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THE BONE-MARROW.

A CYTOLOGICAL STUDY

forming

An Introduction to

The Normal and Pathological Histology

THE PATHOLOGY OF THE TISSUE.

*more especially with
regard to*

Blood formation, destruction, etc.

A Thesis

Submitted for the Degree of Doctor of Medicine in
The University of Edinburgh.

by

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FOREWORD.

FOREWORD.

This ^{volume} ~~thesis~~ represents the results of a portion of the work done during my tenure of the Crichton Research Scholarship in Pathology in the University of Edinburgh, ^{*} ~~^~~ The research ^{was} ~~has been~~ carried out in the Laboratory of the Pathological Department of the University, and to Professor Greenfield my grateful thanks are due, not only for granting me every facility in connection with the experimental part of the work, and for the free use of clinical and post-mortem material from his wards in the Royal Infirmary, but also for the unfailing interest he has shewn, and for much kindly advice he has given me, during the carrying out of my researches in his laboratory.

^{^ * *} I also wish to record my thanks to the Physicians and ^{Edinburgh Royal} Surgeons of the ~~^~~ Infirmary from whose wards I have been permitted to draw the greater number of my cases; and to ^{Professor Beattie of Sheffield,} Drs Shennan, ~~Beattie~~ and Stuart Mc^{Donald,} ^{of Edinburgh} the pathologists of the same institution, and to Professor Muir of Glasgow, for their great kindness and courtesy in allowing me to obtain the majority of the specimens with the help of which my work has been carried out.

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I have to thank Dr George Dean, of the Lister Institute of Preventive Medicine, for the diphtheria and tetanus toxins and antitoxins used in the experimental part of the work.

Lastly, it is my pleasant duty to express my indebtedness to Mr Richard Muir, ^{Demonstrator of} ~~of the~~ ^{Technique in the} Pathological ^{Department of the University,} ~~Department of the University,~~ for the exceedingly beautiful series of water-colour drawings and micro-photographs, by means of which ^{the book} ~~my~~ Thesis is illustrated. That they are the work of Mr Muir is more than sufficient guarantee for their accuracy, and he has my sincere thanks for the unstinted labour and skill which he has expended upon them.

W. E. C. D.

From the Pathological Department.
University of Edinburgh
1907.

GENERAL INTRODUCTION.

The Bone-Marrow

3.

Introductory Remarks:

General Introduction

The vital importance of the functions possessed by the bone-marrow in a state of health, and the rapidity and profound nature of its reactions and variations in disease, are as yet but too little realised in many of the departments of medical science; and in view of the important rôle which it plays in the life of the organism, the physiology and pathology of this tissue call for further research and a fuller elucidation of its structure and functions under both normal and abnormal conditions.

The comparative neglect with which, in the past, the study of the marrow has been treated, is perhaps to be explained by the old and popular idea - and it is an idea which it is even yet difficult to eradicate - that the bones are fixed and permanent structures, which alter only in their size and shape with the growth of the body; and in their structure, or "molecular condition" as it is usually expressed, and "become more brittle" as age advances. In other words, the more obvious, and certainly very important mechanical functions which the osseous system has to perform, have masked, and in great part prevented due attention from being bestowed upon the other equally, if indeed not more vital, rôle which this tissue has to play - that of producing the great majority of the formed elements of the blood.

So far indeed from being unchanging in their structure, the bones are, with the exception only of the blood itself, perhaps the most unstable tissue in the body; and in them alterations may occur not only in the softer parts of their structure - the marrow - but may be produced with most remarkable rapidity in the hard and apparently unyielding osseous portions proper.

When it is remembered that the bone-marrow is the parent tissue of the red blood corpuscles and of by far the greater proportion of the leucocyte series of formed blood elements; and when it is borne in mind with what extreme readiness these cells may undergo variations, both physiological and pathological, - as witness the vast field of knowledge being but now opened up by modern haematology, - an attempt to describe and classify the more essential of these changes in the marrow may help in the elucidation of many of the phenomena of disease, which can be studied at the bedside in the living body, or after death in the post-mortem room, and which constitute one of the most effective and important lines of defence which the organism has at its call in the fight against the inroads of disease.

Again, from a general study of the reactions of the marrow in various pathological conditions, it may here be confidently stated that many diseases which have hitherto been regarded as primary affections of the osseous system, will come in the immediate future rather to be looked upon as the efforts of a blood-forming tissue to react against some general toxic substances or agency, introduced perhaps from without, or manufactured, it may be, by disease in some tissue or organ at another part of the body, the chief changes so produced being usually a rarefaction brought about by an active absorption of bone, in order to allow of the expansion of the proliferating marrow in its interior. In this category will in all probability come to be placed such morbid conditions as the so-called cases of osteoporosis, osteomalacia, osteitis deformans, and analogous diseases, whilst pernicious anaemia, and perhaps also the leukaemias are even now definitely recognised by some writers and teachers as conditions in which the characteristic changes in the blood are brought about as a specific defensive reaction of the marrow, incomplete it may be and perverted, but with its main object the protection of the organism, and the destruction of the hostile substances invading it.

From a study of the marrow, an explanation of the severe secondary anaemias occurring in the course of such diseases as syphilis, lead and arsenical poisoning, Bright's disease, carcinoma and tuberculosis, is to be discovered in the fact that there may occur in these conditions, in addition to the already recognised factors at work in their production, a wide-spread and often profound degeneration of this tissue, a condition which may supervene upon any prolonged or excessive hyperactivity of the haemopoietic functions of the marrow; and to a similar state of exhaustion of the blood-forming glandular system - for such in reality it is - may also be attributed the post-febrile anaemias so typical of acute infective fevers, such as pneumonia, diphtheria, etc., in addition to the destruction of formed elements of the blood due to the increased haemolytic processes which accompany and characterise these diseases.

HISTORICAL RÉSUMÉ.

Historical Resumé:

In 1873, Golgi⁽⁵³⁾ investigated and described the naked-eye and other modifications of the bone-marrow in cases of smallpox, shewing that in this disease an intense reaction is set up, and also that giant cells are found in considerably increased numbers. Five years later, in 1878, Busch⁽²⁰⁾ described a case of osteomyelitis in the dog, in which he found that the marrow in the bones of the limb on the side opposite to that affected by the disease became "red, soft and lymphoid"; and in 1881-4 Grohe⁽⁵⁴⁾ described the condition of the marrow in a large number of diseases. Ehrlich⁽⁴⁰⁾ in his first paper (1878) had described what he termed the specific granulations of blood and other cells, and this great observer and his pupils subsequently proved that the different varieties of granular leucocyte arise from the corresponding granular cells of the marrow, and hence that it is only by an increased functional activity of the latter tissue that the number of these cells can be increased in the blood. In 1895, Timofeiewsky^(*) inoculated dogs and rabbits intravenously with various toxins, and succeeded in producing the appearance of nucleated red corpuscles in ^{the} peripheral circulation, thus

* Quoted by Dominici⁽³¹⁾

proving the presence of a profound reaction in the marrow; and in the following year Trambusti⁽¹²⁹⁾ demonstrated in guinea-pigs the presence of increased activity of the tissue with diphtheria toxin. In the same year Dominici⁽²⁹⁾ shewed that various septicaemic conditions could produce a similar effect, and Ribbert⁽¹⁰⁸⁾ described a diminution in the fat and a corresponding increase in finely granular neutrophil myelocytes. About this time a great number of contributions to literature on the marrow was made, principally by members of the French school, among whom Roger and Josué⁽¹¹¹⁾, Dominici,⁽²⁸⁾ Weil⁽¹³⁴⁾, Jolly⁽⁶⁸⁾ and others have contributed the most valuable work; whilst Haushalter and Spillmann,⁽⁵⁸⁾ investigating the marrow from cases of infantile disease and from experimental conditions produced in young animals; and Pappenheim⁽²⁰⁾, whose researches are chiefly concerned with the origin of the red cells in the embryonic tissue; have also made valuable contributions to the subject. To the work of some of these observers I shall refer more in detail when considering the special departments of the subject into which my paper is divided; and in the same way I shall refer elsewhere to the more important and more recent work which has been done by Muir,⁽⁸⁸⁾

Ferguson,⁽²⁵⁾ Stockman and Charteris,⁽¹²⁵⁾ and
others in this country; and by Jackson⁽⁶⁷⁾ of
Missouri, (working in Leipzig) and Longcope⁽⁷⁹⁾ in
America.

MATERIAL AND METHODS.

B.T.

Material and Methods:

In the course of this research I have cut and examined microscopically the bone-marrow obtained both post-mortem and by operation, from the femur, ribs, and other bones in a set of over eighty representative cases of disease in the human subject; while to obtain a proper idea of the histological appearances of this tissue under normal conditions, specimens obtained from cases of accident have been utilised, careful inquiry being made as to the previous health of the patient. The nature of the cases so employed, together with the ages of the various patients and the duration of the illness, whenever such details were ascertainable, will be found recorded for each case in Appendix I. at the end of this volume.

As may be inferred from the table, the research was at first more or less confined to the investigation of the changes produced in the bone-marrow by pneumonia, and by various conditions allied to it, but as the work proceeded, it was found necessary to extend the scope of it in order to compare and contrast the results so obtained with those found in other diseases.

In each case the following procedure was as far as possible adopted. The upper part of the shaft of the femur was excised, the bone being sawn through at the level of the lower margin of the small trochanter, and at a point usually varying from one to two inches below the level of the centre of the shaft respectively, the piece of bone so removed being found almost invariably to shew all gradations and stages of any pathological condition present. From each end of the piece of bone so excised, several thin transverse sections were then cut with a fine, rapidly revolving circular saw, one of these being fixed by the Kaiserling^{*} method as a naked-eye specimen, and the other pieces being used for purposes of microscopic examination. The remaining cylinder of bone was then sawn up longitudinally in an antero-posterior direction through the line of the linea aspera and dealt with in a similar manner, part being preserved in Kaiserling for purposes of naked-eye demonstration, and part being prepared for section work. Pieces of two ribs, usually the third and seventh, for the sake of uniformity, were also excised, the line of section corresponding roughly with the mid-axillary line posteriorly, and the costo-chondral articulation in front. With the aid

* Pick's formula is preferable, & gives better results.

of strong bone-forceps, the marrow was then forcibly expressed from both ends of the pieces of rib so excised. Film preparations of the marrow so obtained from the femur and ribs were prepared, some being fixed wet in various fixatives, and others dried, fixed by heat, etc. as described later in detail, while other pieces of the tissue were set aside for section work. The method of spreading bone-marrow films employed is of considerable importance, as in some cases difficulty may be experienced in obtaining a uniform layer of the tissue in which the various cellular elements are regularly spread and separated from one another. After trying many different methods of procedure, the best results were obtained by putting the drop or fragment of marrow on a slide and mixing it well with a piece of glass-tubing drawn out until its diameter is about 1-2 m.m. This thin piece of glass tubing is then used to lift a sufficient quantity of the tissue, and, by the method illustrated in the diagram, a thin even film can be made by drawing it over the surface of the slide or coverglass. Many marrows in pathological

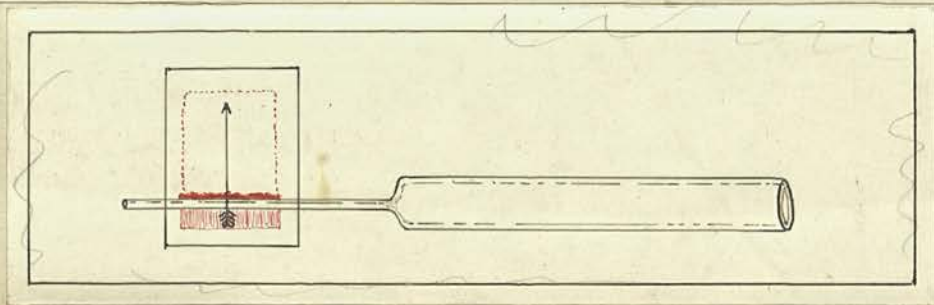


Diagram (1)
 This is a drawing
 of glass tube,
 and the drawing
 if it may be
 improved if
 possible.

conditions are sufficiently fluid to allow of their being thus spread without the least difficulty. Should, however, any such difficulty be experienced, it is usually due to the presence of fat, and it was found that the simple expedient of warming the marrow for a minute or two at body temperature was generally quite sufficient to obviate this difficulty, or should this not be sufficient, the addition of a little warm saline solution is all that is required. Films so prepared were then dropped face downwards into the fixative, and for this purpose I found that a saturated solution of corrosive sublimate in normal saline gave the best and most uniform staining results, a 10% solution of formalin in absolute alcohol also being useful; and these fixatives I also used for the pieces of tissue set aside for section-cutting purposes, the paraffin impregnation method being that usually employed. Dried film preparations were also made, and stained by Jenner's, Leishman's and other methods; or these were fixed by heating for an hour at 115°C. and stained by Ehrlich's triacid mixture, etc. The routine stains employed for wet films and for paraffin sections were methylene blue and watery eosin, methylene blue and alcoholic eosin, ~~and~~ Ehrlich's triacid mixture, ^{and} other stains such as Benda's saffranin and licht Grün process, Pappenheim's pyronin method, picro-erythrosin, etc. In every case where it was possible, the marrow from the

femur was cut without decalcifying, this being inadvisable, even at the risk of each time ruining the edge of the section knife, in order to ensure uniform staining reactions, and to allow of a comparison of the results with those obtained in the case of other tissues. To facilitate the manipulation of red marrow while preparing it for embedding in paraffin, I employed the ingenious method suggested by Muir, ⁽⁹⁰⁾ who expresses the drop of marrow from the rib on to the surface of a small square of paper, and while resting upon this, it is fixed and carried through all the necessary processes, the whole being finally embedded in paraffin together, after which the paper can be easily shaved off with a knife before cutting the sections.

Experimental Work in Animals:

The experimental work in connection with this research has been conducted mainly with rabbits, as the marrow in these animals is specially suitable for the study of the pathological changes so produced; while the marrow from the guinea-pig, rat, mouse and horse has also been examined in various conditions, but for reasons to be more fully explained later, these have not been found so suitable for the purpose. The chief pathogenic substances and organisms employed to bring about alterations in the marrow were diphtheria toxin, the pneumococcus, bacillus coli communis, and the tubercle bacillus, while some of the very acute toxic changes described later were produced by snake venom. Rabbits in good condition and of as nearly as possible the same age and weight have been used throughout, many animals having to be rejected, chiefly owing to the changes found to be produced by coccidiosis and cestode cysts. When death did not occur in the course of the experiment, the animals were killed by severing the spinal cord in the neck. The femora were then dissected out and the shafts split longitudinally, a cylinder of marrow being removed from

the centre of each. One of these pieces was at once fixed for section work, and the other was utilised for observations on the fresh tissue and for preparation of films, the methods of fixation and staining being the same as those already described for the treatment of human marrow.

During the period devoted to this work I have cut and examined more than four thousand microscopic preparations, over three thousand of which are from the human subject, and some eleven hundred from the animals detailed above.

GENERAL ANATOMY AND HISTOLOGY

OF

THE BONE-MARROW.

Before proceeding to the consideration in detail of the cytology, and of the normal and pathological variations that may be found in the relative numbers of the cells in human bone-marrow, it will be well first to give a brief outline of the general anatomy and histology of the tissue, together with a résumé of its more important physiological functions; and in order that we may the more easily understand the somewhat complex structure of the marrow in the human subject, we shall begin our study with an account of the simpler and more easily demonstrated, yet on the whole very similar structure of the tissue as we find it in the bones of some of the lower animals, and of these, for laboratory purposes, the rabbit is most suitable and convenient.

The relative proportions of marrow cells and of fatty tissue are found to vary in different animals within very wide limits. For example, in the case of the guinea-pig, the marrow tissue of the femur is red and soft, being almost entirely cellular in formation, and containing very little fat. On the other hand, the marrow from the centre of the shaft of the femur or other long bone is, in the adult human subject, composed almost entirely of fatty tissue (see photo No. 12); while in the rabbit it occupies a somewhat intermediate position with regard to the

relative proportions of these two constituent elements, as may be seen from photo No. 1.

Richardson
On splitting the shaft of the rabbit's femur longitudinally, it is found that the enclosed cylinder of marrow can be easily separated from the interior of the bone of the shaft, except at the point of entrance of the medullary artery, and also towards the ends of the bone where there are large cancellated spaces into which the marrow passes. From the inside of the shaft itself, however, the marrow easily strips, carrying with it the endosteum, a delicate membranous structure which gives a smooth, glistening appearance to the surface of the marrow, which is normally pinkish red in colour. Considerable care has to be exercised in the extraction of the marrow, as the tissue is soft and friable, and is very easily ruptured. In some pathological conditions it may become almost fluid in consistence, and the colour may undergo considerable variations, becoming in some diseases much redder and in others much paler, than normal. On transverse section, the central blood sinus of the marrow may be seen as a small dark red point. The medullary artery, after passing obliquely through the bone, usually divides into two main divisions, each running towards either

Alp 5

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end of the bone, and gradually coming to take up a more or less central position in the marrow. This central artery gives off smaller branches which pass out radially, occasionally almost at right angles to the parent trunk, sometimes more obliquely; and it may also divide into two or more primary branches, especially as it approaches the more cancellated parts towards the ends of the bone.

The central artery, for the greater part of its course through the marrow, is usually accompanied by a large, very thin-walled venous channel or sinus, which receives innumerable smaller tributaries running more or less radially from the periphery towards the centre of the marrow, a condition which may be very well seen when these channels are engorged with blood in the early stages of the acute diseases, as is shewn in photographs no. 20, 22, 3 and 5, from cases 34 and 61, and experiments 7 and 23 respectively. This sinus may be found partially surrounding the central artery, as described by Roger and Josué, but it often simply runs parallel to, without actually embracing the artery, and may even be at some distance from it. The radial venous channels or capillaries, already alluded to above, anastomose freely with one another and form a very intricate network,

the smaller tributaries of which appear to open directly into the lymphatic spaces in which lie the marrow cells proper. I have been unable to distinguish any difference in the relative positions of the myelocytes and the erythroblasts to these channels, though some observers allege that the former are extra- and the latter intra-vascular in their position, these two varieties of cell appearing, as far as my observations go, to be inextricably intermingled with one another.

In transverse section, the marrow may, for convenience in description, be mapped out into three concentric zones:

1. Central or vascular area, containing the thick-walled medullary or central artery of the marrow, together with the accompanying venous sinus, nerves, lymphatics, and connective tissue.

2. Intermediate zone, or marrow tissue proper, lying between the central and peripheral zones, and consisting of a delicate adenoid reticulum composed of branching and anastomosing connective tissue cells and fine connective tissue fibrils, in the meshes of which lie the marrow cells or myelocytes, etc., to be described in detail later. In this

layer are also found the fat cells which constitute in the rabbit a considerable part of the whole.

3. Peripheral zone, or endosteum, composed of a condensation of the connective tissue framework of the marrow - the delicate, membranous structure to which allusion has already been made in describing the removal of the marrow from the interior of the bone.

This layer of tissue appears to be, both in structure and functions, somewhat analogous to the inner or formative layer of the periosteum; and its cells take an active part, not only in bone formation and regeneration, but also in the absorptive changes which will be described later, when dealing with leucoblastic and other reactions of the bone-marrow.

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VARIETIES OF BONE-MARROW.

1 B. T.

by Van der Stricht⁽¹²⁸⁾, Duval,⁽³⁹⁾ and Stöhr,⁽¹²⁷⁾ and in a specimen of a three months' human foetus kindly lent me by Dr H. D. Shepherd, I have been able to observe the commencement of this process of transformation in the femur and fibula at that early period. Some authors, however, for example Kölliker,⁽⁷⁶⁾ hold that the myelocytes or marrow cells proper are developed directly from the branching myxoid cells of the primitive or embryonic marrow. This however I do not believe to be the case, and my own observations tend to confirm the view that from these cells is formed the adenoid reticulum which forms the framework and is characteristic of the marrow, as well as of the other blood-forming glands.

2. Red, "lymphoid", or formative bone-marrow proper, is found in the short and flat bones, sternum, ribs, vertebrae, etc., and also to a varying extent in the cancellated tissue at the ends of the long bones of the extremities in the adult. In the young child it also fills the medullary cavity of the diaphyses of the last named variety of bone, but as age advances it is gradually in this situation transformed into yellow or fatty marrow. It is in this red bone-marrow that the haemoglobin-holding elements of the blood are developed, as are also the granular

and perhaps some of the other forms of white blood corpuscles; and according as the number of the cellular elements which go respectively to form the red or the white blood cells, may happen to preponderate in the tissue, the marrow may, as suggested by Professor Muir⁽⁹⁰⁾ of Glasgow, be classified as erythroblastic or leucoblastic in type, a varying admixture of these two conditions being generally found present.

In photographs 10 and 11, will be seen the usual appearances presented by sections of healthy human red bone-marrow taken, in this instance, from the rib (Case 72). In the former of these illustrations may be seen the relative number and size of the fat cells as compared with the amount of blood-forming cellular tissue lying between them; and in the second of these photographs the same structures are shown under a higher magnification. In such a microscopic section the various members of the red and the white blood-forming series of cells, to be described in detail later, may be recognised, lying in the meshes of the adenoid reticulum^{which is} composed of connective tissue fibrils and the branching and anastomosing processes of the reticular cells.

