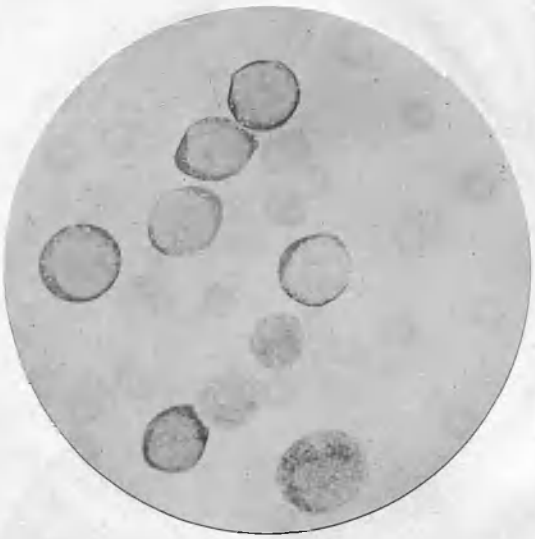


Fig 8

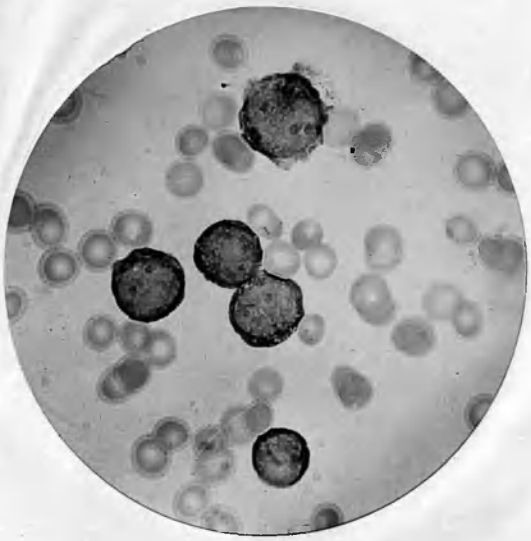


Fig 9

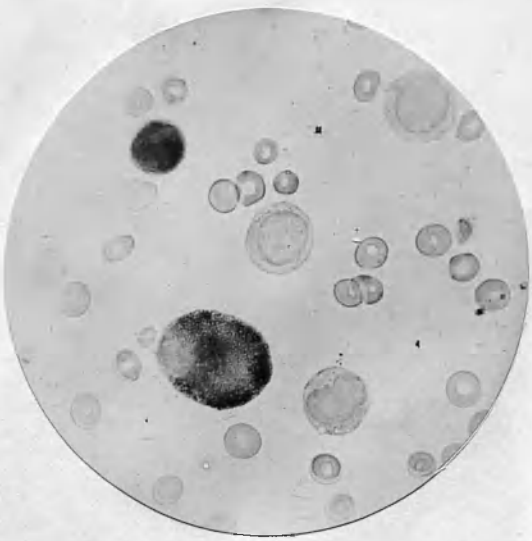


Fig 10

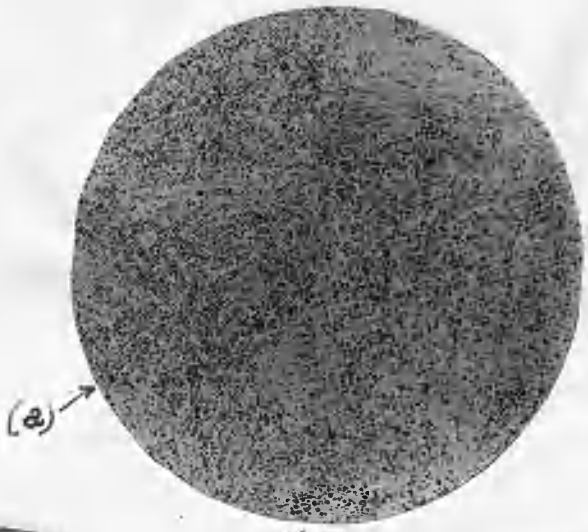


Fig 11

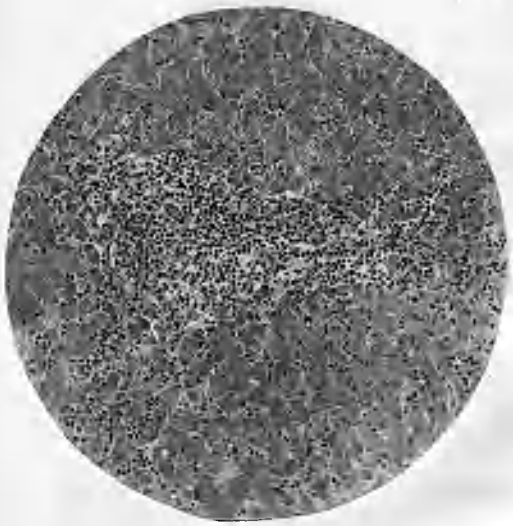