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Not in colour
all through book

3. Fatty or yellow marrow: This variety of bone-marrow is, as far at all events as the formation of the cellular elements of the blood is concerned, an inert tissue. In relation, however, to the general nutrition of the organism, it may possibly be regarded as being of considerable importance, as it is exceedingly well supplied with blood vessels, and is capable of undergoing very rapid alterations in disease. It is highly probable that such fatty tissue should be looked upon not as an inert substance, but rather as a storehouse where anabolic and katabolic processes are constantly at work, storing up and dealing out nutritive material, in accordance with the requirements of the organism.

When this fatty tissue has attained to its full development in such positions as the centres of the shafts of the long bones, it presents the ordinary characters of adipose tissue elsewhere, with the exception that the fat cells lie embedded in a network of branching cells and connective tissue fibrils, which represent the surviving or persisting elements of the original embryonic marrow already alluded to above. These appearances will be found dealt with in greater detail on pp. 159 & 161.

Lying embedded in this retiform tissue, described elsewhere as a true adenoid reticulum, and situated especially towards the periphery of the section, may be found, in the intervals between the fat cells, an occasional nucleated red blood corpuscle or a myelocyte, generally single, but sometimes aggregated into little groups or islets resembling those seen in red marrow, but containing a much smaller number of cells.

Yellow or fatty marrow may be regarded as being formed by a process of physiological transformation or degradation of the connective tissue elements of the red bone-marrow which it replaces, in certain situations where the more active variety of tissue is no longer required. It may here also be noted that this process of fatty transformation may in certain pathological conditions become excessive in amount, for example in chronic alcoholism, malignant and other cachexias, etc., and that red marrow, in situations where it should normally persist, may also undergo this fatty degeneration in varying degree.* Another phenomenon which may be of vital importance to the organism in disease, and which is only now beginning to be at all fully realised and

* Note: See plates VIII and IX. and the corresponding naked-eye and microscopic preparations from two cases of pneumonia accompanied by leucopenia due to fatty degeneration of the marrow (cases 23 & 54).

studied, is the latent capacity possessed by these normally fatty areas of bone-marrow of becoming transformed again into active blood-forming tissue - a defensive reaction by which, in addition to the increased functional activity of the red marrow in its usual positions, more blood cells may be formed, either to replace those destroyed by disease, or to actively combat the micro-organisms or other agents by which the disease is produced; and it is to the study of these changes and the processes involved, that we shall specially turn our attention later, as well as to the consideration of certain abnormal factors by which their occurrence may be prevented.

Pathological

4. Fibroid Marrow: In old persons, especially if debilitated by long standing disease, the connective tissue elements of the marrow may be found in excessive amount, this proliferation and progressive sclerosis being accompanied by a corresponding diminution in the blood-forming elements of the tissue. This condition, as noted elsewhere (p.173), may also be found occurring earlier in life in some pathological conditions, and is specially characteristic of syphilis.

5. Gelatinous Marrow: Gelatinous Degeneration

of the marrow is essentially a retrogressive change in the tissue, and is in no way identical with the condition of the embryonic tissue with which it has been confused by some writers, e.g. Bichat⁽⁶⁾, who says that "L'aspect de la moelle dans ces maladies est mucilagineux, gelatineux, semblable pour ainsi dire à celui qu'elle nous offre dans la foetus." Neumann⁽⁹⁶⁾ regards it as a transformation into a myxomatous tissue, the fat cells being changed into branching mucoid cells lying in a homogeneous mucin-holding matrix, a view supported by Bizzozero⁽¹⁵²⁾ and Torre⁽¹⁰⁴⁾, and others; though Roger and Josue⁽¹¹⁵⁾ are unable to isolate mucin from such marrows. Gelatinous degeneration has been described by many writers as being typical of starvation, inanition, and similar conditions, e.g. Jackson⁽⁶⁷⁾ found it in starved rabbits and birds, the marrow of which became light red and transparent, the fat cells becoming shrivelled and separated from one another by a mass of homogeneous gelatinous material, a condition which he found rapidly returned to normal on re-feeding the animal, e.g. within three weeks in the case of the pigeon.

Gelatinous degeneration may occur as a chronic change in either fatty or red bone-marrow, as a

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primary change in some cases, e.g. in starvation, but
 it more usually follows upon increased activity, and
 subsequent exhaustion of the tissue, e.g. in long
 standing septic conditions, (case 47, from which plate
 XIII. is drawn - see ~~naked-eye specimen and slides~~;
 case 59, from which see plate XIV; photos 44, 45
 46; ~~naked-eye specimen, and microscopic slides~~;
 and also case 68 - all these being cases of septi-
 caemia with ulcerative endocarditis); tuberculosis;
 malignant disease (Case 22, carcinoma of stomach in
 man of 22, with secondary spread throughout the marrow
 of the entire osseous system; case 59, sarcoma of
 kidney in man of 61,) etc. Stockman and Charteris⁽¹²⁵⁾₍₁₂₆₎
 in an experimental research on the action of various
 drugs upon the bone-marrow of animals, found that the
 prolonged administration of lead, mercury, and arsenic,
 gives rise to gelatinous degeneration, following upon
 a condition of hyperactivity of the tissue; and
 Professor Muir describes its occurrence in a case of
 chronic Bright's disease, and in a patient suffering
 from "marasmus without assignable cause."

In most authors' descriptions of this form of
 change, the marrow is stated to have a "red, gelat-
 inous character", (Muir 91); or to be "light red

and transparent" (Jackson 67); but an examination of the specimens from the cases detailed above - (22, 47, 59, 68, 69) - will shew that the appearances vary almost indefinitely, and depend, not so much upon the change itself, as upon the previous condition of the tissue in which it occurs. Thus in the specimen from case 22, the gelatinous tissue lying between the nodules of new growth will be seen to be almost colourless and very transparent; in case 59, in which the central part of the marrow is affected, it closely resembles fatty tissue in appearance (see Plate XIV.), but is slightly more translucent than normal yellow marrow; in cases 68 and 69, it has a mottled appearance, due to a mixture of red with colourless transparent tissue; and in case 47, the whole marrow has an intensely dark red colour (see plate XIII.)

The typical microscopic appearances of chronic gelatinous degeneration are shewn in photographs 50 and 51, from a case of intestinal carcinoma, and in these the fat cells can be seen in process of absorption, their place being taken by a more or less homogeneous red-staining substance which appears to be laid down in a concentric manner around them, or

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which is more probably formed by a process of degeneration occurring in the protoplasm of the fat cells themselves, a change which gradually spreads inwards from the periphery of the cell, as may perhaps be better seen in the next case. At some parts of this substance very thin delicate fibrils may be seen running in all directions, forming an interlacing network, and it is frequently possible to trace these to the bodies of certain small cells with rounded nuclei, of which they appear to be the processes. It is probable that these cells are in reality the reticular and possibly also the fat cells which have undergone this myxomatous form of degeneration.

In the areas invaded by this change, the blood-forming marrow elements gradually disappear; and all stages of this process may be found in the same microscopic section, often presenting, especially in the earlier stages, a very patchy and irregular appearance. The vascular channels appear to remain permeable to a much greater extent than might be expected from the atrophy of the other elements, and frequently considerable congestion, and even haemorrhages may be found in such gelatinous marrows.

As already stated, I have found that gelatinous degeneration of the marrow may be found not only as

a chronic, but also as an acute change; and photographs 44, 45 and 46 illustrate its occurrence in a case of ulcerative endocarditis of less than three weeks duration (Case 59.) In these specimens the same phenomena as those already described are found to occur, but with considerably greater rapidity; and the resulting "gelatinous substance" is much less dense in its structure, and is composed almost entirely of the fine, loose network of branching fibrils, the meshes of which are apparently filled with some fluid material. The fat cells are evidently in process of rapid absorption, and are undergoing the degenerative change from their periphery inwards, the earlier stages of this process being well shewn in photograph 45 from this case, where the periphery of the fat cells is seen to be becoming transformed into this myxomatous substance, in some parts finely fibrillated and in others somewhat granular in appearance, the central clear areas being the parts of the cells in which the fat still remains unabsorbed. A further stage of this condition is shewn in the next photograph (no. 46), where one fat cell has been almost, and several others have been completely, transformed by the change.

Gelatinous degeneration is thus a change in the

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marrow characterised by the progressive diminution of its blood-forming constituents, and by the absorption of fat, the cells containing the latter undergoing a species of myxomatous change characterised by the development of a network of fibrils and branching processes. The condition may be either acute or chronic, primary or secondary; and is of considerable importance in its bearings on the question of the occurrence of defective leucocytosis in acute and other diseases.

THE REACTIONS

of

THE BONE-MARROW

in

DISEASE.

THE REACTIONS OF THE BONE-MARROW IN DISEASE.

It is a matter of some surprise that, in certain monographs upon the bone-marrow, we find that a normal standard is deduced from the examination of the tissue obtained from cases which have died of various acute infective diseases, such as pleurisy, pneumonia, etc.; and again, as in Villy's⁽¹³²⁾ paper on "The Bone Marrow of Cancer Patients", the result of much careful work is somewhat lessened in value by the fact that, in his descriptions of the marrow in carcinoma, no account is taken of the effects produced on the marrow by the acute terminal conditions bringing about a fatal issue in many of his cases, e.g. pneumonia, septic conditions such as bed-sores, abscess of the lung, etc.

We shall study first the sequence of changes occurring in certain of the acute infective diseases of comparatively short duration, which are accompanied by leucocytosis, or other blood change, e.g. pneumonia, acute septicaemias, etc. Next we shall take up the changes found in cases of longer duration, such as rheumatic fever, ulcerative endocarditis, and more prolonged septicaemias; and lastly, the subacute and chronic changes occurring in the bone-marrow.

Some of the changes brought about by these diseases are degenerative in character, others again are reactions of the marrow by which its blood-forming capacity is increased - either quantitatively, or qualitatively, but most usually in both directions - in order that the special blood elements required by the organism may be supplied to assist it in its struggle against the invading organism or toxin. Again, the two conditions of increased functional activity and degeneration may be found together, the more chronic degenerative changes usually supervening as the direct result of such hyper-activity, and subsequent exhaustion of the tissue, as has already been explained when considering gelatinous degeneration of the marrow (p. 29). The minute cellular changes in the structure of the marrow in disease are more fully detailed in the section of this volume dealing with the cytology of the tissue (pp.53 to 174).

The reactive changes occurring in the bone-marrow may be classified into two great groups - the leucoblastic and the erythroblastic - according as the change is characterised by the increased production of blood cells of the white or of the red series; and the former of these types - the

leucoblastic - may again be subdivided into four sub-groups, according as the reaction is specially characterised by an increase of the neutrophil, eosinophil, basophil or hyaline cells; and the latter - the erythroblastic - into two sub-divisions, the normoblastic and megaloblastic, according to the predominating character of the nucleated red cells which are proliferating. It is extremely rare, however, to find a condition of the marrow in which only one such variety of cell proliferation occurs alone, and it much more frequently happens that, when the tissue reacts in disease, it presents a varying admixture of several of these types.

The varieties or types of marrow reaction may be tabulated as follows:-

Types of Bone-Marrow Reaction.) *for os 6-10 col*

I. Leucoblastic: *s. col*

1. Neutrophil.
2. Eosinophil.
3. Basophil.
4. Hyaline.

II. Erythroblastic: *s. col*

1. Normoblastic.
2. Megaloblastic.

I. LEUCOBLASTIC REACTION OF THE BONE-MARROW: *1) Review*1. Neutrophil Leucoblastic reaction: *ne*

The type of leucoblastic change which we shall find it most useful to investigate somewhat more fully than the others, is that characterised by an increased production of the neutrophil series of cells, a condition which is found in the majority of acute infective diseases, whether these be local, (e.g. inflammation, abscess formation, etc., due to staphylo- and strepto-cocci, B.coli, and other micro-organisms); or general conditions such as pneumonia, septicaemia, etc., the result upon the marrow tissue being in general the same in both sets of conditions, varying with the intensity, quality and amount of the irritant, rather than with its position or point of attack.

Before considering in detail the alterations found in the bone-marrow, it will be necessary to give a short account of the process known as inflammatory leucocytosis, and to discuss some of the possible factors concerned in its production.

Inflammatory Leucocytosis. *1) Review*

Various theories have been advanced to explain the production of inflammatory leucocytosis, or the transitory increase of certain of the white cells,

occurring in the blood in most of the acute infective diseases, and in other similar conditions. Various chemical substances in solution have been found to attract or to repel certain of the protozoa, (Stahl^{*}) and to this phenomenon Pfeiffer^(*) gave the name chemiotaxis - positive or negative according as the protozoa approach or avoid the substance employed. Pfeiffer also found that bacteria can be similarly influenced by the chemical constituents of nutritive substances, and he and numerous other observers have established the fact that leucocytes can in like manner be influenced by certain bacterial and chemical bodies, positively or negatively as the case may be. This so-called "chemiotactic action" of bacteria and their products upon leucocytes was found by Leber^(*) to be capable of being exerted at a distance, and thus the presence of increased numbers of leucocytes in the circulating blood came to be regarded as due to their being attracted or drawn from the blood-forming organs by positive chemiotaxis; whilst in the leucopenia or absence of leucocytosis found in typhoid fever and in measles, the bacterial products are supposed to exercise a negative or repellent action upon the cells. It is not my intention here to discuss the question of how this increased output of blood elements by the bone-marrow is brought about,

* Quoted by Ewing (45)

except in so far as an explanation may be found in the proliferative and other changes in the tissue which accompany it. Whether this proliferation and increased output of cells is due to direct stimulation of the marrow itself, or is merely a regenerative effort on the part of the tissue, in order to replace the cells which have been withdrawn from it, it is extremely difficult to determine; and again, the question whether the chemiotactic substances bringing about these processes in the marrow are produced by the bacteria themselves, or are derived from the leucocytes destroyed by them, is a problem of considerable difficulty, and it is not unlikely that all of the factors mentioned may assist in varying degree in the production of these phenomena.

Whatever be the nature of the cause of this reaction in the blood-forming tissues, the series of phenomena which accompany or rather which comprise the actual process, usually follow a somewhat definite order, and the first phenomenon to appear, whether in marrow which is already red in type, or in fatty marrow which is about to be transformed into such active haemopoietic tissue, is an increased supply of blood to the part. For the sake of simplicity, we shall first consider the condition as it may be

seen occurring in the fatty or yellow bone-marrow of the femur, and reference should here be made to the series of water-colour drawings illustrating the various stages of the process in the interior of the shaft of the femur (plates VIII. to XV.) ; and to the corresponding microscopic ^{preparations} and photographs.

The naked-eye and microscopic specimens from a case of "osteoporosis" in the horse (N.E. Specimen No. 1, and slide no. 12) ^{were} will be found to present a key to the series of changes occurring in a marrow undergoing leucoblastic transformation, and in these all gradations, from yellow or pure fatty marrow up to almost complete metamorphosis, ^{could be} will be found in the one section. On passing from the unchanged fatty marrow towards the redder part of the section, a broad belt of congested tissue ^{was} is first encountered, forming as it were an advance guard in the progress of the army of cells which is taking part in the invasion of the territory undergoing transformation. As this congested area ^{was} is traced onwards it ^{was} will be seen ~~that~~ that the venous capillaries which comprise it and which lie between the fat cells, gradually ^{do} shew more and more marked engorgement with blood; but that, just before the margin of the leucoblastic area ^{was} is reached, this congestion or engorgement ^{was} is

found to have considerably subsided. Another point to be noted as ^{one} we pass ^{ed} outwards, ^{was} is the increasing numbers of erythroblasts and myelocytes to be seen among and around the red cells, very scattered and scanty at first, but, as ^{one} we proceed ^{ed} farther, ^{one from} we find these coming to form cords and islands of steadily increasing dimensions, in which the formative cells come to constitute an ever-growing, and the erythrocytes an ever-diminishing, constituent element; until finally, on approaching the periphery of the marrow, large fields or areas ^{were} ~~are~~ seen, composed almost entirely of haemopoietic cells, the myelocytes or leucoblasts being represented by the lighter, and the erythroblasts or formative red cells by the darker stained nuclei, among which ^{could} ~~may~~ also be seen numerous pigmented phagocytes and giant cells, such ^{are} as ^{described} elsewhere: while the eosinophil series ^{was} is represented, in the horse, by cells containing granules of a very large size. The section also ^{ed} shews degeneration of the vascular elements of the tissue, more especially the arteries, but these need not farther detain us here.

From the foregoing description of the microscopic appearances presented by this specimen, the corresponding phenomena in the naked-eye preparation ^{could} ~~can~~

^{then} now be more fully appreciated, and the sequence of changes traced out, beginning with the pure fatty area of yellow marrow on one side of the section, and passing from this across the band or zone of first increasing, and then subsiding, congestion, due to engorgement of the venous capillary spaces; and then reaching the intense red coloured area which exhibits^{ed} a gradually increasing and finally complete degree of leucoblastic - and also in this case, but to a much slighter extent, erythroblastic - transformation.

The different stages of this process may be found in many such specimens of marrow, not as a sequence of events which may be seen spreading with a sharp margin in one marrow section - all gradations of the change being present at one and the same time - but as phenomena affecting simultaneously large areas of marrow as regards time. Thus the whole section may shew in the earlier stages of this process only the preliminary congestive condition (as is seen in photos 20, 22); or over wide areas this preliminary congestive phenomenon may be found subsiding, and different degrees of comparatively uniform leucoblastic transformation may be seen. In other words, all or large parts of the marrow may undergo identical gradations of the change simultaneously; or

on the other hand, the transformation may commence at one part of the marrow and spread slowly into the neighbouring tracts of fatty tissue, the part first affected shewing a more advanced stage in the process.

The latter of these methods, i.e. the simultaneous transformation of large areas of tissue, is that usually found in the bone-marrow of young subjects, for example in children, (see slides from cases 34, 42, 61, 62, 63, 64, etc.) in whom the tissue is still in varying degree red or lymphoid before the onset of the disease, as is also the case in the marrow of the rib in the adult.

The method first described, i.e. that observed in the specimen from the horse, is in the adult human subject the one more usually observed in the transformation of yellow into red marrow, e.g. in the shaft of the femur. Here the process is generally one of local spread from situations where red marrow cells are already present, for example in the parts towards the ends of the bone, and also in the cancellous spaces in the medullary cavity next the compact bone. Hence it is for example in the naked-eye specimens from cases 14a; 33, (plate X.); 49, (plate XI.) 38, (plate XII.) 59, (plate XIV.) and several others - that this change may be observed in longitudinal

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sections, to be spreading gradually downwards from the upper, or upwards from the lower end, towards the centre of the shaft of the bone; whilst in transverse sections (e.g. as is illustrated in Plates X., XIV., and XIII.) the process begins at the outer margin of the marrow, in the cancellated tissue next the compact bone, and gradually creeps inwards from the periphery, forming a ring of leucoblastic change gradually increasing in thickness as the invaded fatty tissue within the ever-narrowing circle of its inner margin becomes less and less in amount, and is at length completely transformed (as in plate XIII.) should the disease be of sufficient duration to bring about the entire leucoblastic transformation of the marrow, as is shewn in plate XIII., and in many of the other specimens examined.

During this process of transformation of yellow to red bone-marrow, two other very important phenomena now to be described, may be noticed, and the explanation of these is to be found in the fact that the marrow is contained within a closed space - the medullary cavity - surrounded upon all sides by **hard**, **inexpansible**, bony walls. More space is required to allow of the proliferation of the marrow cells, and this is provided in two different directions,

firstly, by the progressive diminution in size, and, if necessary, total disappearance of the fat cells of the yellow marrow; and secondly, by the rapid absorption of the spicules and partitions of cancellated bone towards the interior, and later also at the periphery of the medullary cavity; and this process may even bring about a considerable amount of erosion of the inner aspect of the compact bone beyond this, should the reaction be sufficiently prolonged and intense - a condition which is beautifully illustrated in plate XIII. from a case of chronic septicaemia of about a year's duration (Case 47). From the lower portion of this specimen I have carefully washed away the proliferated marrow tissue, and it will be observed that the walls of the medullary cavity are smooth and eroded, all the spicules of cancellated tissue, as well as some of the compact bone, having been removed by absorption : a thin osseous sheath with imperfect walls being left around the medullary artery at its point of entrance into the marrow. To this erosion and absorption of bone allusion has already been made in the description of the section-cutting procedure adopted in this research; and it is owing to the fact of this partial or complete absorption of the cancellated bony tissue in the central part, and sometimes even throughout

almost the entire extent, of the marrow, that it has usually been possible to cut the microscopic sections without previously decalcifying the tissue, the latter method of procedure being exceedingly liable to injure the finer structural details and staining reactions of the delicate marrow cells.

The progressive stages in the absorption of fat may be easily followed both in the naked-eye specimens, and in the microscopic slides and photographs; and the process has already been partially described when dealing with the invasion of the fatty marrow by the leucoblastic change, the fat disappearing earliest in the parts first attacked, i.e. towards the ends and at the periphery of the medullary cavity, and persisting longest towards its centre. (See plates XI. and XII). Similarly, under the microscope, the fat cells are seen to become progressively diminished both in number and in size, whether the change occurs in red or in yellow marrow, the process simply being less complete or occupying a somewhat longer time in the latter position. (See Photographs 12 , 13 , 18 , for the progressive absorption of fat in the yellow marrow of the femur: and photographs 10 , 24 , 26 , 28, for the same change occurring in the red marrow of the rib).

As already stated elsewhere, this leucoblastic transformation of the marrow, a phenomenon of such vital importance in enabling the organism to resist the inroads of disease, may in certain cases be defective or even absent. Such deficiency in the completeness of this reaction, or its entire absence, may be found in the case of old and debilitated persons, or at an earlier period in certain individuals weakened by alcoholic excess, syphilis and many other similar conditions; and if these patients are attacked by some acute infective disease such as pneumonia, the haemopoietic tissues may shew little or no reaction, no hyperleucocytosis is brought about, and there may be even an actual diminution of the number of leucocytes in the blood - conditions known respectively as aleucocytosis and hypo-leucocytosis or leucopenia. In such cases there is usually a rapidly fatal issue, and in plates VIII. and IX. are shewn specimens of yellow bone-marrow in which practically no leucoblastic change is present. (Cases 23 and 54 - both patients suffering from acute lobar pneumonia with leucopenia.) The microscopic preparations of these bone-marrow shew almost nothing but pure fatty tissue containing a few engorged venous channels and capillaries. In other words the condition is one of fatty degeneration of the marrow, a pathological change

Ordway

which has been described on p. 26.

Another cause of such imperfect reaction of the marrow, and the consequent deficiency in the production and output of leucocytes and red blood corpuscles, is to be found in the occurrence of gelatinous degeneration of the tissue, a condition described in detail on p. 28 ; while in certain intensely acute toxic conditions, extensive areas of haemorrhage may be found in the marrow, as is shewn in plate XV. and the corresponding microscopic preparations and photographs, obtained from the femur of a girl (case 67), who rapidly succumbed to an attack of acute streptococcal septicaemia, possibly supervening upon diphtheria. In this case large numbers of streptococci are to be seen in the marrow tissue, plugging many of the smaller vessels, and setting up intense inflammatory and necrotic changes around them (see photograph No. 42).

In almost all cases of acute pneumonia and septicaemia examined, organisms were ^{very frequently} usually found in the bone-marrow on microscopic examination, occasionally in very large numbers, as in cases 11, 12, 18a, 67, and in many others; and cultures made from the marrow of such cases were usually found to shew typical growths of the organism causing the disease, for example, staphylö- and strepto-cocci, bacillus coli communis,

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bacillus typhosus, pneumococcus, etc.

The other subvarieties of leucoblastic change will be more conveniently dealt with when considering the special variety of cell involved; and the headings are merely recapitulated here in order to facilitate a reference to the descriptions of these which will be found in the cytological section of this volume.

2. Leucoblastic Reaction, characterised by special increase of the Eosinophil Granular Cells. (see p.92).
3. Leucoblastic Reaction, characterised by special proliferation of Basophil Granular Cells (see p.104), a condition specially found, in addition to the other typical changes, in myelogenous leukaemia, in which mast-cells are present in greater abundance than in any other disease. (see p.102).
4. Leucoblastic Reaction, characterised by increase in the numbers of the pre-myelocytes or large basophil non-granular cells.

In many of the diseases accompanied by neutrophil leucocytosis, the parent cells of the neutrophil myelocytes, as well as the latter cells themselves, are usually found in greater numbers; and this is

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especially the case when the production of the leucocytosis has to be kept up for any length of time, as for example, in cases 44(chronic pyaemia), 69 (sarcoma of kidney - see plate 1, figs.8 and 9), which may be taken as types of two important groups of disease accompanied by long-standing leucocytosis, viz., chronic septic conditions, and malignant disease. (see page 72).

II. ERYTHROBLASTIC REACTION OF THE BONE MARROW.

Reichert's

II. ERYTHROBLASTIC REACTION OF THE BONE-MARROW:

not 1. Normoblastic in type: got specially after haemorrhage; in most conditions of secondary anaemia; in exophthalmic goitre, etc.; and in prolonged septic diseases characterised by any considerable amount of red cell destruction, the change representing the attempt on the part of the bone-marrow to replace the cells which are deficient. (see page 115).

not 2. Megaloblastic in type: most typically seen in the bone-marrow in cases of pernicious anaemia. In precisely the same way* as normoblasts may be healthy or may exhibit pathological variations in disease, so are megaloblasts capable of being classified as normal and abnormal in their structure and appearances. The megaloblasts of pernicious anaemia I regard as belonging to the latter category - in other words, they, and still more the gigantoblasts found in this disease, are pathological variations from a normal cell type - the healthy megaloblast or early developmental form of nucleated red cell. As will be described in detail later, in the cytological section dealing with these cells (page 125), megaloblasts are found increased in a very large number of conditions, both acute and chronic, and all transitions

between them and the normoblast can be seen. (see page 128.).

A brief note may here be added that in the earlier stages of proliferation of the marrow, whether the reaction be ultimately leucoblastic or erythroblastic in character, all varieties of blood-forming cells are found increased in numbers. As the change proceeds, however, the special type of cell required by the organism gradually comes to predominate in numbers, and the other varieties of cell may either remain almost unchanged, or may undergo diminution, or only a comparatively slight relative increase.

Again, if the disease becomes chronic, and the reaction is prolonged, for example, in the chronic cases of ulcerative endocarditis and other septic-aemias of my series, the leucoblastic reaction may be imperfectly sustained, and an erythroblastic condition may be superadded, probably brought about by the presence of a secondary anaemia caused by the long continuation of the disease. (e.g. cases 9, 47, etc.). It may also be noted that the earlier stages of increased production of red cells, e.g. in regeneration after haemorrhage, are usually accompanied by a more or less marked leucocytotic condition of the

blood, a phenomenon to be explained in a precisely similar manner to that described above, i.e. to a stimulation of all the marrow elements to increased proliferation, before the erythroblastic type of change is definitely established.

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CYTOLOGY OF THE MARROW.

In stained sections and film preparations of red or blood-forming bone-marrow in the human subject, the following varieties of cells may be distinguished:-

I. BLOOD-FORMING CELLS:- *Sp. 02.11.11.11*

A. Leucocyte Series: *Sp. 03.11.11.11*

(a) Non-granular cells, with basophil protoplasm:

1. Large.
2. Small:

a) Cells similar to, but smaller than, the large variety.

b) Cells identical in appearance and in staining reactions with the small lymphocyte of the blood.

(b) Granular Cells:

1. Neutrophil (corresponding to amphophil in the rabbit.)

i. Myelocytes, with large, rounded or oval nucleus.

- (a) Larger variety,
- (b) Smaller variety.

ii. Intermediate Cells, with indented or horse-shoe shaped nucleus.

iii. Polymorphonuclear Cells, or leucocytes.

2. Eosinophil:-

- i. Myelocytes.
- ii. Intermediate Cells.
- iii. Polymorphonuclear Cells or leucocytes.

3. Basophil:-

(a) Mastcells:-

- i. Myelocytes.
- ii. Intermediate Cells.
- iii. Polymorphonuclear Cells.

- (b) Cells resembling the eosinophil myelocytes, but with granulations staining with the basic dye.

B. Haemoglobin-holding Series: *Proseria*

1. Normoblasts → normocytes or ordinary red blood corpuscles.
2. Megaloblasts $\begin{cases} \leftarrow \text{megalocytes.} \\ \leftarrow \text{normoblasts (?) } \end{cases}$

II. GIANT CELLS:- *Proseria*

Mononucleated = "Megakaryocytes"
 Multinucleated = "Polykaryocytes"

III. CELLS OF CONNECTIVE TISSUE TYPE:- *Proseria*

- i. Fat Cells.
- ii. Cells of the reticulum.
- iii. Various forms of phagocytic cell (pigment cells, etc.).
- iv. Ordinary Connective tissue cells passing in with vessels, etc.

IV. ENDOTHELIAL CELLS, lining the walls of capillaries, blood sinuses, etc.,

55.

- i. Found in their normal positions in the vessel walls.
- ii. Found proliferating, taking on phagocytic functions, etc.

I. BLOOD-FORMING CELLS:A. Leucocyte Series:(a) Non-Granular cells, with basophil protoplasm:1. Large Variety:

Of the cells which belong to the leucocyte or white series, that which is found in greatest numbers in red bone-marrow in a state of health, and also in the majority of pathological conditions, is the "neutrophil myelocyte" of Ehrlich, (Plate I., figs, 1, 2, 3, etc.) who was the first to recognise and demonstrate the fact that ^{from} this variety of cell is derived the polymorphonuclear neutrophil leucocyte of the blood. This neutrophil myelocyte is, according to Dominici, ⁽³²⁾ in its turn developed from a large mononucleated cell, with basophil protoplasm which contains no granulations. The nucleus of this "non-granular basophil myelocyte" (Plate I., figs. 10, 11, 12) is usually of considerable size, and somewhat rounded or oval in shape; and in appearance it is vesicular, with a definite perinuclear membrane to which are attached several large granules of chromatin by a delicate nuclear meshwork, which may also shew either one or two very definite nucleoli.

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According to Dominici, the protoplasm of this cell is stained a uniform dark blue, but according to my own observations, this staining reaction varies within somewhat wide limits, all gradations from a definite blue to a pale pink being obtainable with methylene blue and eosine, the protoplasm being usually very definitely reticular and often enclosing clearer spaces which give the cell a somewhat vacuolated appearance. (Plate I., figs 10, 11, 12). All transitions between this cell and the ordinary neutrophil myelocyte can be seen, and in preparations which are not specially stained to demonstrate the granules, it is often difficult and indeed sometimes impossible to distinguish between them, a fact which may be easily realised by a reference to Plate I. and by a comparison of the non-granular cells depicted in figs. 10, 11 & 12, with the mononuclear myelocytes shewn in figs. 1, 2 and 3 respectively. These parent or primitive cells are, under certain circumstances, e.g. in long-standing leucocytosis, etc., as will be described later, frequently greatly increased in number. Shortly after Dominici published his paper upon the occurrence of these cells, his results were independently confirmed by Pappenheim⁽¹⁰⁷⁾, who also

states that these basophil granule-free cells resembling large lymphocytes are the earliest blood-forming cells to be found in the marrow, and that by their proliferation in the embryonic tissue, are formed not only the neutrophil myelocytes, but also the haemoglobin-bearing series, the eosinophils, and the other forms of white cell. In a film preparation made from the rib marrow of a case of sarcoma of the kidney (Case 69), I have been able to trace very definitely all transitions from this basophil non-granular cell to the ordinary neutrophil myelocyte, and some of these stages will be found depicted in Plate I., figs. 8 and 9. The marrow films from which these figures are drawn, were fixed in saturated corrosive, and stained with triacid and methylene blue (Hewes' method) and with methylene blue and watery eosine respectively. Cell 1, fig.8, shews one of these primitive cells in division, and cell 2 may be taken as a typical example of the basophil non-granular cells in this case. The nucleus has the characteristics already described, and comes to resemble very closely that of its neutrophil granular derivative; the cell body has a slightly mottled appearance due to the presence of a definite proto-

plasmic reticulum which contains as yet no granulations. This cell then proliferates, and the next stage in the development of the resulting cells is the appearance of the typical neutrophil granules in the protoplasm (Cells 3 & 4, fig.9). These may appear first in a somewhat irregular manner, being in cell 3, fig.8, seen scattered scantily, and diffusely throughout the still blue-staining protoplasm, or again they may be found forming denser masses at certain parts of the cells, sometimes near or around the nucleus(as in cells 3 & 4, fig.9), or again at the periphery or in some other part of the protoplasm, giving an irregular mottled red and blue appearance to the cell body (fig. 9, cell 5.).

In the same preparation there are also present all gradations between these large blue "pre-myelocytes" and much smaller cells, which closely resemble lymphocytes in their characters, and which probably represent the so-called "indifferent lymphocyte" of Wolff (136) which he describes as being "larger than the small lymphocyte of the blood, with a rounded or somewhat indented nucleus and non-granular cytoplasm, which is less basophil than the nucleus, while in the "differentiated lymphocyte" the cytoplasm is more basophil than the nucleus." (See fig.9, cells 8, 9, 10 and 11.) Whether this "undifferentiated lymphocyte"

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has, in the adult, anything to do with the formation also of the erythroblast or not is a very difficult question to solve. In the specimens from this case those cells certainly closely resemble each other; but I am as yet not prepared to hazard any definite opinion on the matter. These cells will be more fully discussed in the next paragraph. By utilising as a guide the somewhat exaggerated changes seen so definitely in this case, we have the key to the development of the neutrophil myelocyte under normal conditions. Precisely similar phenomena, but in a less degree, can be seen occurring in normal and in other bone-marrow, though never so distinctly as in these specimens, the various staining reactions which I have employed not having so far succeeded in demonstrating them so beautifully as in this case.

2. Small non-granular cells with basophil protoplasm.

This variety of cell, which occurs in very varying proportions in the different specimens which I have examined, is usually ^{almost} indistinguishable from the small lymphocyte as it is found in the blood or in lymphoid tissue. It is found scattered irregularly among the other marrow cells, or is sometimes aggre-

gated into small patches or areas which in many respects closely resemble the usual structure of lymphoid tissue elsewhere, the cells lying in the meshes of a definite adenoid reticulum. Lymphoid patches of this description are usually somewhat scanty in normal marrow, throughout which these lymphocyte-like cells are generally scattered irregularly; but in certain pathological conditions they may be found in much greater numbers, for example in typhoid fever, tuberculosis, etc., and in lymphatic leukaemia they are found replacing almost entirely all the other varieties of marrow cell. These facts seem to lend colour to the assertions of Dominici⁽³⁴⁾ who holds that the haemopoietic tissues are "built on a plan represented by a combination (with phases of variable development) of two great varieties of tissue, lymphoid and myeloid, intermingled in one histological complex, the haemopoietic tissue proper." Both of these types of tissue occur side by side, or intermingled with one another in the marrow, but whether the same holds true of the spleen and lymphatic glands is doubtful, and my observations in this direction are not yet sufficiently extensive to justify any definite statement on the subject. Fig.13, plate I.

shews a small lymphoid patch occurring in the marrow of the rib from a case of septic pneumonia (Case 16), and in the upper part of the same figure are shewn a series of small lymphoid cells from the same section, stained with methylene blue and eosine, and in figs. 14 and 15 the same variety of cell is depicted, the stains being Ehrlich's triacid mixture, and haematoxylin and eosin respectively. Similar small areas of lymphoid tissue will be seen in slide W.I.3. from case 54, a pneumonia with leucopenia, the specimen being obtained from the fatty marrow of the humerus. Reference has already been made to the small basophil non-granular cell or undifferentiated lymphocyte of Wolff, from which we have concluded that it is probable that the larger basophil pre-myelocytes are formed, and it seems likely that these cells differ in some respects from the adult or small lymphocyte of the blood as described by this observer, and it ~~also~~ appears at all events possible that the former is also the primitive or parent cell from which the latter is derived, if not in the adult, at all events in the embryo, and also in certain pathological conditions. From the study of the marrow in several cases of lymphatic leukaemia (51, etc.) it appears likely that the small lymphoid cells found "infiltrat-

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ing" the marrow as well as the other haemopoietic organs, liver, kidney, etc., belong to the more primitive or undifferentiated variety of cell, the condition being in reality a reversion to the embryonal type of blood formation, as has been asserted by Müller. ⁽⁹⁴⁾ Another point of interest in this connection is the relatively greater number of lymphocytes found in the blood in infancy, and also the extreme readiness with which lymphocytosis is produced by many of the diseases occurring in the early years of life, there being a tendency apparently for the blood-forming tissues to "revert" and produce cells of an earlier type, and the younger the subject, the more readily is this found to occur. ⁽⁶⁶⁾ Hutchison, in his Goulstonian Lectures upon "The Disorders of the Blood and Blood-forming Organs in Early Life", calls attention to the fact "that the non-granular cells are present in the blood of the infant in markedly larger absolute numbers than in the adult, and corresponding to this the adenoid tissue which is concerned in the production of these cells is extremely abundant, active and widely diffused," and he concludes "that although this specialisation of function on the part of the two great blood-forming tissues - the marrow and the adenoid tissue - has

taken place in the child as in the adult, yet the former, standing nearer to the period of foetal life, may be expected to be able under stress of disease to revert more easily to the foetal methods of blood-formation."

In other words, he proves, in another connection, that the lymphocyte is the more primitive cell, and appears earlier than the granular myelocyte, a fact which, taken along with the data which I have already given, forms a strong clinical argument in favour of the possibility that the latter variety of cell is derived from the former, i.e. that the non-granular basophil cell may be correctly regarded and designated as the "pre-myelocyte" or parent cell of the neutrophil granular series of cells.

(b) Granular Cells.

The first observer to lay special stress upon the occurrence of cell-granulations was Professor Ehrlich, whose work on the subject is recognised as being of extreme importance. Ehrlich has studied at great length the different varieties of granules, which he regards as "specific" of certain cells, and his classification of these according to their stain-

ing reactions with certain of the aniline dyes is now generally accepted, and his triacid staining mixture is still much used for the identification of the various cell-granulations. This mixture contains three dyes, orange γ , acid fuchsin, and methyl green; and with it the nuclei are stained greenish (See Plate I., figs. 2 and 5; plate IV., fig. 2); the red blood corpuscles, a tint varying from red to orange; the oxyphil granules, amber or copper red (Plate I., fig. 5); and the neutrophils reddish violet (Plate I., figs. 2 and 8); while the granules of the mast cells remain unstained and stand out as clear, bright, colourless, vacuole-like bodies. Ehrlich's classification of granules is as follows:-

1. α -granules, staining with acid dyes.
(e.g. in the coarsely granular oxyphil or eosinophil leucocyte and myelocyte, Plate I., figs. 3, 4 and 5.)
2. β -granules, staining with both.
(e.g. in the amphophil polymorphonuclear leucocyte and myelocyte in the rabbit.)
3. γ -granules, staining with the basic dye.
(e.g. in the basophil or mast cells, Plate I., fig. 7, cell 8.)
4. δ -granules, staining with the basic dye,
(e.g. small basophil granulations in certain mononuclear cells, Plate I., fig. 7, cells 1-6)
5. ϵ -granules, staining well with neither acid nor basic dyes, but best demonstrated by one of neutral reaction.
(e.g. in the neutrophil polymorphonuclear leucocyte and neutrophil myelocyte, Plate I., figs. 1-3).

Risso

While Ehrlich and his school regard these reactions as absolutely specific, it has been found that within certain limits they vary. Arnold,⁽²⁾ for example, says, "that a subdivision of bone-marrow cells according to their contained granules is as yet impossible, because the same granulations are found in different forms of cells, and dissimilar granulations in the same cell."

Milroy and Malcolm,⁽⁸⁷⁾ using injections of nucleic acid in rabbits and guinea-pigs, found that "there was a gradual alteration in the staining affinities of the oxyphil granules, which become finally purely basophil. This change in reaction is probably due to the fact that the granules are composed of nucleo-proteid with a more or less easily detachable albumin, and the first action of nucleic acid is to remove the albumin, or rather to cause the cell to give up the albumin, that is combined with the more acid residue - nuclein. This residue reacts more strongly acid than the complex nucleo-proteid, and hence is stained with methylene blue, and as it is less easily dissolved (or digested) it remains for some time after the albumin has disappeared. The change is most marked in the amphophil granules,

"because in them the combination between nuclein and albumin is a looser one than in the coarsely granular oxyphil cells." (Milroy and Malcolm, loc.cit., p.128.)

The conclusions arrived at by these authors, whose experiments shew how readily the granules may undergo changes in their chemical composition and staining reactions, are amply confirmed by the observation of their behaviour in the human marrow under certain pathological conditions; but until a better classification of the cells of the blood and bone-marrow is adopted, we must fall back upon that of Ehrlich as the best practical method yet suggested, and we shall now proceed to the consideration of the various granular cells found in the red bone-marrow.

1. Cells with neutrophil granulations:-

As already indicated, the finely granular neutrophil myelocyte arises from the non-granular basophil cell described above, a fact which is strenuously denied by many authors*, and both of these cells may in certain circumstances, I hold, be found simultaneously and under the same pathological con-

* "A fundamental distinction from the large mononuclear cells lies in the fact that the protoplasm exhibits a more or less numerous neutrophil granulation." (Ehrlich - Myers' trans., p.77.)

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ditions in the circulating blood. Türk,⁽¹³⁰⁾ in 1898, in his account of the alterations occurring in the blood in certain of the acute diseases, describes the appearance of a peculiar variety of cell to which he gives the appellation "Reizungsformen" or stimulation forms - non-granular mononucleated cells with basophil protoplasm and a nucleus which contains a distinct chromatin network. These are commonly included by most observers among the large lymphocytes, and according to Ehrlich they may "possibly form an early stage of development of the nucleated red blood corpuscles, as the deeply staining and homogeneous protoplasm seems to indicate." (40) (Myers' trans. loc.cit., p.80.) Ewing⁽⁴⁵⁾ states that they are probably ancestral forms of megaloblasts and result from abnormal stimulation of blood-forming tissues. While not denying the possibility of this supposition, a point which will be more fully discussed later, I venture to suggest that these "Reizungsformen" of Türk are in reality simply the earlier forms of myelocyte already alluded to above, under the name of large basophil non-granular cells. As far as I can make out from a careful study of these stimulation forms, as they occur in the blood in pneumonia, etc.,

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they are identical with the pre-myelocyte forms found in the marrow in greatly increased numbers in many of the acute diseases which are accompanied by neutrophil polymorphonuclear leucocytosis; and the fact, recognised by Türk himself, as well as by Ehrlich and others, that these "Reizungsformen" often occur "simultaneously with, and under the same conditions as the myelocytes," is a very strong argument in favour of my supposition, and that they are due, as Türk suggests by the term he has applied to them, to the excessive stimulation of the blood-forming organs (in this case, the bone-marrow), brought about by the increased demand by the system for neutrophil polymorphonuclear leucocytes, and hence also by a demand for the parent cells from which these are formed, an explanation which appears to me much more probable than that they are connected with the formation of cells of the red series, as suggested by Türk and Ehrlich.