Fig 12

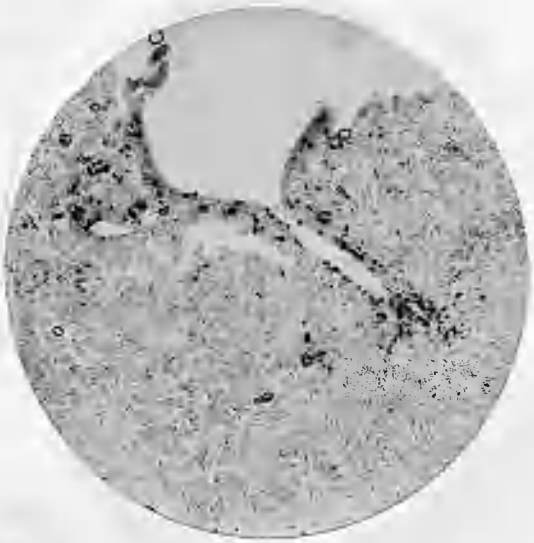


Fig 13

Fig 14

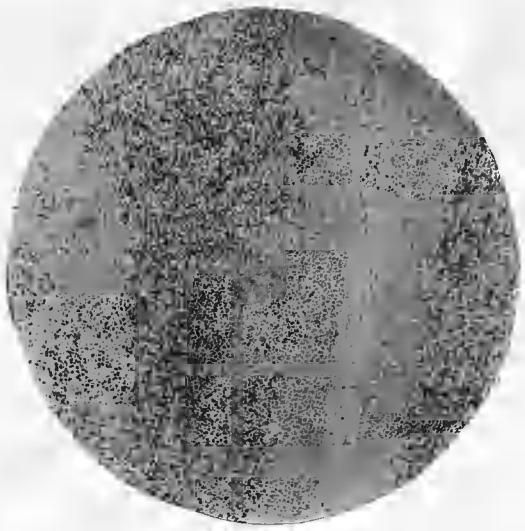


Fig 15

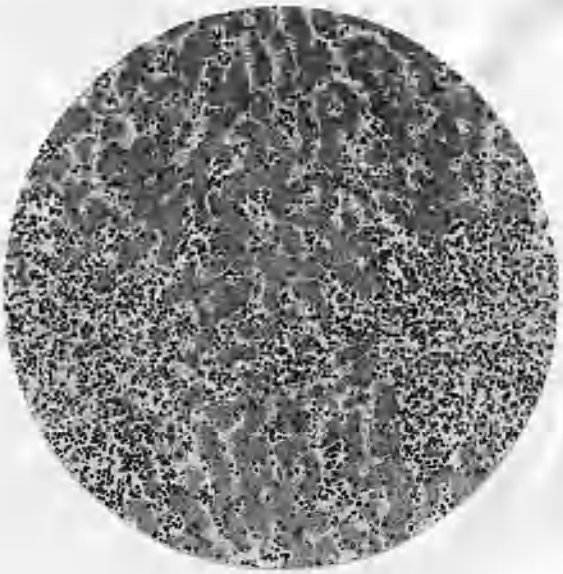


Fig 16

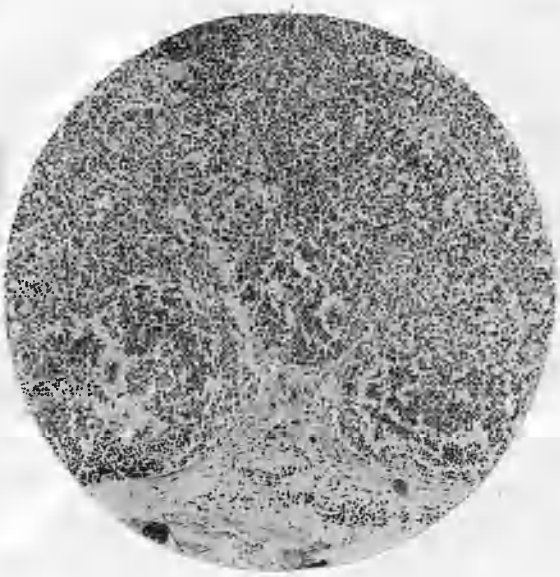


Fig 17