It is no longer necessary to discuss whether the polymorphonuclear leucocyte is, or is not, derived from the granular myelocyte, as this has now been amply proved to be the case. Ehrlich⁽⁴⁰⁾ has shewn that the production of leucocytosis, more especially polymorphonuclear leucocytosis, is one

of the most important functions of the bone-marrow, and his results have been amply confirmed since by numerous observers, notably by Goldscheider and Jacob⁽⁵²⁾, Roger and Josué,⁽¹¹⁵⁾ Pappenheim,⁽¹⁰¹⁾ Arnold,⁽²⁾ Dominici,⁽²⁸⁾ Haushalter and Spillman,⁽⁵⁸⁾ and many others abroad, while in this country the most valuable work on the subject has been done by Muir⁽⁸⁸⁾ of Glasgow, and his late assistant Dr Ferguson,⁽⁴⁶⁾ now of Cairo.

The neutrophil myelocyte of Ehrlich or, as it may be termed, the myelocyte proper, is the member of the leucocyte or white cell series which is usually found in greatest numbers in the red marrow. It is extremely variable in size. Occasionally it may attain to a diameter of nearly 30μ , as in the specimen figured in Plate I., figs. 8 & 9, or again myelocytes may be found no larger than an ordinary small lymphocyte, i.e. 9 or 10μ or even less in diameter. The average size of these cells may however be taken as lying somewhere between 12 and 15μ . They are therefore, broadly speaking, larger cells than the derivative polymorphonuclear leucocytes, whose diameter may be averaged at about 10 or 12μ , while the intermediate cells, to be described shortly below, form a group which bridges across the interval be-

tween these two great series of cells found in the marrow. (See Plate I., figs. 1, 2, 3, etc.)

The myelocyte proper is usually a somewhat large cell with a single, relatively large, rounded, or somewhat oval nucleus, centrally, or occasionally somewhat eccentrically placed. With Ehrlich's triple stain (Plate I., fig. 2, cell 1.) this nucleus presents a very pale homogeneous appearance, but if stained with methylene blue (Plate I., fig. 1, cell 1), or with haematin (Plate I., fig. 3, cell 1.), it is seen to be composed of a definite perinuclear membrane and nuclear network, the chromatin showing frequently as somewhat ^{large,} irregularly shaped granules. It is in fact, as already stated above, identical in form and in staining properties with the nucleus of the larger non-granular pre-myelocyte, as can be easily seen from the accompanying microscopic preparations (Slides W.I.-6, 8 & 9), and the figures already alluded to in Plate I. This nucleus is embedded in a reticular cytoplasm, whose special feature is the possession of fine neutrophil granules (Ehrlich's ϵ -granules), which can be best seen in preparations stained with triacid (Plate I., fig. 2), or with some modification of the Romanowsky stain (e.g. Jenner's or Leishman's). These granules are small in size, but are usually

omit/

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found to vary somewhat, not only according to the method of fixation, but even in the same film and in the same cell. I have also observed considerable variation in size in different individual cases, and in certain pathological conditions, and it may here also be added that the variations, e.g. uniform enlargement or diminution in size of the cellular elements, not only of the marrow but of the other organs and tissues of certain cases, have formed a noticeable feature in the examinations I have carried out during this research. For example, in the case of sarcoma, to which I have already alluded (No. 69), the cells of the various organs were remarkable for their large size, a peculiarity not limited to the myelocytes, etc., figured in fig.9, Plate 1. As a general rule it may be accepted that the larger myelocytes are the younger or less mature forms found in the process of formation of the polymorphonuclear leucocytes, though this is by no means invariably the case. These by their division form myelocytes of smaller size, which in turn become members of the intermediate group of myelocytes, characterised by their indented or horse-shoe shaped nuclei, see figs. 1, 2 and 3, cells 2 and 3 respectively.

Muir

In many marrow sections it is somewhat difficult to find any definite plan or method on which these cells are topographically arranged, and they frequently appear - especially in proliferated marrows - to be irregularly intermingled. Sometimes, however, one may observe the arrangement described by Muir,⁽⁹⁰⁾ where the polymorphonuclear or adult cells can be seen nearest to the vascular spaces, i.e. to the blood stream, the myelocytes of largest size being farthest from the circulating blood, and forming, as it were, germinal centres from the periphery of which the adult polymorphonuclear leucocytes are cast off, or rather emigrate, as they are produced, or as they are required by the system; the smaller myelocytes and cells with indented and horse-shoe nuclei, i.e. the intermediate or transitional forms, being arranged, though often somewhat irregularly, from the central germinal area outwards, in the order of their development, the younger forms occupying the more central, and the maturer cells the more peripheral position between the parent myelocytes and the polymorphonuclear or adult cells derived from them.

In normal, but still more markedly in pathological, states associated with leucocytosis and pro-

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liferation, beautiful mitotic figures and nuclear divisions are seen very frequently among those neutrophil myelocytes, some of which are illustrated in the lower groups of cells in Plate I., figs 1 and 3.

Roger and Josué⁽¹¹⁵⁾ affirm that karyokinetic figures are rare, even in proliferating marrow, but this I have no hesitation in stating not to be the case, as

in the preparations from many of my cases they were found to be very numerous (e.g. cases 28, 69, etc.), and in this way my results agree with those of Jolly, Muir, and others.

In all probability the cause of their not being found by some authorities is the fact that mitosis is a comparatively rapid process, and examination must be made for them very soon after death, or better still in marrow from operation specimens obtained during life and rapidly fixed. Perhaps the best material for studying these changes and also the developmental phenomena to be seen in marrow cells, is that obtained from the small pieces of rib resected in cases of empyema and prepared in the ways already indicated.

Insert addition

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division of the granules, or whether they are formed

de novo from the reticulum of the daughter cells in order to bring them up to the full complement, I am unable to say, though the latter supposition appears the more likely. They seem, however, to be in very

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close relationship to the cytoplasmic reticulum, and actual attachment to this in the case of the coarsely granular eosinophil cell has been described by Arnold, (1 to 3) Heidenhain, (63) Gulland, (56) and others, while other observers deny that this is so, and regard them more as metaplastic products lying free in the cell protoplasm, from which they can be discharged when required in the form of a secretion. This scattering of cell granules, however, appears to me to be a phenomenon which is caused ~~rather~~ by the death and disintegration, and also in many cases by the actual rupturing, of the cell during preparation of the film, rather than by a process of active discharge as alleged by Audibert, (4) Mesinescu, (86) Bonne, (17) Jolly, (70) and others, whose descriptions appear to indicate that the process is one of cell destruction, although the other interpretation ^{is} put upon them by these authors themselves; while the results of Milroy and Malcolm (87) seem ~~rather~~ to indicate that disappearance or modification of the staining reactions of granules is rather to be regarded as an intracellular chemical change, by which they may become variously altered or undergo actual solution.

Another point of extreme importance is the question of at what period the cells of this series acquire the amoebic motility so characteristic of the

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adult polymorphonuclear leucocyte, and also how much this power of movement is responsible for these cells leaving the marrow as they ripen. Muir^(90,91) states that along with the changes in the nucleus (i.e. with the assumption of the polymorphonuclear type) amoeboid activity is acquired, the finely granular marrow cells being according to his observations, non-amoeboid, while the finely granular leucocytes are actively so; a position also taken up by Ehrlich, who states that, "in contradistinction to these polynuclear neutrophil elements, these mononuclear forms shew no amoeboid movement on the warm stage." This however, I do not find to be the case, and for the purpose of studying the neutrophil myelocytes in a living state with regard both to motility and to phagocytosis, I have carefully watched hot-stage preparations of fresh bone-marrow from the rabbit, and also of blood from cases of myelogenous leukaemia, sometimes for many hours at a time, and from these observations I have no hesitation in saying that the mononucleated myelocyte is - under these conditions at all events - not only slightly motile, but is also somewhat feebly phagocytic for particulate substances, such as carmine and vermilion, and also for strepto-

and staphylococci, both living and dead. For the purpose of studying and testing these activities, fresh preparations of marrow from the rabbit's femur, and also specimens of leukaemic blood were incubated for various periods with the above mentioned substances and organisms, and the preparations then stained according to the method described by Leishman⁽⁷⁸⁾ of Netley. The polymorphonuclear leucocytes were very active in all cases, and took up large numbers of pigment particles and of the living and dead cocci employed. The intermediate neutrophils were less actively phagocytic, and took up a moderate number of organisms, but very few particles, while the mononuclear myelocytes were feebly, but distinctly phagocytic for the cocci, but took up almost no pigment granules. In ~~slide (tray VI) (Case 60), in~~ ^{fresh} which the blood from a patient suffering from myelogenous leukaemia, ^{(Case 60),} was incubated for half-an-hour with living staphylococci, ^{and} many of the polymorphs ~~will~~ ^{were} be seen crowded with the organisms; in some of the intermediate cells as many as a dozen cocci ^{might} may be found; while in the myelocytes proper ^{might} may be seen from three or four up to perhaps six or seven at most. In the marrow films ^{from similar cases} the ~~same~~ phenomena were observed, analogous phagocytic

but in a less degree, shewing ~~apparently~~ that the marrow cells in leukaemic blood are apparently capable of assuming some of the functions of the adult leucocyte, feeble, it is true, but distinctly present, the organisms being seen surrounded by a digestive vacuole, and in many instances being in process of disappearance by intra-cellular digestion. It is interesting to note that the mast cells, which are very numerous in this specimen, and also the eosinophils, appear to take no visible part in the phagocytosis, though the latter cells are, in the fresh specimens, very actively amoeboid; and it is also of course possible, as has been suggested by some authors, that they may assist in the process chemically, by means of their secretions.

In this series of experiments, the incubations were carried out in each case with living, though not very virulent, strains of staphylo- and streptococci; with cultures of the same organisms killed by boiling; and with normal saline suspensions of vermilion and of carmine; and it was uniformly found throughout that all the neutrophil cells - polymorph, intermediate, and myelocyte alike - attacked and engulfed them most readily in the order named, a fact which may be explained on the chemiotactic theory by supposing that the living cocci possessed or

*ie. the living cocci more readily than the dead cocci,
& both of these more readily than vermilion or carmine,*

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secreted some substance with a greater attraction for these phagocytic cells than did the dead organisms, while the latter in their turn were attacked with greater readiness than the more inert particles of carmine and vermilion, a point which is of some interest in connection with the relative powers which these bodies possess of doing harm to the tissues which it is the function of the phagocytes to protect - a fact which demonstrates the selective capacities of these highly-endowed cells.

See A The digestive capabilities of the varieties of neutrophil cell were also found to be roughly parallel with the number of cocci which they had englobed, and hence also with the stage of development reached, many of the more active polymorphs shewing only the faint outline of the organisms remaining, and in many cases merely the clear digestive vacuole-like spaces representing the position of the cocci which had been ingested and which had disappeared by process of intra-cellular digestion, occurring in this experiment within a period of less than the half-hour during which the preparations were incubated at blood temperature. Many of the polymorphs shewed the included cocci in all stages of disappearance, the cells apparently continuing to englobe fresh organ-

cott.

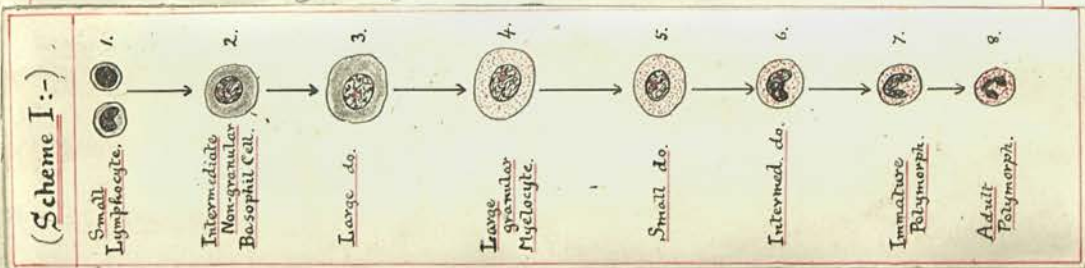
isms as those previously taken up were digested, only a few of the phagocytic cells themselves shewing very marked signs of disintegration within the limits of the time specified. As the process is traced backwards through the less mature cells of the series, i.e. in the immature polymorphs, intermediate cells, and rounded mononuclear myelocytes, we find the process becoming gradually less active and less complete, but yet distinctly present, even in the youngest cells which are the first to develop neutrophil granules - namely, the myelocytes proper - and these cells, both in leukaemic blood and in marrow preparations, may therefore shew, on the warm stage, at all events, a foreshadowing of the functions typical of the adult polymorphonuclear leucocyte or microphage derived from them, these functions becoming more and more marked as the nucleus assumes the polymorphonuclear type.

As this transition from myelocyte to adult leucocyte becomes more complete, the nucleus becomes progressively more and more convoluted and irregular in shape, until it finally assumes the typical and familiar type found in the polymorphonuclear leucocyte. While this change is proceeding, there also occurs a "condensation" or some such analogous change,

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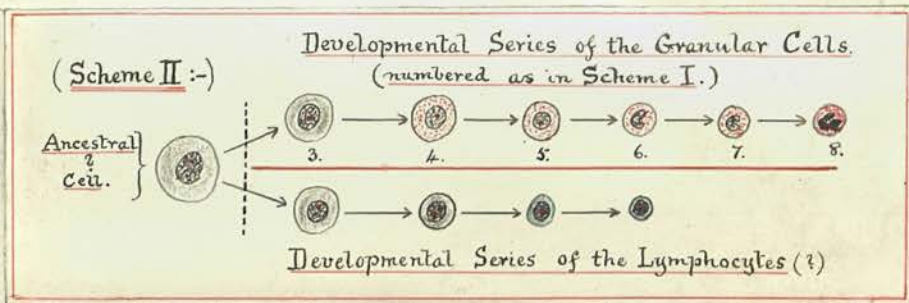
whereby the chromatin is found to stain much more intensely, while a somewhat similar change is found in the cytoplasm which gradually becomes less and less basophil, and also in the granules which it contains, a condition which is sometimes described by certain writers as a "ripening of the granules", whereby they stain more deeply with the acid (e.g. eosin), and less and less with the basic dye. It may here be added that the term "neutrophil" used by Ehrlich is in reality somewhat erroneous, as they stain faintly but distinctly with acid dyes, such as eosin; and therefore a more accurate term for them would be finely granular oxyphil cells, as suggested by Kanthack⁽⁷⁵⁾ and others.

We have thus traced in the adult human subject the development of the polymorphonuclear leucocyte as it is found in the blood, and this ^{has been} ~~may be~~ diagrammatically represented thus:- in Plate - , Scheme I.

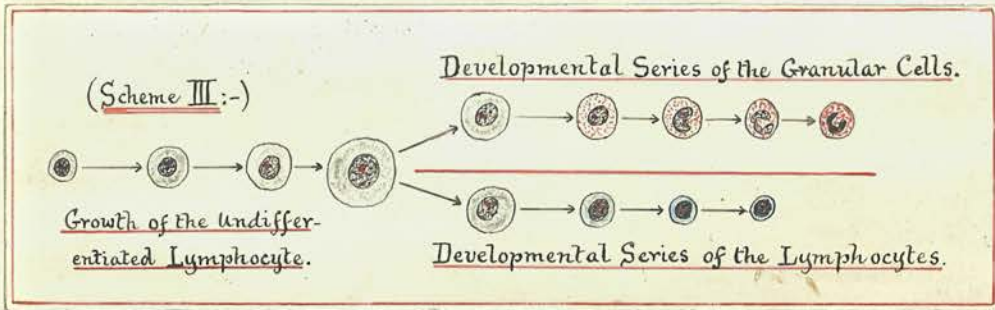


I am yet in some doubt as to whether the small undifferentiated lymphocyte of Wolff may not possibly

be a derivative rather than a progenitor of the larger form of non-granular basophil cells, as one finds mitotic division occurring most frequently in the latter variety, in which case the larger cells would be the earliest type of cell found in the adult marrow, and our diagram would then take the form: *shown in Plate - , Scheme II.*



I am inclined, however, to believe that Scheme I. is the correct one, and that by readjusting and combining them both; *(see Scheme III)* we indicate the most probable method of development, both of ^{the} neutrophil series and of the differentiated lymphocytes, viz., by regarding the undifferentiated lymphocyte as the parent cell of the pre-myelocyte and myelocyte series, and also of the differentiated lymphocytes which are found in comparatively small numbers in normal, but are increased in certain pathological, marrows.



We may now briefly discuss certain pathological conditions under which some of these cells may find their way into the circulating blood. In a state of health, the polymorphonuclear leucocyte is the only member of the neutrophil granular group which enters the blood stream. It is somewhat beyond the scope of this paper to investigate fully the process by which this migration is effected, as my work does not help to throw any new light upon it, and it will be sufficient to state here that the so-called chemiotactic theory, although very unsatisfactory and incomplete, is, so far, the best that has yet been advanced, especially if taken in conjunction with the researches of Metchnikoff upon phagocytosis, - the chemiotactic and the phagocytic theories being complementary to one another rather than antagonistic, as so many writers on the subject have alleged. It will suffice for the present to mention, that in a large number of diseases, the marrow is stimulated

to produce and send forth certain of its cells in greatly increased numbers, and we may now consider the changes thus brought about in the neutrophil series. The most frequent form of active leucocytosis is that in which the polymorphonuclear neutrophils are chiefly concerned, i.e. the mature of fully-formed cells of this group. Whatever be the cause at work which brings about this increased emigration of those elements from the bone-marrow, it is evident from clinical experience that in the different diseases in which it occurs, and even in cases of the same disease, the resulting leucocytosis varies very widely, both quantitatively and qualitatively, in different individual cases. The reasons for this variation may be sought for in many directions, and we may classify them roughly into two principal groups, both of which are of vital importance:

1. The relative quality, intensity, amount and period of action of the stimulating substance.
2. The vitality of the blood-forming tissue, and the age, general condition, etc., of the patient himself.

In the consideration of the first of these great factors, we may again divide the substances stimulating the haemopoietic tissues into two great varieties,

according as they act under physiological, or under pathological conditions. In the former group, we may place the causes of the leucocytosis occurring after exertion, cold bathing, etc., during digestion and pregnancy, and normally found in the blood of the infant at and just after birth; whilst into the latter or pathological group we may put those of toxic and inflammatory, post-haemorrhagic, ^c~~a~~ca^hctic, ante-mortem, therapeutic and experimental leucocytosis. Of these, it is the leucocytosis of toxic and inflammatory conditions that we shall more fully study in connection with the alterations found in the marrow, and for this purpose that occurring in pneumonia and in various septicaemias will be found most convenient. When the infection or intoxication is mild, and the individual has been previously healthy, the leucocytosis may be slight - say 10,000, 12,000 or 14,000; when the disease is less mild and the patient's resistance somewhat lowered, the leucocytosis may be moderate in amount, say 18,000 or 20,000, or more. Again, if the infection is severe, and the resistance good, the result may be a very decided rise in the leucocyte count, of 30,000 or 40,000; but if the resistance be lowered, say from previous ill-health, alcoholic habits, etc., the rise may either

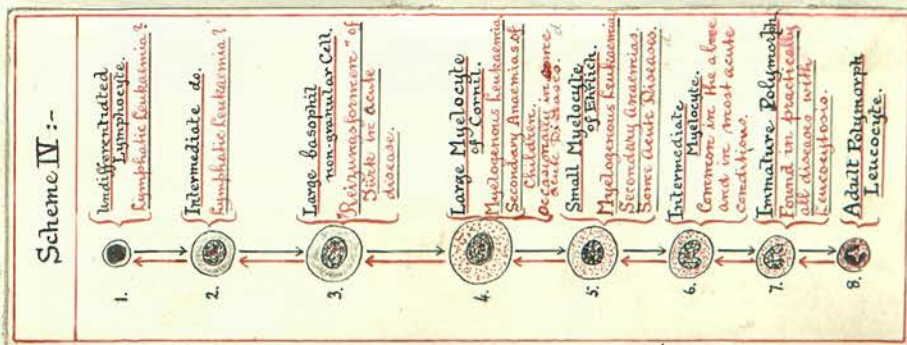
be very excessive (60,000, 70,000 or even more), or in a certain class of cases, especially if the virulence of the organism be great, and the vitality of the patient much impaired, there may be no rise in the number of leucocytes at all - there may even be a lowering of the count, a condition known as leucopenia or hypoleucocytosis. So far, we have only considered the total numbers, i.e. the quantitative reaction involved; but there may be also very important qualitative variations in different cases, occurring not only with regard to the relative numbers of the different species of blood cells, such as the eosinophils (to be considered later), lymphocytes; and neutrophils, but there may be very important disturbances among the varieties of cells which have been described as belonging to the neutrophil group. In the majority of the milder infections and inflammations, the numbers of the polymorph or adult neutrophil cells alone may be affected, and indeed, many writers apply the term "polymorphonuclear" or "polynuclear" leucocytosis as synonymous with that occurring in these conditions. This term, however, is very often a misnomer and for it the term "neutrophil" should be substituted, as in many cases in which it occurs, some of the more immature

cells of the neutrophil series are found in the blood. Türk,⁽¹³¹⁾ in his very complete study of the condition of the blood in the acute infective diseases, was one of the first to draw attention to the fact that a considerable number of myelocytes may be found, quite apart from the leukaemic condition, in which, according to Ehrlich's earlier researches,⁽¹⁰⁹⁾ they were found in the human subject only. Rieder demonstrated the occurrence of these cells in several leucocytotic bloods; Türk in pneumonias sometimes found as many as 10%, and more of the neutrophils to be composed of myelocytes, the increase usually occurring about or just after the crisis of the disease; and Engel⁽⁴³⁾ in 1896 showed that large numbers of myelocytes (3.6 to 16.4%) may be found in serious or fatal cases of diphtheria occurring in children. More recently various observers have noted the occurrence of myelocytes in many other conditions, notably the remarkable myelocytotic condition of the blood in smallpox described by Roger and Weil⁽¹²¹⁾, Courmont and Montagard⁽²⁵⁾, Ferguson,⁽⁴⁶⁾ and others, where the bone-marrow appears to be no longer capable of producing adult leucocytes, "ceasing", as Ehrlich describes it, "to

represent an incubator where the parent cells of the leucocytes mature and transform themselves into polymorphs". Myelocytes may also be found, but in much fewer numbers, in such conditions as the primary and secondary anaemias, especially in pernicious anaemia, v. Jaksch's anaemia, and in the anaemias following syphilis, malignant tumours, etc.

In bloods shewing even a slight degree of leucocytosis, and occasionally even in apparently healthy cases, one may find a few immature polymorph cells; and where the leucocytosis has attained any marked degree, we almost invariably find not only immature polymorphs but also numerous transitional neutrophil myelocytes. In other words, as the stimulus which brings about the leucocytosis increases in amount (e.g. in pneumonia, septicaemia, etc.), or according as it becomes altered in quality and comes to resemble, say that of small-pox, so do we find that progressively less and less mature cells

Smith



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See p. 88.

of the neutrophil series may find their way into the blood ~~serum~~^{stream}, adding to, and sometimes even coming almost to replace, the adult or fully developed polymorphs. Thus we may find that, as this occurs, these cells, speaking somewhat generally, tend to pass into the blood stream with a readiness proportionate in degree to their stage of development, i.e. in the reverse order to that which is shown in the above diagram, viz., adult polymorphs, immature polymorphs, intermediate myelocytes, small myelocytes (sometimes known as those of Ehrlich) and large myelocytes (described by Cornil^(24a) as occurring almost exclusively in myelocythaemia).

Another factor which may aid in the production of a leucocytosis characterised by the presence of a smaller or larger proportion of immature cells, is the actual prevention of maturation of the younger cells of the series by some special quality of the substances or toxic material causing the disease; and this, together with a stimulation of these younger cells to greater proliferation, doubtless accounts for such remarkable conditions as those found in the blood in the leukaemias, though the nature and origin of the specific poison at work in this group of diseases yet remain unknown, and other

factors yet more obscure may play a part in their causation. As already suggested, while considering the large mononuclear basophil cells from which I have shewn that the larger granular cells are directly derived, it seems only natural to expect that, should the stimulus be sufficient in degree or in quality, these cells will also sometimes appear in the peripheral blood; and I am of opinion that these indeed constitute the "Reizungsformen" of Türk, a suggestion which appears to me much more probable than that they belong to the red series of blood cells; though the possibility of the two cells being somewhat closely allied from a developmental point of view must also be borne in mind.

Again, as we have seen in the foregoing paragraph, should the stimulus be so constituted as to call forth the neutrophil myelocyte in very excessive numbers, we may quite well obtain the clinical picture of a myelogenous leukaemia, and the disease therefore appears to be in reality nothing more than a very specialised form of leucocytosis brought about by some specific toxic agent acting in precisely a similar manner to those already considered; and by again passing backwards one, or perhaps two, more steps in our developmental series, we have

before us a partial explanation as to the nature of the so-called "lymphatic" forms of the same disease, as already suggested while considering the small mononucleated cells found in the marrow, a tissue which, in the series of such cases which I have examined, appears to be equally, if not more profoundly altered than the lymphatic tissues proper, though whether this is due to an infiltration with lymphoid cells, or to "inhibition" of normal development, or to "stimulation of younger forms" and "reversion to an embryonic type of blood-formation", I have found it impossible to determine; and these phrases, so often used by many writers on the subject as if they were actual explanations of the cause, instead of being merely some of its attributes, are at present used at random and are simply a confession of our ignorance, as to its real nature and mode of action.

EOSINOPHIL CELLS.



2. Eosinophil Cells:

97 9'

These cells are in their development, function, morphology, and general distribution, both in health and in disease, absolutely distinct from the members of the neutrophil series, nor have I ever found any indication that they may arise from the latter group of cells, as averred by Gulland,⁽⁵⁵⁾ who describes transitional forms between neutrophil and eosinophil granules in the blood cells of embryos.

The eosinophil cell is by no means specially characteristic of the blood or of bone-marrow, and it is found widely distributed in various connective tissues, in certain parts of the alimentary canal, in the omentum, spleen, lymphatic glands, and many other positions. It approaches much more to the connective tissue type of mesoblastic cell than does the neutrophil polymorphonuclear leucocyte, which is a true blood cell, and is liable, when it leaves the blood stream, to be englobed and destroyed by other cells; while the eosinophils are apparently able to wander with immunity through many of the tissues. In my observations upon the occurrence of eosinophils in the bone-marrow I have failed to find any definite ratio between the numbers of these cells as they occur in this tissue and in the blood, the marrow appear-

ing to be only one of the many sites where these cells may proliferate.

In the eosinophil cells of the marrow there can be traced a definite series of changes almost precisely similar in character to those occurring in the neutrophil cells, and there can thus be distinguished an eosinophil myelocyte, intermediate myelocyte, and polymorphonuclear leucocyte, illustrations of which may be seen in Plate I., figs. 4, 5 and 6. The eosinophil myelocyte, like its neutrophil analogue, varies considerably in size and shape. In stained specimens (e.g. in Plate I., figs. 4, 5, 6, cells 1, 1, 1,) the nucleus is rounded or somewhat oval in shape, and is usually somewhat smaller and may also stain less deeply than the nucleus of the corresponding neutrophil cell, though the staining reactions vary considerably with different dyes, and in some cases a pyknotic condition may be found, usually however indicating approaching mitosis, or some form of nuclear degeneration. The nucleus possesses a definite nuclear membrane, chromatin network and nucleoli, etc., and is very similar in appearance to that of the neutrophil myelocyte. The nuclei of the intermediate eosinophil myelocytes are also somewhat similar to those of the other set,

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passing through the same series of changes in outline and becoming first slightly indented and then horse-shoe-shaped, and finally approaching the polymorphonuclear type as they become more mature. They are generally however distinctly smaller in size, both absolutely and in proportion to the magnitude of the cells in which they lie, nor do they usually stain so deeply as the corresponding nuclei of the neutrophil cells (See Plate I., cell 2, in figs 4, 5 and 6 respectively, and compare and contrast with the intermediate cells shown in figs. 1, 2 and 3.) The nucleus of the polymorph eosinophil cell never attains to the same complexity of shape and structure as that shown by the neutrophil leucocyte, not usually exhibiting more than two or at most three lobulations, which have a somewhat coarse reticular structure. The protoplasm of all three members of the eosinophil group of cells is chiefly remarkable for the presence of large, more or less rounded, granulations, usually comparatively uniform in size and in their distribution throughout the cell body, though sometimes varying considerably in different individual cases and in certain pathological conditions. These coarse eosinophil granules (α -granules of Ehrlich) are, as the name implies, strongly

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(40)

oxyphil in their staining reactions, but as Ehrlich himself has pointed out, the younger or less mature granulations may shew a more marked affinity for the basic dyes, and may stain bluish-red or even pure blue with eosin and methylene blue (loc.cit. p.109). Malcolm and Milroy⁽⁸⁷⁾ have experimentally demonstrated that the coarse oxyphil granulations may, in the rabbit and guinea-pig, become definitely basophil after the injection of nucleic acid, and I have found that the same phenomenon occurs, though in less marked degree, in rabbits inoculated with diphtheria toxin and other substances, whilst I have also found the same condition in the eosinophil cells of human bone-marrow in several cases of pneumococcal and other septicaemias. In a few instances I have found cells (Plate I., fig.7, cells 1-6) which are morphologically identical with the eosinophil cells among which they lie, and from which they only differ in the colour of their granulations, which stain as indicated in the figure, of a bluish-red to ^apure blue tint with eosine-methylene-blue. These cells are absolutely distinct in their characters from the ordinary granular basophil or mast cell, one of which has been inserted into the diagram by way of contrast (Cell 8), and I am inclined to regard them as eosino-

phil cells which are undergoing or have undergone some pathological or other change analogous to that described by Milroy and Malcolm.

As already stated, the eosinophils form a series absolutely distinct from the neutrophil cells, differing widely from these both with regard to their development, and in their behaviour to "chemiotactic" substances. They are found in the embryo at a much earlier period than the neutrophils, and they have been described by Gulland⁽⁵⁵⁾ and Schaffer⁽¹²⁴⁾ as occurring in the lymphatic glands and in the thymus before the formation of the bone-marrow.

In the circulating blood, they are found to be increased in a large number of conditions which have been very fully studied and recorded by many writers. Under normal conditions they usually vary from one to three or four per cent., while they may be greatly increased in many acute and chronic skin diseases, bronchial asthma, myelogenous leukaemia, certain diseases due to intestinal parasites, and in many other conditions much too numerous to mention here. More important for the present purpose is their relative diminution or even entire disappearance from the circulating blood in certain of the acute infective fevers, a condition usually explained as being

due to "negative chemiotaxis", the substances which in these conditions are supposed to attract the neutrophils; being also credited with the property of repelling the eosinophils, and so preventing their passage into the blood. This is notably so in the majority of cases of acute lobar pneumonia, and also in many other toxic and septic conditions (with the somewhat remarkable exception of scarlet fever), the reappearance of these cells, often in greatly increased numbers, being usually a favourable symptom in the course of the disease. In the majority of the fatal cases of pneumococcal and other acute septicaemias which compose my series, the eosinophil cells have been either uniformly diminished or are [entirely absent from the circulating blood. On the other hand, the condition of the marrow with regard to these cells has been found to vary within very wide limits, as may be seen in the accompanying table, ^{*} a study of which will only justify us in concluding that in some cases of acute streptococcal septicaemia the eosinophils may be found to be entirely absent; in cases of acute lobar pneumonia accompanied by leucopenia, these cells may be exceedingly scanty in the marrow; but in young subjects, on the contrary, they may be found

* See Appendix II. at the end of this Volume.

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in greatly increased numbers, as they also are in certain cases where death supervenes rapidly in very acute septicaemic conditions. (Cases 61, 62.)

Any further conclusions other than that the numbers of eosinophil cells in the marrow may vary within very wide limits in the acute diseases under consideration, and that these bear no ascertainable relation to the number found in the circulating blood, would appear, from a consideration of this set of cases at all events, not to be justified at present. I have however found that in specimens of bone-marrow from certain cases of more chronic disease, the eosinophil cells may be enormously increased in numbers. This is specially the case in a small set of tubercular conditions in children aged 7, 7, and 14 years, the cases being tubercular peritonitis, meningitis, and acute general miliary tuberculosis respectively. A considerable increase was also present in all the marrows from a series of five cases of exophthalmic goitre; in the majority of cases of malignant disease (six cases); and in all my cases of pernicious anaemia, six in number, the eosinophil cells were present in very large amount. The most enormous increase, however, in the cells of this series, I found to be present in the bone-marrow of a child of

nine who died from a condition resembling acute myelogenous leukaemia of three weeks duration, and of an infant which probably suffered from congenital syphilis, which succumbed to an attack of diphtheria at the age of three months. In the case of a child of eleven, who died in the third week of uncomplicated typhoid fever, they were very scanty, and in a child of three years, which died of diphtheria supervening on tuberculosis of the mesenteric glands, there were also remarkably few eosinophils present in the marrow. It is of interest here to note that some authors believe that there exists some peculiar relation between the occurrence of lymphocytosis and eosinophilia, (Ewing, ⁽⁴⁵⁾ who quotes the experiments of Grawitz with tuberculin, and Kurloff in splenectomised animals, which produced a lymphocytosis followed by eosinophilia), and the appearances found in some of my cases, e.g. the tubercular conditions in children, etc., appear to lend some support to this belief. Opie ⁽⁹⁸⁾ believes that there is a direct relationship between eosinophil cells and nutrition, their numbers becoming decreased by starvation, and increased when food is again given, while the occurrence of a post-digestive lymphocytosis, reaching its maximum some four hours after a meal, is now a well-established fact.

Muir (91) finds that "the eosinophils, both myelocytes and their polymorphonuclear derivatives, occur in somewhat varying proportions," and he adds that in the cases of empyema which he examined, "they were never, however, increased relatively to the neutrophils, although there was usually an absolute increase, and in two cases this was distinct." My results are therefore, on the whole, in accordance with those of this observer, though in some of my cases the increase appears to have been more marked. I find that in the very early cases of pneumonia, i.e. during the first day or two of the fever, though none of the cells may be present in the circulating blood, the increase of eosinophils in the marrow may be distinct (Cases 61, 62); during the height of the disease, they are still absent from the circulation, and their behaviour in the marrow is somewhat uncertain, being sometimes increased or unchanged, but more usually diminished in their relative proportion to the neutrophil cells, while later on there is a critical, or post-critical rise in their numbers, both in the marrow and in the blood, a condition which may also be found in some of the other septicaemic diseases.

The occurrence in the blood of eosinophil myelocytes in any considerable numbers is a condition of extreme rarity, except in cases of myelogenous leukaemia; they are also said to occur in v.Jaksch's anaemia, and in myxoedema, whilst Türk⁽¹³⁰⁾ has found them in some of the acute infective diseases, but very rarely, and always in very small numbers, though Weil⁽¹³⁴⁾ has observed as many as 1% in the blood of small-pox cases.

BASOPHIL GRANULAR CELLS.

(a) Mastzellen or Mast Cells.

(b) Cells resembling eosinophil myelocytes in all their morphological characters, but with basophil instead of oxyphil granulations.

3. Basophil Granular Cells:

(a) Mast-zellen or Mast-cells.

Many of the questions in connection with these cells are surrounded by great obscurity, and this has been considerably increased by the laxity with which many authors apply the term "Mastzellen" to any cells containing basophil granulations, whether these be coarse or fine, and also quite irrespective of whether the cells in question are blood or connective tissue cells. The term should only be applied to the one or to the other variety of cell, and not indiscriminately to both, and for the present I shall use the term Mastzell or Mast-cell to designate the type which may be found in the circulating blood, in numbers varying from 0.28% (Canon²²) in health, up to as many as 140,000 (Taylor*) per c.m.m. in myelogenous leukaemia. Ehrlich⁽⁴⁰⁾ is of opinion that these cells are exclusively derived from the bone-marrow, of which, he states, ~~it~~^{they} form a normal constituent; and in arriving at this conclusion, he considers that it is sufficiently proved by the fact that a leucocytosis of mast-cells occurs in myelogenous leukaemia. In my own observations I have found the occurrence of mastcells to be of considerable rarity in the marrow in the conditions

* Taylor, quoted by Ewing. (45)

under which I have examined it, and I have not yet been fortunate enough to obtain the marrow from a case of myelogenous leukaemia. Upon this result, however, I do not lay any special stress, as recent observers have found that the granulations of the mast-cell are very soluble in water, and that alcoholic solutions only should on that account be used for their demonstration. I have therefore no special observations to record in regard to these cells, with the exception of the fact that I have found them abnormally increased in number in the blood of acromegalic patients (four cases) reaching sometimes nearly as high as 2%, a fact which, as far as I can ascertain, has not hitherto been recorded. A mast-cell from a case of myelogenous leukaemia is illustrated on Plate I., fig.7, cell 8, for comparison with the next variety of cell to come under consideration; and, in the incubation experiments performed with the blood from this case, to which allusion has already been made in connection with the phagocytic activity of the neutrophil series of cells, it may here be added that neither the mast-cells, which occurred in very great numbers, nor the eosinophils, took any visible part in phagocytosis. The mast-cell shewn in the figure was fixed dry, and hence

the nucleus appears considerably larger than in specimens fixed wet (c.f. the nuclei of the neutrophil and eosinophil cells depicted in the lower row in figs. 2 and 5 of the same plate. The nucleus is usually polymorphonuclear in character in the adult blood cell, and rounded or oval in the younger members of the series. In the protoplasm are found embedded the characteristic large and small basophil granules which generally exhibit a certain amount of "metachromasia", i.e. do not show the pure bluish colour of the basic dye, but usually stain with a slight purplish tinge, especially with thionin blue.

(b) Cells resembling the eosinophil myelocytes in all their morphological characters, but with basophil, instead of oxyphil, granulations (Plate I., fig. 7, cells 1 to 7.)

These cells occur in small numbers in a moderate proportion of my cases, e.g. in the rib-marrow of two apparently healthy individuals, aged 53 and 60 (cases 71 and 72.); and in a very few of my more acute cases, for example in No. 24, an acute lobar pneumonia, from which the three upper cells (1-3) in fig. 7 are drawn; and also in case 16, a septic pneumonia with meningitis, from which cells 4-6 in the

same figure are taken. In the two last cases the leucoblastic changes in the marrow were very slight in degree, and the acute disease had apparently supervened upon some chronic condition, there being in Case 24 a considerable amount of interstitial pneumonia, perhaps syphilitic in origin, and in Case 16 a history of obscure abdominal trouble. The large granular basophil cell (No.7) in the lower right-hand corner, is from No.69, the case of sarcoma of the kidney, from which figs 8 and 9 of the same plate were taken, as already described. From a careful study of these cells, especially as they are seen in the preparations from case 16 (q.v.) I can find, with regard to their size, shape and nuclear characters, and in the number, size and distribution of their granulations, no point in which they differ from the eosinophil coarsely granular myelocytes among clusters of which they usually lie; and indeed, after a prolonged search, several cells were seen in which the granules showed intermediate tints between the bright red of the oxyphil, and the definite blue of the basophil cells, and I therefore regard the latter as being in reality members of the eosinophil series, the granules of which have undergone some pathological change analogous to that described by

Milroy and Malcolm⁽⁸⁷⁾ in their paper on the action of nucleic acid, ~~and~~ a condition which may possibly indicate that these cells are still comparatively immature, as evidenced by the shape of their nuclei, which belong to the mononuclear myelocyte type. In no case was this change observed in the older cells, e.g. the polymorphonuclear leucocytes, of the eosinophil series. In the specimen from which cells 4-6 are drawn, the red tint of the nuclear staining is due to the fact that this bone-marrow had been decalcified with nitric acid - a process which, I may add, would have destroyed these granulations had they been identical with those of the mast-cell previously described. (Plate I., fig.7, cell 8.)

DEGENERATIVE CHANGES

occurring in the Cells of the

LEUCOCYTE-FORMING SERIES.

Degenerative changes occurring in Cells of the
Leucocyte-forming Series.
(Plates II. & III.)

Picta ant.
l. c. (centra)

In addition to the proliferative and other changes described elsewhere, various degenerative phenomena, due to the action of the bacterial and other toxic substances producing or accompanying the disease, are of practically constant occurrence in the cells of the bone-marrow, and similar changes may also be found in many of the cells of the circulating blood. These degenerative conditions may be found more marked in the nuclei of the cells, or again, they may chiefly affect the ^{*Cylo*}~~protoplast~~plasm. Usually however, if they are found to any marked degree in one of these situations, the other parts of the cell are almost invariably affected.

In order to study these changes under the most acute conditions possible, I have prepared and examined a number of bone-marrow specimens from rats, rabbits and guineapigs killed by varying doses of snake venom*, and a typical field from one of these is figured in Plate II., where the remarkable ef-

* For some of these animals I am indebted to my friend Dr Hunter of the Physiology Department, who has been conducting a research on the action of snake venom upon the production of haemolysins and precipitins in the blood.

of the University of Edinburgh

fects of the poison upon the nuclei of the white cells is beautifully illustrated. In this specimen there is perhaps some slight increase in the number of cell divisions occurring in the members of the myelocyte series (e.g. cells 22 and 23), but the principal effect which the venom appears to have exerted is the production of a very peculiar change in the shape of the nuclei of these cells. This may be described as the assumption by the nucleus of ^a spiral or rosette-like appearance, varying in complexity and resembling in some ways a very exaggeratedly polymorphonuclear type. As it is by the shape and characters of the nucleus that we are enabled to classify the different members of the neutrophil series, it is somewhat difficult to determine whether it is the younger or the older cells of this group which are most affected by this peculiar change. The appearances, however, are exceedingly suggestive of the possibility that these cells, under the special action of this poison, tend in the degeneration so produced, to become rapidly altered in the same direction as that towards which they normally develop. In other words, the inherent morphological tendency of the nucleus to become polymorphous in character appears to be accelerated and greatly exaggerated in degree in this type of change - a condition which may also sometimes be noted in the leucocytes of the blood in

many of the acute diseases, where the nuclei of the polymorphonuclear cells may appear abnormally convoluted and complex in their structure; and may shew a much greater number of lobes or subdivisions than is usually seen in the healthy polymorph. In this figure (plate II.) cells 1 to 3 are ordinary neutrophil myelocytes; cells 4 to 16 shew nuclei varying in the complexity of their structure from that of the slightly indented and horseshoe-like nuclei of the intermediate myelocytes, up to the intricate rosette-shaped bodies described above. The latter are, in reality, probably composed of one long thread-like structure coiled up within the cell in the manner described; and Nos. 17, 18 and 19 shew this body arranged in a somewhat more open spiral manner.

The other more usual types of cellular degeneration are also found occurring in the myelocyte series in acute and chronic diseases, and specimens illustrative of these conditions are shown in Plate III.

These changes may in general be divided and classified - according to the manner in which they are found to affect the nucleus - into the two great subdivisions, karyorrhexis accompanied by condensation or pyknotic changes in, and fragmentation of, the chromatin, and by its subsequent disappearance by

solution or phagocytosis; and karyolysis, characterised by swelling up of the nucleus and solution of its chromatin, occasionally accompanied by, but more frequently without, any preliminary pyknotic stage.

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In figs. 1 to 4 of plate III. are shewn illustrative groups of myelocytes exhibiting varying degrees of degeneration by karyorrhexis, a condition which is found in many of the acute diseases. In fig.1, from a case of acute lobar pneumonia, the nuclear network is seen to be in process of breaking up, and its chromatin becoming aggregated into irregular particles or masses. These may exhibit no special plan in their arrangement, but they are sometimes found dotted very regularly over the whole nucleus, as in Cell 1; or, more frequently, they are seen arranged in a somewhat symmetrical manner around the periphery of the nucleus, attached apparently to the inner surface of the perinuclear membrane. A somewhat similar stage of nuclear degeneration is depicted in fig.2, from another case of pneumonia, in which however, the protoplasm of the cells is somewhat more affected than in the first figure. The subsequent phases of karyorrhexis are illustrated in the two following figures - figs.3 and 4 - the

former of these being from a case of very acute septicaemia, probably pneumococcal in origin; and the latter shewing the effects of experimental inoculation with diphtheria toxin upon the bone-marrow of the rabbit. In fig.3, the chromatin particles are of considerable size, and they also in this case illustrate the tendency which they frequently exhibit of becoming aggregated round the margin of the nucleus within the perinuclear membrane; and thus, when the latter becomes dissolved, they are found scattered free in the cell protoplasm in which they may undergo solution. This plate (figs.1 to 4) also illustrates the disappearance of the nucleolus, and the progressive breaking up of the nuclear network and perinuclear membrane; and these figures also shew polymorphonuclear leucocytes undergoing similar pyknotic and fragmentative changes. The protoplasm of the myelocytes is also specially liable to degeneration under the toxic action of organisms and their products. This change may usually be first observed as a process closely similar to that of cloudy swelling. The ~~protoplast~~^{cyto}plasm becomes swollen up and the cytoreticulum altered in some way, which may cause it to stain more deeply and sometimes irregularly, giving what is frequently but erroneously described

as a "granular" appearance to the cell (see fig.1, plate III.), a condition which must not be confused with that which is sometimes found, especially in certain more chronic diseases, where the actual granules are increased in number, or in staining capacity, e.g. as is shown in fig.6 of this plate, and also in plate I., figs.8 and 9.

Vacuoles may now appear in the protoplasm, and these may increase in number and size until the whole cell-body shows merely as a scanty, shred-like network, before it finally becomes broken up and disappears. (For the successive phases of protoplasmic degeneration see figs. 1, 2 and 5 respectively, in the order named.)

The changes seen in the degeneration of myelocytes by karyolysis are depicted in figs.5 and 6 of the same plate, and these illustrate the two methods by which this may be brought about. The nucleus (fig.5) usually becomes swollen and vacuolated in appearance, its network gradually breaking up, and the chromatin being either dissolved in the karyoplasm or becoming altered in such a way chemically that it no longer takes on the basic dye. This "hydropic" condition of the nucleus becomes more and more marked, until finally the nuclear membrane disappears and the whole nucleus becomes dissolved in the protoplasm.

In fig.6, the chromatin has become diffused throughout the nucleus, perhaps by solution in the nuclear juice; and the whole structure is cloudy and indistinct, and does not stain sharply. This condition is frequently due to post-mortem change, when the tissues have had time to become altered before fixation, or again it is sometimes due to the latter process being imperfectly carried out. It is, however, also a comparatively common mode of degeneration during life, and the nucleus then gradually disappears by process of solution in the cytoplasm.

The question as to which of the granular cells is most readily affected by such toxic conditions, is one of some importance, and the relative liability of the different varieties of myelocyte to degenerative changes appears to vary somewhat in different cases. Frequently it is the younger or earlier members of the neutrophil series that are found to be most affected; but it is a somewhat remarkable fact, noted also by Muir,⁽⁹⁰⁾ that, though many of these cells in the marrow tissue may in some cases shew marked degenerative changes, the myelocytes seen free in the blood stream and found in the circulation may present a comparatively normal and undegenerated appearance.

The eosinophil cells, and also the small lymphocytes, appear capable of much greater resistance to the action of most toxic agents; and these cells will frequently be found almost unaltered in appearance and staining reactions, even when the degenerative changes in the neutrophils are very marked indeed. The most usual alteration visible in the eosinophil myelocyte in such circumstances is a condensation and pyknosis (see plate III., fig.1, cell 14 and fig.3, cell 23), or more rarely fragmentation (fig.3, cell 24), of its nucleus. Sometimes there may be considerable breaking up of the protoplasm and scattering of the granules, but this is generally an artificial appearance produced in making films, and is not usually very marked in sections of the marrow.

I. BLOOD-FORMING CELLS (continued):

B. Haemoglobin-holding Series.

- I. Normocytes and Normoblasts.
- II. Megalocytes and Megaloblasts.

Phenol

I. BLOOD-FORMING CELLS (Continued):

B. Haemoglobin-holding Series:-) *Lgh amb. no. 7*

I. Normocytes and Normoblasts:) *centre*

In the adult human subject, in health and probably also in disease, the only site of formation of the haemoglobin-holding or red cells of the blood is the red bone-marrow, and hence it is to a study of this tissue that we must look for light upon the origin and development of these cells. The adult red blood corpuscle occurs in very varying proportions in the marrow, the number of these cells depending not only on conditions of health or disease, but also upon the age of the individual; and similar variations are naturally also found in the nucleated cells from which they are derived, whilst in the different bones from the same case, and even at different parts of the same bone, the relative numbers of the white and of the red cells are found to vary within very wide limits.

The first observers to establish the fact that in extrauterine life the marrow is the site of the formation of the red blood corpuscle from pre-existing nucleated cells, were Bizzozero (8) and Neumann (95)

who, working independently, arrived at this important conclusion in the year 1868. This discovery has been uniformly confirmed by the later observations of a great number of other writers, and the subject which at the present day gives rise to most divergence of opinion in this connection is the origin in turn of the nucleated red cells or "normoblasts" as Ehrlich has termed them, from which the adult red corpuscles are derived.

Three varieties of nucleated red blood corpuscles or erythroblasts are recognised by Ehrlich,⁽⁴⁰⁾ and also by most other observers - the normoblast, the megaloblast, (including the gigantoblast of pernicious anaemia), and the microblast, a cell differing only from the normoblast in that it is distinctly smaller in size. These different varieties of nucleated red cell may be roughly stated to correspond in size respectively with the normocyte, the megalocyte and gigantocyte, and the microcyte found in the blood. The normoblast (Plate IV., figs 1-9, etc) is usually of about the same size as an ordinary red blood corpuscle, viz. about 7 or 8 μ in diameter. The cell is generally rounded in outline, or it may shew some slight irregularity in shape, usually due to the methods of preparation, or to the compression of neighbouring cells; and occasionally from the

latter cause, when these cells are actively proliferating, they may form small areas with a tessellated or polygonal appearance in the individual cells, due to mutual compression from lack of room for expansion. The usual staining reactions of the normoblast are illustrated in Plate IV., figs.1-4. Fig.1 is stained with methylene blue and alcoholic eosin, and, in comparison with this, fig.4 shows the cells from the same case, stained with methylene blue and watery eosin, both the haemoglobin-holding "discoplasm" and the nucleus being coloured much more intensely with the former staining combination than with the latter. Fig.2. shews the usual reactions with Ehrlich's triacid mixture in wet film preparations, and in fig.3 the stain used is haematoxylin and eosin. In fig.1, and to a less marked degree in fig.3, may be seen the very intense colouration of the nucleus with the basic dye, so very characteristic of the nucleated red corpuscle, and very aptly described by Roger and Josué⁽¹¹⁵⁾ as resembling a "drop of ink" in appearance. The nucleus itself is usually centrally, or slightly eccentrically placed, and is rounded in shape, its diameter varying roughly from about one third to one half the total diameter of the cell in which it is contained. In less deeply stained spec-

imens, e.g. in the water-soluble eosin preparation depicted in fig.4, the nucleus is seen to possess a definite perinuclear membrane and nuclear network, to which are attached larger or smaller very deeply staining granules of chromatin; and in some preparations and with certain staining reagents, it is sometimes difficult to distinguish the nucleus of the normoblast from that of the small lymphocyte, from which however it differs in the somewhat remarkable fact that it never possesses a nucleolus. With the triacid mixture the nucleus of the normoblast usually stains a pale, more or less homogeneous green or bluish-green colour (fig.2), though in dried films various appearances may be got, many of which I regard as artifacts due to the process of dessication, (see fig.9a and b.), as they are not found to occur in wet fixed films. Normoblasts may find their way into the circulating blood in many conditions where there is stimulation of the bone-marrow, and for an enumeration of which special works on haematology may be consulted.

A point which may be more fully discussed here is the much debated question as to how the erythroblast loses its nucleus and becomes an erythrocyte or non-nucleated blood corpuscle. Several theories

have been put forward to explain the somewhat obscure phenomenon, the most important of these being:-

- I. Expulsion or extrusion of the nucleus.
- II. Solution and disappearance of the nucleus in the protoplasm.
- III. "Budding" or extrusion of a portion of the cell substance.
- IV. Other theories, such as that of Maximow,⁽⁸⁵⁾ who is of opinion that, though the body of the nucleus is extruded, a small portion always remains in the shape of fine basophil granules in the protoplasm. Other observers believe that the nucleus persists, but loses its colour reaction, etc., etc. Foà's⁽⁴⁸⁾ statement that red cells are formed by endogenous formation in giant cells is due merely to ignorance of the ordinary elementary phenomena of phagocytosis.

The first of these theories, that of extrusion of the nucleus, was originally advocated by Rindfleisch,⁽¹¹⁰⁾ and later by Howell⁽⁶⁵⁾, also by v. der Stricht⁽¹²⁸⁾, Dominici⁽²⁸⁾ and other writers, including Ehrlich,⁽⁴⁰⁾ who accepts both the theory of extrusion and also that of solution. Muir^(90 & 91) finds that the nucleus may become small and compact, or may become fragmented, but that it is ultimately extruded and is taken up by the phagocytic cells. In some of my own preparations, I have observed the apparent occurrence of extrusion in all its described stages, but this has only been the case in films and smears; and in the sections of the marrows from the

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same cases, I have never been able to find the least evidence to correspond with the phenomena apparently demonstrated by the former method of preparation, and I have therefore come to the conclusion that these are artificially produced by mechanically rupturing the cells in the process of making the films.

W. B. Ross

The second theory, that of the disappearance of the nucleus within the cell-body by process of fragmentation (karyorrhexis), solution (karyolysis) or by both methods combined, appears to me, from my own observations on the cells in question, and also from a consideration of analogous phenomena as they occur pathologically in other cells of the body, to be the true explanation of this much debated question. The first change seen to occur in the nucleus of the normoblast is a process of condensation whereby it becomes smaller in size and stains even more intensely than before (Plate IV., fig.4, cells 1, 2, 3.) A minute bud-like projection is then seen to be formed at the circumference (Plate IV., fig.5, cell 1), apparently hollow at first and composed of perinuclear membrane. A narrow constriction or neck is then formed at the point of attachment to the nucleus, and at the same time the bud becomes filled with chromatin. (Plate IV., fig.5, cells 2 and 3).

This bud then proceeds to enlarge at the expense of the nucleus and its chromatin, until ultimately it may even equal it in size (cells 4 and 5), the two divisions remaining attached by the thread-like neck. This is not a process of true nuclear division, and in cell 6 may be seen two daughter nuclei which have been formed by true mitosis (such as is depicted in the same plate in cells 11, 12 and 13, in figs. 1 and 4 respectively), in which the uniting thread-like structure is absent. In other instances, instead of the small bud growing to a size equal to that of the diminished nucleus, other secondary buds may be formed, either from the first bud, giving a clover-leaf or shamrock-like appearance to the projection (cells 7, 8, 9, 10); or from the neck; or from the original nuclear body (Cells 11 and 12). These secondary buds then enlarge at the expense of the nucleus and its contents, and finally a somewhat irregular rosette or group of rounded nuclear fragments is formed (Cells 13 and 14). These may become still farther subdivided, or may now proceed to lose their chromatin, or at all events ~~their~~ *its* affinity for the basic stain, a process by which they at any rate become invisible by the ordinary staining methods. Specimens shewing this loss of chromatin

in two cases of acute disease by which the process is rendered more apparent - are shown in fig.8 of the same plate. Normally this disappearance only takes place after very complete fragmentation, but in disease it may be found occurring at all stages, as will be seen from a study of cells 2 to 11 in fig.8, in their order of enumeration, No.1 of the same series shewing a practically unchanged normoblast, and Nos.10 and 11 depicting the last change that can be demonstrated by this staining method, where the colourless "ghosts" of the nuclei or their fragments, are alone visible, and may still be seen to retain the nuclear outline, before their ultimate disappearance in the cell body. Some of these fragmentation changes in the nucleus of the erythroblast are not unusually mistaken by some observers for true nuclear division, and this fact accounts for the frequency with which cells of this description, when met with in the blood, are reported as having two, three, four or even more nuclei. True mitosis is rarely encountered in the healthy normoblast, even in the marrow, and good specimens of such division are shewn in figs.1 and 4, cells 11, 12, and 13 in each figure. In many diseased conditions, however, the two processes of division and fragmentation, though essentially dissimilar in nature, often appear to go hand in hand,

a phenomenon which illustrates the well-known pathological fact that cell-division is frequently stimulated by the abnormal conditions present in many diseases (Fig.7a), and that degenerative changes may supervene in one or other, or in both of the resulting daughter nuclei (cells 2 and 3), before the division of the cell-body can be accomplished. Fig.7b. shews the fragmentation changes which take place in greatly increased amount in most of the acute diseases; and fig.6 represents some analogous nuclear changes - both karyorrhexis and karyolysis - as they occur in pernicious anaemia. The majority of the phenomena shewn in fig.9, a dried film, stained in (a) - with triacid, and - in (b) - with triacid reinforced with methylene blue, are, I hold, artificial, as already stated above, and very many observers, whose works I have read, describe and figure changes of this nature in their writings - a consideration which very materially adds to the difficulty of the subject. An example of this may be seen in the illustrations of Cabot's "Clinical Examination of the Blood" (21) in plate IV., shewing "varieties of nucleated red cells", ~~with~~ the appearances in which are mostly artifacts.

The third theory of any importance in connection with the transformation of the erythroblast into the erythrocyte, is that of Malassez⁽⁸²⁾, who believes that the latter cell is produced by the extrusion of a portion of the cytoplasm of the parent cell; while Engel⁽⁴⁴⁾ holds that it is formed from the megaloblast by a similar process of "gemmation", neither of which suppositions needs detain us, as they^{both} are, as far as my observations go, entirely without foundation in fact.

(Degenerative changes in the cell body of the normoblast will be considered later, along with those occurring in the other forms of red cell.)

2. Megaloblasts and Cells intermediate
between the megaloblast and
normoblast.



2. Megaloblasts and Cells intermediate between
the megaloblast and normoblast.

In examining preparations of bone-marrow for normoblastic cells such as I have just been describing, one cannot but notice the occurrence of many cells which are very similar to them in many respects, but which possess a nucleus whose staining reactions are not so intense, and in which both the nucleus and the cell-body are of somewhat larger size, the latter measuring from perhaps 8 to 10 μ in diameter. These are probably less mature forms of normoblast in which the condensation or pyknotic change preliminary to fragmentation of the nucleus has not yet occurred. In these forms, true mitosis and the presence of daughter nuclei are of much more frequent occurrence than in the smaller normoblast proper, though still a comparatively rare phenomenon; and in the haemoglobin-holding series of cells we can, in a precisely similar fashion to that already worked out for the leucocyte series, trace the development of the red-cells from larger cells containing more voluminous and more faintly staining nuclei. Before discussing these gradations at greater length we shall first consider the occurrence and characters of the megaloblast, the presence of which in the circulating blood is, in extrauterine life, entirely pathological.

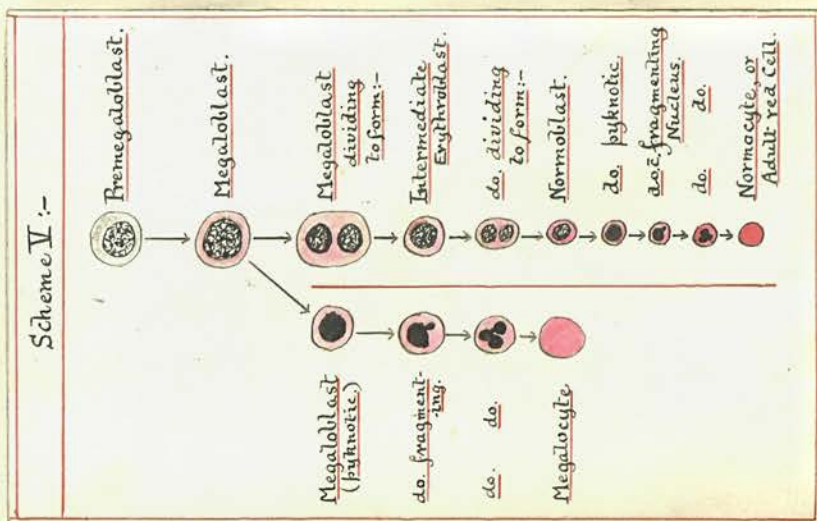
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The megaloblast is a nucleated red cell, which, as the term implies, is of larger size than the normoblast, varying in diameter from 10 to 20 μ , being designated by the name "gigantoblast" when it exceeds the latter dimensions (see Plate IV., fig. 11, from a case of pernicious anaemia, No. 50.) The usual descriptions of this cell are taken, by the majority of authors, from specimens occurring in dried films of the blood in pernicious anaemia, of which condition it forms one of the most constant and remarkable features. It occurs, however, as I shall show immediately, as a constituent of the bone-marrow in a large variety of other conditions, in which its appearance may differ widely from that of those occurring in the blood in pernicious anaemia, whilst we have already demonstrated that in dried preparations, delicate cells and their still more delicate nuclei are flattened out and distorted, and this may lead to many misconceptions with regard to their size, shape, and internal structure, which in dried films may often no more resemble the corresponding living cell than does the dried-up mummy its counterpart, the living human body. The megaloblast - and I shall describe its appearances from wet-fixed films and sections as it occurs in

many conditions in the human bone-marrow - is a cell of the dimensions already stated, though in wet films it may frequently be found somewhat smaller in size. In Plate IV., figs.10 to 18, will be found drawings of these cells as they occur in the marrow in various conditions, fig.10 being taken from a preparation made from the marrow of the femur of a full-time healthy child which died from suffocation. Cell 1 in this figure may be taken as a representative member of the megaloblast group, and it will be seen, from a comparison with the normoblasts in the same case (figs.1-4) that it is in some respects merely the magnified image of the smaller cell. The nucleus is large in relation to the size of the whole cell, and typically stains much less intensely than that of the normoblast. It shews a definite perinuclear membrane and nuclear network studded with dark staining chromatin granules, and it also resembles that of the smaller cell in possessing no true acid-staining nucleoli. In other specimens of this cell the nucleus may be found in a pyknotic condition, fig.10, cell 2., which may be preliminary either to true nuclear division (cell 3) or to fragmentation changes (cells 5, 6, 7 and 8), which are closely analogous to those

described above in the formation of the normocyte. This fragmentation is only got in health, during ~~the~~ intrauterine life, and for a very short time after birth, megalocytes being, apart from disease, found in the circulation only at that early period, the normal fate of the megaloblast being mitotic division and the formation of smaller cells of the nucleated red group (Cells 4 and 10, in fig.10); and I hold that these cells form one great developmental series whose function it is to produce the erythrocyte or adult red blood corpuscle. Ehrlich⁽⁴⁰⁾ strenuously denies that this is the case, holding that the two types of cell are wholly distinct, and he states that "all researches which try to obscure or totally deny the distinction between megaloblasts and normoblasts are wrecked by the simple clinical fact that in pernicious anaemia the blood is megaloblastic," an argument which I hold to be no more valid than if one were to deny that neutrophil polymorphs formed a series distinct from the neutrophil myelocytes, because the latter occur in myelogenous leukaemia. Ehrlich himself admits "that it is often difficult to decide whether a particular cell is to be regarded as a specially small megaloblast or a large normoblast. In such cases one would naturally search the

preparation for perfect forms of haematoblasts, and for the presence of free nuclei or of megalocytes, in order to obtain an indirect conclusion concerning the cells in question." Moreover, megaloblasts are well known to occur in the blood in various secondary anaemias, notably in those due to the presence of bothriocephalus latus, ankylostoma duodenale and other intestinal parasites, and they are also found in myelogenous leukaemia, etc. In other words, I regard the presence of megaloblasts in the blood as a phenomenon precisely analogous to the appearance of neutrophil myelocytes and of the basophil. "Reizungsformen" of Türk in the circulation, both conditions being due to pathological stimulation, either specific in quality, as in pernicious anaemia, and the leukaemias; or excessive in amount, as in some of the acute diseases, bringing about a perversion or disturbance of the haemopoietic functions of the marrow, whereby it loses its faculty of forming adult, and sends forth immature blood cells, in both cases passing backwards along the developmental chain, as already fully explained in the case of the neutrophil leucocyte series of cells (See *Plate 5*, Scheme 4, and compare with schemes 1, 2, & 3, pp. 81, 82, 83)



That this is fact and not mere theory may be easily seen by a study of the various bone-marrow preparations from which figs. 10 to 18, plate II. are drawn; and in the great majority of specimens from my series of eighty cases, undoubted megaloblasts can be found, in some it is true with comparative rarity, but in many, on the other hand, for example in those of exophthalmic goitre (fig. 12), malignant disease (fig. 18), tubercular conditions (fig. 16), acute and chronic septic conditions (figs. 15 & 14), acute lobar pneumonia (fig. 17a), and typhoid fever (17b), and also in many other acute and chronic conditions, they occur with comparative, and even great, frequency; and the same is even more true of the nucleated red forms bridging over the alleged developmental gap between the megaloblast and the normoblast. Mitotic division may be seen occurring with comparative frequency in the larger or earlier cells

of this series, and as already remarked, true nuclear division, as distinguished from fragmentation, is of comparatively rare occurrence in the normoblast. (Plate IV., fig.10, cell 3; fig.11, cell 11; fig.12, cell 11; fig.13, cell 11, monaster stage; fig.14, cells 6 and 7; and fig.18, cell 6; and here I may again call attention to the fact that these specimens are fixed wet, and hence appear smaller in size than they otherwise would if fixed by drying.)

Ehrlich⁽⁴⁰⁾ has noted with regard to pernicious anaemia that the presence of megaloblasts in the marrow does not necessarily mean that they are also to be found in the blood, and he is unable to give any reason why they should be found free in the circulation in some cases of pernicious anaemia and not in others, though present in the marrows of both in very large numbers.

A short description may here be given of the changes observable in the cell-body or discoplasm of the various members of the nucleated red group, as they occur in the course of their normal development or maturation, and also in certain pathological conditions. It may be laid down as a broad general principle that, in health, as the nucleus becomes smaller and more pyknotic as the process of cell

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division goes on, and the cell approximates more and more to the normoblast and normocyte, a parallel increase is found in the amount of haemoglobin present in the protoplasm of the cell-body, as evidenced by its taking on a progressively deeper tint with eosin and other stains having a special affinity for this substance; and whilst on the other hand, as one traces these cells backwards through the younger or less mature members of the series, i.e. the megaloblasts with large pale nuclei (figs. 10 and 14, plate IV) - the amount of haemoglobin present is found to become progressively less and less until finally it can no longer be distinguished, the protoplasm now taking on a definitely bluish reaction, a condition which is considerably exaggerated in some conditions of disease where the cells are being rapidly and imperfectly formed, e.g. in the specimens illustrated in fig. 16, 17a and 18, from cases of tubercular meningitis, pneumonia and sarcoma respectively, and also in many other conditions. This phenomenon has been termed "polychromatophilia", and is regarded by some authorities, e.g. Ehrlich and others, as a sign of "coagulation-necrosis", and approaching death of the cell. Many observers, however, are of opinion that, as it is chiefly found in conditions where

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red cells are being rapidly and imperfectly generated, this alteration is really due to the immaturity of the resulting cells, which are richer in primitive blue-staining protoplasm and comparatively poorer in red-staining haemoglobin, the explanation which, so far as I am able to judge from my own observations, is the correct one, the condition differing entirely from that found present in the adult red blood corpuscles in many anaemias, and in most of the acute diseases which are characterised by rapid destruction of pre-existing haematoglobin, where the pale de-haemoglobinised cells may be almost colourless or may stain a somewhat brownish tint.

In certain diseases, notably in pernicious anaemia, the opposite condition to that described above may be present, and in fig.11, plate IV. will be found specimens of megal- and giantoblasts which contain more than the usual proportion of haemoglobin, a phenomenon for which I can find no adequate explanation. In this specimen may also be seen several other interesting and important degenerative features, for example, greatly increased karyorrhesis, poikilocytosis, and the appearance of cracks and vacuolisation in the cell body said to be

due to necrobiotic changes, etc., for fuller descriptions of which reference may be made to works upon pernicious anaemia, of which these phenomena are specially characteristic.

There is much difference of opinion as to which members of the red cell group are in adult marrows the earliest or most primitive form of cell. In the early embryo the red cells are derived from special mesoblastic cells or "blood-islands" in the vascular area; and the endogenous or intracellular theory of their formation in the so-called vasoformative cells, previously supported by Schäfer* and still held by a few observers, has been gradually abandoned, and is now, I believe, regarded by Professor Schäfer himself as a phenomenon bearing quite another interpretation. In later embryonic and in extra-uterine life, we have already seen that in the bone-marrow mitotic division is to be found occurring in the nucleated red cells, with considerable rarity in the case of the normoblasts, but with comparative frequency in that of the earlier or larger intermediate cells and megaloblasts; and we have in turn traced the latter variety of cell back to a colourless "pre-megaloblast", to coin a word analogous to the "pre-myelocyte" already used. This large pre-

* See Quain's Anatomy, Tenth edit., 1893, Vol. I., Part II., p. 218.

megaloblast closely resembles in many respects the large basophil pre-myelocyte referred to, but in the former cell I have not yet been able, with the ordinary staining methods, to demonstrate the presence of the nucleolus so characteristic of the pre-myelocyte and of the lymphoid cells generally; and this, together with certain other minor differences in nuclear structure, prevents me from stating definitely that in the adult an ancestral cell common to the red and to the white series has been found, though I am inclined to believe that it may possibly exist, or that it may be found in some pathological reversions to a more primitive type of blood formation such as the leukaemias, etc. What I do hold is that the gap separating the most primitive cells to which I have succeeded in tracing the two developmental chains, is a very small one, and the link uniting them may at any time be discovered. The "Reizungsformen" of Türk already alluded to when discussing the earliest forms of myelocyte, appear to me to be the cells which at present most closely unite the features of the two great cell groups, red and white, and it seems probable that they, or some variety of cell closely allied to them, may come to be looked upon as the common ancestor of both in the adult bone-marrow.

II. GIANT CELLS.

1. Polykaryocytes.
 2. Megakaryocytes.
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Hudson

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II. GIANT CELLS.

) *Pica ant. caps*

1. Polykaryocytes.

2. Megakaryocytes.

*Pica ant. l.c.
centre*

One of the most remarkable and characteristic phenomena in the cytology of the bone-marrow is the presence of cells which sometimes attain to a very large size, and which shew considerable variations in the shape and structure both of their cell-body and of the nucleus. Many authors distinguish two types of these cells, the polykaryocyte or multi-nucleated, and the megakaryocyte, or large mono-nucleated variety, a view first held by Bizzozero,⁽⁷⁾ and supported by many other writers, e.g. Howell,⁽⁶⁵⁾ Foà,⁽⁴⁹⁾ and more recently by Jackson⁽⁶⁷⁾, but denied by such authorities as Ranvier,⁽¹⁰⁵⁾ Duval,⁽³⁹⁾ and Renault,⁽¹⁰⁷⁾ (quoted by Jackson⁽⁶⁷⁾ in his paper.)

The latter observers regard the megakaryocytes as being merely younger forms of the larger polykaryocytes, or "multinucleated" variety. ^{*}(the view which from my own observations appears to be the correct one.) These cells very readily undergo degenerative changes and also become rapidly altered after death, and it will be convenient to describe their structure from freshly fixed sections made from the rabbit's bone-marrow, in which, as also in the kitten's, very

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* Addition - p 136.

It is best to restrict the term "polykaryocyte" to the multinucleated phagocytic cells or "osteoclasts" found especially during the development of growing bone, and in other conditions in which the bone requires to be modelled and partially reabsorbed by means of these cells. Abnormal varieties of polykaryocyte are also to be found in some tumours of bone, more especially in the case of the well-known "myeloplaxes" of myeloid sarcomas. The term "megakaryocyte" on the other hand should only be applied to the peculiar giant cells possessing the characteristic and highly complex but single "basket-nuclei" which now fall to be described somewhat more fully than the polykaryocytes, ^{the latter being} ~~which are~~ ^{which} cells ~~belong~~ to a type by no means restricted to the Bone-Marrow.

The megakaryocytes very readily undergo degenerative.....

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good examples may be found in large numbers; and then, later, I shall indicate the chief points wherein these cells differ from those found in the human subject.

In Plate V. may be seen a set of drawings made from serial sections of the same giant cell, and the appearances here shewn illustrate one, at all events, of the sources of error to be found in the descriptions of some authors, viz. the extreme variations which may be presented by different sections of the same cell and its nucleus. Serial section 4 in this plate shews a full-grown giant cell cut across as nearly as possible in its equatorial or largest diameter, and hence demonstrates the appearance of the nuclear section at this point -- a complete ring-shaped body in many such cells, but occasionally shewing a break in its continuity, as is shewn in Plate VI., fig. 1b., cell 2, from the human subject. This so-called "ring-nucleus" is composed of a very varying number of more or less rounded or convoluted lobes, each of which in section may in itself resemble a more or less complete nucleus composed of a definite perinuclear membrane and very evident nuclear network shewing blue-staining nodes of chromatin, which are frequently aggregated towards the peri-

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phery of the nuclear section; and the nuclear network usually contains one or more very large, bright, red-staining nucleoli. As can be very easily seen from a study of serial sections, and often even from the same section, these are not small, independent nuclei, but are in reality the component parts of one large and very complex structure, hollow and irregularly globular in formation, and containing in its interior part of the protoplasm of the cell - the "endoplasm", as it has been called by Heidenhain. This communicates with another area of protoplasm similar in structure and appearance, which forms a somewhat lightly stained zone immediately around the nucleus, these two parts of the cytoplasm being continuous with one another through an irregular opening in the "basket-work" of the nucleus, a fact which accounts for the presence of a break in the continuity of the usual ring-like sections already referred to above (fig. 1b., cell 2, plate VI.) The fact that the whole nuclear structure resembles a slightly incomplete hollow globe or sphere will now enable us to interpret the apparently innumerable varieties of nucleus seen in the giant cell, as will be readily understood by a reference to plate V., in which section 4 is, as already stated, equatorial, the other sections being made parallel to this, and at increasing distances from it on either side - becoming

first smaller circles of the sphere (Section 5); then passing tangentially through the wall of the nucleus, and hence appearing as a solid mass of nuclear lobes (sections 1, 2, 3 and 6); and finally coming to pass through the cytoplasm entirely external to the nucleus (section 7). It is thus evident that, unless our observations are carefully controlled by the making of serial sections, the extremely varied appearances shewn by different sections of one cell, may lead to serious misapprehension in the interpretation of any one single section. Thus section 1 in the figure might very well be mistaken for a young or small mono-nucleated form; and sections 6, 2, 3 and 4 might be looked upon as successive stages in the developmental career of such a cell; and section 7 as the degenerated remains of another giant cell from which the nucleus has disappeared.

The protoplasm of the fully formed giant cell may be differentiated, as can be well seen in section 4 of this plate, into three distinct, more or less concentric zones, the outermost being a somewhat pale-staining, irregular, delicately reticular structure, which evidently plays some very important part in the phagocytic activities of the cell, as may be seen from the numerous cell-inclusions contained

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within it. In fig.3 are shewn a nucleated red cell, two intermediate myelocytes, and a degenerating polymorph, which have been englobed; whilst in section 4 there are in this outer zone a large intermediate myelocyte, and the faint degenerated remains of two other cell-inclusions. In sections 5 and 6 are two polymorphs which are undergoing intracellular digestion, a process which may apparently be completed in this outermost zone of the giant cell; or again, in other cases the included cells may be taken further into its interior, as is shewn in the case of the two ingested cells seen towards the lower part of section 4, where an intermediate myelocyte and a polymorph have passed, or more probably have been drawn, into the next protoplasmic zone of the giant cell. This second zone varies considerably in thickness in different cases, and appears to possess a somewhat dense fibrillated structure which stains more deeply than the other parts of the protoplasm. Some of these giant cells are very irregular in outline and often shew branching processes of considerable length, by means of which they appear to anastomose with similar processes given off by other giant cells in the neighbourhood, and also apparently with those of the smaller reticular cells of the marrow

from which they appear to derive their origin, a condition also described by Wegener⁽¹³³⁾; and by Jackson⁽⁶⁷⁾ in his paper upon the reticular framework of the marrow. In the interior of these processes it is often possible to trace prolongations of this fibrillated middle protoplasmic zone which is at present under consideration, and the appearance of this layer in section 4 seems almost to suggest that these fibrils may be endowed with some contractile powers, or at all events with a capability of movement among the surrounding reticular protoplasm, as can be seen in this section from ^{their} position around the two englobed cells which appear to be in process of being drawn in by the outer of the two bands into which the fibrils have become divided at this point. The innermost protoplasmic zone or endoplasm of the giant cell has already been partially described. It immediately surrounds and is found between the individual lobes of the nucleus, and it also lies in the interior of this basket-like structure, the extra- and intranuclear portions being usually continuous, as already mentioned, through an opening in the nucleus which is seen cut across (Plate VI., fig. 1b, cell 2.) This endoplasm usually stains somewhat lightly with the aniline dyes, but is distinctly darker than the

outermost zone of the giant cell. In appearance it is definitely reticular, and, in the part lying within the embrace of the nucleus, very numerous centrosomes have been described by Heidenhain,⁽⁶⁴⁾ who states that these bodies take an important part in the nuclear division, a process which he believes to be an indirect one by mitosis. Arnold⁽¹⁾ describes what he terms division by "indirect fragmentation", in which the chromatin becomes dissolved in the nucleoplasm which then stains diffusely and intensely; after which, the chromatin becomes aggregated into masses at certain points where it forms itself into young nuclei, and the cell protoplasm divides. From my own observations I should think it probable that these phenomena so described are in reality the pyknotic changes which are very common in the nuclei of degenerating giant cells, while the "young nuclei" are in all likelihood englobed cells such as are figured in Plate V. The now well recognised phenomena which comprise the process of phagocytosis have also been mistaken by numerous observers for "endogenous cell formation", and such an origin within the giant cells of the marrow has been alleged, not only for leucocytes, but also for the red blood corpuscles, for example, by

Pugliese⁽¹⁰⁴⁾, who also found the giant cells increased in the marrow after splenectomy), Denys⁽²⁶⁾, and others. Heidenhain⁽⁶³⁾ denies that giant cells possess phagocytic activities, and regards the appearances seen microscopically as being due to an invasion of these cells by polymorphonuclear leucocytes, a view which is manifestly erroneous, as the included cells can be seen to be not only polymorphs, but also myelocytes, and nucleated and non-nucleated red cells, all of which can be observed in various stages of intra-cellular digestion. What is described by Muir⁽⁹⁰⁾ as being invasion of the giant cells by polymorphs when the former cells have degenerated and are breaking up, is of course another matter, and there thus appears to be a mutual relationship between the phagocytic activities of the polymorph and of the giant cell, whereby, if one of these becomes abnormal or effete, it is attacked and digested by the other. Giant cells thus seem to be specially concerned with the destruction of polymorphonuclear leucocytes and other cells of the same class, though they may ingest a few red corpuscles; but in the species of phagocytic cell to be described immediately, we shall find that exactly the opposite is the case, viz., that they are chiefly concerned with the destruction of the red corpuscles,

and of the white cells only to a very much less degree.

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The giant cells of human-bone marrow do not usually attain to such large proportions as do those found in that of the rabbit, and I have also had much greater difficulty in differentiating their cytoplasm into the three protoplasmic zones already described. This however is very probably due to the fact that most of my specimens of the marrow in the human subject have been obtained from post-mortem cases, some of them, however, only an hour or two after death; and the homogenous appearance of the protoplasm, shewn for example, in cells 1 and 2 of fig. 1b, plate VI. may possibly be thus accounted for. The nucleus of the human cell does not usually become such a large and complicated structure as in the case of the rabbit, but in other respects the general characteristics of these cells and their nuclei are very closely analogous both in morphology and mode of growth.

Mitotic division appears to be the method by which the giant cell nucleus proliferates and increases in size, but before discussing this process, it will be well first to determine the origin and earlier life history of these cells, and in

doing so it is very necessary to bear in mind the indefinite variety of appearances which may be exhibited by different sections of the same cell, and hence the necessity of studying and comparing these by means of serial sections.

The histogenesis of the giant cells is a question which has given rise to much discussion, and many theories have been brought forward to explain their origin. Bredichin⁽¹⁸⁾, for example, believes that they arise from osteoblasts, a view also held by Kölliker⁽⁷⁷⁾ and Howell⁽⁶⁵⁾; while Ranvier⁽¹⁰⁵⁾ Renault⁽¹⁰⁷⁾ and others, regard them as springing from myelocytes; and Wegener⁽¹³³⁾ looks upon them as being derived from the walls of capillaries. Jackson⁽⁶⁷⁾ concludes that pigment cells and polykaryocytes arise from cells of the marrow reticulum, and that, after their functions are performed, they may become retransformed into reticular cells; and he also believes that megakaryocytes are enlarged myelocytes, and have nothing to do with the framework of the marrow.

After a prolonged study of the younger forms of the giant and pigment cells of the marrow, I have come to the conclusion that the polykaryocytes, ^{e.g. osteoclast,} are derived from the reticular cells as stated by

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* Addition p. 145-

With regard to the determination of the origin of the "basket-cells" or megacaryocytes, much greater difficulties exist. In the earlier stages of my investigations I thought that there was, in some of my preparations, definite evidence that these cells were developed from cells of the reticulum; but lately, especially from consideration of the careful work of Goodall on this subject, I have come to doubt the accuracy of my previous observations; and I prefer to regard the question as still sub judice, i.e. as to whether the megakaryocytes are derived from cells of the myelocyte-leucocyte series; or from cells of the connective tissue type, e.g. cells of the reticulum - the confirmation of either of these contentions requiring farther evidence.

Jackson; but that the megakaryocytes are in reality only younger forms of the same cell; and that the pigmented and other varieties of phagocytic cell to be described later are in all probability formed from endothelial cells, and also perhaps from the cells of the reticulum, and possibly from the large mononucleated cells of the blood after emigration of these from the vessels.

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Addition here

I had at first great difficulty in determining whether ^{these} the giant cells do not also arise from endothelial cells, for example ^{from those} of the capillary walls, as suggested by Wegener (133), and fig. 2 in Plate VI. illustrates a condition which might readily give rise to this supposition. Parts of one giant cell (cell 11) are seen cut across on both sides of the longitudinal section of a capillary filled with blood cells, and on tracing out the relationship of the parts as shewn by the neighbouring serial sections, it is found that the giant cell is wrapped almost, if not entirely, round the capillary, of whose walls it almost seems to be a component part. On careful examination, however, the very delicate endothelial cell plate may be seen in section, forming a thin band-like structure running along the inner side of the giant cell, which it thus separates from the lumen. This at first appeared to be definite proof

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that the cell was not of endothelial, but of connective tissue ^{or other} origin, as the endothelial cells of the capillaries usually lie with their nuclei bulging somewhat inwards into the lumen of the vessel, and shew the delicate band-like structure passing along the margin of the protoplasm on the side of the cell body which is furthest from the blood stream, (e.g. cells 2 and 3 in the figure); but on tracing out the section of the same capillary, I found several endothelial cells with their nuclei and protoplasm outside the endothelial plate (Cell 4), a condition which appears to occur more readily when those cells are proliferating. I am not therefore in a position to entirely deny the possibility of Wegener's view being at all events partially correct, as the majority of authors appear to do, but in the greater number of the specimens in which I have been able to trace out the development of these giant cells, I have found that ^{, although I still prefer to leave the question an open one, they at all events} it appears to arise by proliferation and enlargement of certain cells of the adenoid reticulum, some of the characters of which I have mentioned above when discussing the lymphocyte-like cells of the marrow. One of these reticular cells may divide and form two or more slightly larger mononucleated

cells such as are shewn in Plate VI, figs.1., 4 & 5, and small groups of such cells may be found scattered irregularly in sections where these cells are multiplying. Of these little groups, one or more members may then proceed to enlarge to a greater extent than the others; the nucleus takes on a new and incomplete method of division by mitosis, and young giant cells, such as are shewn in figs.4 and 5, (fig.4, cells 3', 3", and 4"; fig.5, cells 2 and 3) are formed.

As may be well seen in fig.5, plate VI., the younger cells possess protoplasm with a very definite basophil staining reaction, but as they enlarge and become more and more mature giant cells, the cytoplasm develops a progressively greater affinity for the acid dye, as may be seen in cells 4 and 5 of this fig., and also in Cells 1 and 2 in fig.1 of the same plate. The cells making up the analogous developmental series shewn in fig.4 were, unfortunately, taken from two different cases, in one of which (cells 1", 2", 3" and 4") there was present a considerable amount of protoplasmic degeneration, causing the eosin staining to become more marked than it usually is in the earlier cells of the series, the usual blue tint of the staining reaction being depicted in cells

1', 2' and 3' of this figure. In some of the younger forms, it is often very easy to distinguish the centrosome lying embedded in the protoplasm immediately external to the nucleus, as is shown in Cell 1' of this set, and these bodies are found to take a very important part in the nuclear division.

This process of incomplete division by mitosis is an exceedingly complicated one, and I have not yet succeeded in tracing out and following all its successive stages - some of which are shown in fig.6 of the same plate. These changes must be carefully distinguished from degenerative conditions such as are illustrated below in fig.7, in which various phases of pyknosis and karyorrhexis are seen occurring, previous to the disintegration and disappearance of the cell.

The cells in question (fig.6) which appear to be undergoing complicated mitotic changes, are taken from the femoral marrow of an apparently healthy rabbit, in which giant cells were present in great abundance. I shall not refer to these changes in detail, as I am not yet fully satisfied as to the precise order in which the phenomena occur, and shall simply indicate what appears to be the main outline of the process. The first stage of this complicated

division seems to be an aggregation of the basket-like nucleus of the giant cell already described, into a more compact, solid mass, such as is shown in cell 1 of the figure. This process of condensation may continue until the whole nucleus becomes much reduced in total bulk, and during which process ^{the} staining reaction of the chromatin becomes more intense. (Note: It is possible that the stage figured in Cell 2 may be a somewhat later one in the process, and may represent the phase which follows division and rearrangement of the chromosomes.) The nucleus now proceeds to arrange itself in one long continuous convoluted thread-like structure, which gradually becomes thinner and more condensed, and which shows the chromatin becoming aggregated into little rounded masses dotted at regular intervals along its entire length, the red nucleolar staining having now disappeared (spireme or prophase). This thread or spireme, when the condensation of the chromatin is complete, then breaks up (metaphase) into short segments resembling chromosomes, which become re-arranged into a dense rounded globe in the centre of the cell (anaphase), after which it is probable that the resulting basket nucleus is rebuilt upon a more complex plan, by a process resembling that by which

the original nucleus underwent a transformation into chromosomes, i.e. by stages similar to those just described, but occurring in the reverse order. As already stated, this complex process is a very difficult one to follow and interpret, and the above is submitted merely as a preliminary sketch which may have to be somewhat modified after farther study of its phases in different animals. This intricate process of nuclear rearrangement rather than division, is not accompanied or followed by any corresponding division of the cell-body or telophase, the protoplasm simply increasing in bulk as the nucleus becomes larger and more complicated.

Smith
The giant cells of the marrow are, as already indicated, exceedingly liable to degenerative changes under the action of various toxic substances, and in acute disease especially they are seen to be structures of a very delicate nature. These degenerative changes may be roughly divided into two groups, those shewing pyknosis of the nucleus preliminary to karyorrhexis; and those producing swelling up and vesiculation of the nucleus, followed by disappearance of the nuclear network, and karyolysis or solution of the chromatin. The former of these

methods of degeneration is the one more commonly observed in the acute toxic diseases, and also in many other conditions, and post-mortem changes in these cells may also frequently account for the pyknotic and fragmented state of the giant cell nucleus. In fig.7, plate VI., may be seen a series of cells illustrating this type of nuclear degeneration, taken in this particular instance from a case of acute rheumatism, though practically any preparation from the marrow of cases say, of acute pneumonia or septicaemia will be seen to exhibit the same phenomena. The nuclear network disappears, and the whole nucleus may become aggregated into one dense irregular mass of deeply stained chromatin; or again, it may break up into a very varying number of unequal and very irregularly shaped smaller masses, which may then undergo further fragmentation, before finally becoming dissolved in the protoplasm, or being taken up by polymorphonuclear leucocytes which invade the degenerating giant cells as described by Muir. (90)

The other method of nuclear degeneration which may be found in giant cells is that characterised by the swelling up and disappearance of the nucleus and nuclear network, and by the solution of the

chromatin; in which case the nucleus, instead of shewing the pyknotic changes described above, becomes progressively paler and more broken up, a condition illustrated in fig.8, plate VI., the specimen being from a case of chronic Bright's Disease. The extreme rapidity with which giant cells may undergo degeneration has been well shewn by a series of experiments performed in rabbits with diphtheria toxin, and also with the pneumococcus, bacillus coli, etc.; the degenerative changes being in some cases well marked within six hours of inoculation, and being often very complete within twenty-four or forty-eight hours. I am as yet unable to differentiate the factors which determine whether degeneration will be brought about by pyknosis, or by the method characterised by a progressive loss of the chromatin colour reaction without any preliminary condensation. It is possible that these may depend on the amount of the toxic material present, as well as upon its quality, and I have observed in my experiments upon rabbits that pyknosis seems to occur more readily with the pneumococcal, and the second variety with the diphtheritic, poison; though it appears as yet impracticable to lay down any definite law governing these processes.

The protoplasm of the giant cells shews parallel degenerative changes, usually becoming more "granular" in appearance, owing to changes in the protoplasmic reticulum, which exhibits an appearance comparable to that of the cloudy swelling which is seen in the protoplasm of other highly endowed cells, for example, those of the liver or kidney. The differentiation of the cell-body into three distinct protoplasmic zones becomes quickly lost, and the cytoplasm comes to present a uniform ground glass-like appearance, and often stains more intensely with the acid dye, e.g. eosin. It may then gradually become broken up and disappear, or it may become invaded and removed by other phagocytic cells, chiefly polymorphonuclear leucocytes, as described by Muir.⁽⁹⁰⁾ Occasionally the protoplasm, instead of presenting this increased affinity for the acid dye, becomes progressively fainter in its staining reactions, shewing multiple vacuolation of its substance and a ragged, irregular condition of its edges, and gradually disappearing completely, leaving only the isolated and degenerated nucleus or its fragments to mark the position which it previously occupied.

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The precise nature of the functions performed by giant cells is a matter of considerable obscurity. The theory that they are concerned with the production, by "endogenous formation" of leucocytes and red blood corpuscles, is of course due to an ^{incomplete} ~~ignorance~~ ^{knowledge} of the ordinary appearances found in phagocytosis. Some observers allege that "occasionally a nucleus of the giant cell surrounded by a certain amount of protoplasm, separates from the giant cell to form a myelocyte." (Roger and Josué).⁽¹¹⁵⁾ Weil,⁽¹³⁴⁾ in his thesis upon the blood and haemopoietic tissues in smallpox, says that "parts of the giant cells sometimes become detached by gemmation, which probably form mononucleated cells with indifferent protoplasm." These phenomena may probably also be explained as being some of the earlier phases of phagocytosis, or possibly as being due to the appearances presented by neighbouring cells of the reticulum, which, as has already been described above, are frequently attached by their branching protoplasmic processes, to similar branching offshoots from the giant cell, with which they anastomose. Kölliker⁽⁷⁶⁾ holds that, after the development of bone is complete, giant cells may either disappear, or may, if required, become transformed

into osteoblasts. Wegener⁽¹³³⁾ thinks that they develop into fibrous connective tissue, or perhaps into bone-marrow cells; whilst Jackson⁽⁶⁷⁾, holding that the polykaryocytes are developed from reticular cells, is of opinion that they are concerned with the absorption of bone, and that, after their functions are performed, they may change back into cells of the reticulum.

The most obvious, though probably not the most important, function of the giant cell is that of phagocytosis, the phenomena of which have already been described and illustrated (p.139 and plate V.); but it may here be repeated that the activities of this cell usually seem to be directed against the neutrophil polymorphs and their relatives, in the destruction of which they appear to take an important part. This function becomes greatly exaggerated in certain pathological conditions, in which the numbers and sometimes ~~also~~ the individual size of the giant cells are increased to a varying extent. That giant cells are ~~also~~ concerned in the absorption of bone is also extremely likely, as is seen not only in the well-recognised osteoclasts of growing bone and in certain diseases, but ^{also} they appear to assist in the enlargement of the medullary cavity when the marrow requires greater

room for proliferation in many of the acute and chronic conditions in which leuco- and erythroblastic transformation of the marrow occurs. Thus I find them on the whole somewhat increased in number and size in the pneumonias and septicaemias, (e.g. cases 7, 12, 26, 38, 42, 54, 56), though less frequently they may remain unaltered or become considerably diminished (e.g. in cases 4, 24, 25, 30, 67). The diseases in which I have found greatest increase are exophthalmic goitre (e.g. case 43); myxoedema with septic termination (case 46); a case of acute, supervening upon older-standing, rheumatism (case 49); and also in most tubercular and in some malignant cases.

Here, a few words may be said with regard to the occurrence of tubercular giant cells in the bone-marrow, and in Plate VI., fig. 3, will be seen a very good example from a case of acute general tuberculosis in a boy of 14 (case 41). This example is taken from a very small tubercular nodule of which the giant cell composes the greater part; and as can be well observed in the illustration, this cell is of very large size and contains an enormous number of nuclei, each of which shews a very definite structure and possesses a large, deeply

red-staining nucleolus. These nuclei appear to belong to cells of the marrow reticulum which has proliferated at the point of invasion of the tubercle bacilli, and perhaps also to large mononucleated leucocytes which have emigrated from the vessels, and which by their aggregation and fusion with one another appear to form the large tubercular giant cell, which in the very earliest nodules may be noticed to be composed of only a few of these smaller cells, some of which may still shew their cell outline with comparative distinctness. The whole cell in this specimen is surrounded by a zone of red staining fibrin, among which are numerous proliferating reticular cells, some of which are becoming fused with the large central giant cell. The tubercular giant cell is therefore quite distinct from the normal polykaryocyte, both with regard to its structural peculiarities and its method of formation, as can be verified by comparing, in plate VI., the cells represented in figs. 3 and 1b.

III. CELLS OF CONNECTIVE TISSUE TYPE.

1. Fat Cells.
2. Reticular Cells.
3. Various Forms of Phagocytic Cells, Pigment Cells etc., other than the forms of Giant Cell already considered).
4. Ordinary Connective Tissue Cells.

III. CELLS OF CONNECTIVE TISSUE TYPE.

1. Fat Cells:

The amount of fatty tissue in the bone-marrow is extremely variable, being found to alter with the age, health, and state of nourishment of the individual; and also in the different bones examined; facts which ^{were} ~~will be~~ more fully dealt with when considering the alterations of the marrow in disease; the quantity of the fat present varying inversely with the activity of the tissue in the production of blood cells. The fat cells themselves present the usual characteristics, and in microscopic sections prepared by the paraffin process, they shew the characteristics of fatty tissue elsewhere - a large, clear, vesicular part from which the fat has been removed by the various solvents employed, and - if cut across in the section - the usual signet-ring nucleus and scanty protoplasm. In a state of health, the size of the fat cells varies within the usual limits, but in conditions where the fat is in process of absorption their average diameter may become much diminished, until finally they may regain their primitive appearance of connective tissue cells with rounded

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Pica Antib. caps

} centre - Pica l.c

nuclei, and come to be indistinguishable from cells of the reticulum from which they are in all probability developed, as is described by Neumann⁽⁹⁷⁾, Bizzozero⁽⁷⁾, Fleming⁽⁴⁷⁾, Denys⁽²⁶⁾, Renaut⁽¹⁰⁷⁾ and Jackson⁽⁶⁷⁾)

In some cases of fatty degeneration of the marrow, individual fat cells may be seen to be much larger in size than normal, as well as being found in greater numbers, for example, in some of the cases suffering from acute disease with leucopenia, and in some malignant cases. The fat cells also become progressively diminished in size in gelatinous degeneration, which is usually described as being a chronic form of degenerative change, though I have found it in cases of comparatively short duration, and I describe elsewhere (p. 32) a condition which I regard as an acute variety of this change in the connective tissue and fat cells of the marrow.

(Case 59, see photos Nos. 44, 45, 46.) .

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2. Reticular Cells.

2. Reticular Cells:) *centre - Pica Amb l.c.*

These are branching connective tissue cells, the characters and development of which have been very fully studied and described by Jackson, (67) and which, according to him, are derived directly from the branching cells of the embryonic marrow. In the interior of these cells are developed fibrils such as are found in the reticular cells of other lymphoid organs, whilst connective tissue fibres are also laid down outside these cells, and form an interlacing network amongst which the branching and anastomosing reticular cells lie embedded - in other words, the marrow possesses an adenoid reticulum precisely analogous to that of the spleen or of the lymphatic glands.

From the primitive or embryonic marrow cells, therefore, are developed the reticular or connective tissue cells of the adult marrow, and by specialisation of certain of these reticular cells are formed the fat, and probably also the ^{polykaryocytes} giant cells found in the bone-marrow, as well as certain members of the very important cell group which now falls to be described.

The question as to whether the megakaryocytes are derived from the reticular cells is, as already indicated, still to be regarded as not definitely settled.

3. Various forms of phagocytic cells,
(Pigment Cells etc., other than the
forms of Giant Cell already considered).

3. Various Forms of Phagocytic Cells, (Pigment Cells, etc., other than the forms of Giant Cell already considered.)

175
Pica ant. Centre

In almost every marrow preparation from each of my series of eighty cases, there are present varying numbers of phagocytic cells, and in some of these cases phagocytosis is seen to be one of the very important functions of this tissue. Professor Muir⁽⁹¹⁾ states that "phagocytosis in the bone-marrow is relatively a secondary matter, and in this respect a contrast may be drawn with the splenic pulp." (loc.cit., p.380), but with this view my results do not entirely agree, as in many of my cases phagocytic cells are seen to be very numerous and active - a condition fully confirmed by my experiments upon rabbits with diphtheria toxin and ^{with} various organisms, in which phagocytosis was found to be almost as active in the bone-marrow as in the splenic pulp.

The presence of pigmented cells in the marrow of various animals has been noted by Robin^(110a) and Arnold⁽²⁾ (amphibians); by Bizzozero and Torre⁽¹⁶⁾ in various reptiles; by Palladino⁽⁹⁹⁾ in the horse; and by Dobrowolsky⁽²⁷⁾ in connection with the blood-vessels in a five-months human foetus, and in a man of eighty; while Ponfick⁽¹⁰³⁾ describes them as occurring in gelatinous human marrow. (Jackson).

Richardson
Jackson⁽⁶⁷⁾, working with normal tissues, has never found them in mammals, but finds them present in reptiles and amphibians, and sometimes in birds and fishes.

With regard to the nature of these cells in the animals named, in comparison with that of those found in man, I am unable to speak from personal observations, but from the descriptions given by some of the above authors it appears likely that in many of the lower animals, e.g. in amphibians and reptiles especially, they are true pigment cells; whilst I find that in human marrow they are rather to be regarded as phagocytic cells concerned with the removal and destruction of effete or abnormal blood cells, more especially red blood corpuscles, the contained pigment representing the haemoglobin-content of these cells after their intracellular digestion by the phagocytic cell. The pigment may also, but to a much less extent, be formed outside these cells in blood destruction and haemorrhage, being afterwards engulfed by them as preformed pigment, but I believe the former method of pigment production to be the more usual one in the bone-marrow.

(Sup 41)

The phagocytic cells of the human marrow present very many different appearances, some of which are

represented in plate VII, and it will considerably simplify the task of their description, to indicate first the nature of the cells from which they are found to originate. In the earlier stages of my work I had considerable difficulty in determining whether these pigmented and other forms of phagocyte arose from the endothelial cells of the capillaries and venous sinuses of the marrow, or from the branching cells of the adenoid reticulum, the appearances in some sections appearing to warrant the former, and in others the latter hypothesis. Now, however, after a prolonged series of observations both in man and in the rabbit, I have come to the conclusion that both methods of formation are found, and indeed may frequently be seen occurring in the same specimen. In plate VII., fig.1, is represented the section of a blood capillary, ^{the} endothelial cells of which are proliferating. These cells have undergone a process of enlargement somewhat analogous in appearance to that of "cloudy swelling", and they closely resemble the enlarged endothelial cells which may be seen in the walls of capillaries in a tissue undergoing inflammatory changes; or on the surface of serous membranes such as the pleura or peritoneum, when attacked by a similar condition. The endo-

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thelial cells first become swollen up and more rounded in outline, and shew a more "granular" appearance of the cell body, due to changes in the reticulum of the cytoplasm, which render it unusually distinct; they may then undergo proliferation, showing mitotic figures and various stages of cell division and multiplication; and lastly, they may become detached from their usual position, and become amoeboid and actively phagocytic. These phenomena have been noted by other writers as occurring in blood vessels elsewhere, for example, by Mallory, (84) who has observed the actual migration of these proliferated endothelial cells into the surrounding tissues; whilst Beattie, (5) in experimental peritonitis, notes the occurrence of similar cells both within the lumen and also outside the walls of blood-vessels in the omentum, and some of the large branching cells which he figures (loc.cit, plate IV. fig.2) very closely resemble the phagocytic cells which I have observed in some analogous conditions in the marrow.

Fig.1, plate VII. represents this proliferation of endothelial cells of the blood vessels in the marrow from a case of acute streptococcal septicaemia (and possibly diphtheria), occurring in a girl of 16

who had previously been somewhat anaemic, perhaps from chlorosis. In this figure (fig.1, plate VII), can be seen the swelling and proliferation of the lining endothelium (cells 1, 2, 3), and also some of the proliferated cells which have migrated from the capillary, as described by Mallory (cells 4, 5, 6). These cells are actively amoeboid and phagocytic, and may take up enormous numbers of red blood corpuscles and other cells. They may sometimes attain to a very large size, and frequently shew two or more nuclei in the same cell (fig.8), occasionally forming large multinucleated syncytial masses (fig.4), which may englobe and destroy various blood cells in very large numbers - as in Case 52, a patient who died of puerperal septicaemia from which this specimen is taken - every field containing large numbers of these cells in every stage of phagocytic activity, and showing intracellular digestion in all its phases occurring within the substance of their protoplasm.

In addition to the endothelial origin of these cells described above, and of which I can find no record in the literature of the subject, it is also extremely probable that similar phagocytic cells may arise from the adenoid reticulum of the bone-marrow, the branching cells of which have been described on page 161. In Plate VII., fig.2, are depicted

a series of such cells from a patient with severe secondary anaemia following or accompanying aneurism of the aorta, due in all probability to syphilis. Here the pigmented cells appear to arise directly from the reticular cells, some of which may be seen to be spindle-shaped in section, whilst others, more especially the larger cells, are irregularly rounded in outline, and frequently shew long branching processes. The protoplasm of these cells is usually very pale and indeed often almost entirely invisible, the whole cell shewing simply as a rounded or irregular mass of englobed blood cells or pigment granules, around which the outline and structure of the phagocyte itself, may often be seen only with difficulty (e.g. fig.6, cell 11).

As these phagocytic cells enlarge in size, I find increasing difficulty in differentiating those of endothelial from those of reticular origin; and, indeed, from a comparatively early stage in their growth, it is frequently impossible to do so, their protoplasmic and nuclear characteristics and their functional activities being apparently identical. One description will therefore serve for both varieties of cell. The protoplasm, as I have already noted, is usually extremely pale, but occasionally it stains a very slightly deeper tint with eosin.

It is finely reticular in structure, frequently containing numerous vacuoles, probably digestive in nature; and is often prolonged into long branching processes or pseudopodia, similar to those found in some phagocytic cells in other tissues, e.g.

Ranvier's ⁽¹⁰⁶⁾ Clasmatocytes found in the omentum, and the cells described by Beattie ⁽⁵⁾ in peritonitic exudates, etc. Enormous numbers of cell-inclusions, sometimes amounting even to hundreds, may be found within the protoplasm of these cells, and it is probably from the red cells thus englobed that the pigment so often found in them is derived (see figs. 2, and 5 to 10, plate VII). This pigment is usually of a light canary yellow tint, though sometimes rather more orange in colour, and it stains pink or red with watery, but remains unstained with alcoholic eosin. It may be found in the protoplasm in fine granules, but is more frequently aggregated into larger irregular masses, and occasionally one of these may even almost fill the entire cell. (figs. 9, 10, plate VII.)

M. P. Henson
The nuclei of these phagocytic cells are exceedingly characteristic, and their general appearance is represented in plate VII, figs. 3, 4, 6 and 8.

They can best be demonstrated in film preparations;

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as in sections, owing to the considerable size of these cells, the nuclei are frequently not encountered in the part of the cell cut across, and hence no nuclei may be visible, or cell-inclusions may be taken for the true nucleus. This structure is usually very symmetrically oval in outline, though it may occasionally be slightly distorted by the pressure of cell-inclusions or pigment (fig.5). Its most characteristic feature, however, is the extreme paleness with which it is stained by the basic dyes; and apparently the more actively phagocytic the cell becomes, the more marked is this pallor in staining reaction and poverty in chromatin observable in the nucleus. Another very constant characteristic phenomenon in the nuclei of these cells is the presence of one, or more usually two, very definite, red-stained nucleoli, often situated towards both poles of the nuclear oval, and sometimes giving a remarkably symmetrical appearance to that structure, which also possesses a very open and faintly staining nuclear network which often shews a fine stippled or dotted appearance along the lines of its meshes.

These cells are almost invisible in dried film preparations, and can scarcely be distinguished in specimens fixed by heat and stained by Ehrlich's

methods; and in sections, for example those from Case 52, although present in such enormous numbers, as is shewn by the wet-fixed films, they may almost escape notice; and these facts probably explain why attention has not previously been drawn to the presence of these cells by other writers, who either pass by the phenomena of blood destruction in the marrow as insignificant; disregard it entirely; or only mention it as occurring to any marked degree in pernicious anaemia.

In certain cases these cells may develop phagocytic activities specially directed against certain other members of the marrow or blood cell series, as is well shewn in fig.3, plate VII, from a case of tubercular peritonitis of 11 days standing, in which the eosinophil cells of the marrow are remarkably numerous, and are being taken up and digested by these phagocytic cells in considerable quantities.

Phagocytic cells of this description are developed in large numbers in the marrow whenever there is any great amount of blood destruction occurring in the organism. Pigment cells, and cells containing erythroblasts and red corpuscles, are seen in very great numbers, for example, in pernicious anaemia, (fig.5? and 66, plate VII.,) and also in other old-

standing anaemias (fig.2); but they are frequently present in equal, and even in greater numbers in some of the acute diseases, e.g. in many of the septicaemias (figs.4 and 9), and pneumonias, especially in certain of my cases of the latter disease characterised by the presence of leucopenia (figs.7 and 8). In acute disease, they may be found present even at very early stages, for example in Case 62, a septicaemic condition, probably pneumococcal in origin, where death supervened in $21\frac{1}{4}$ hours; and I have found that a similar phenomenon also occurs under experimental conditions, e.g. in the marrows of a large series of rabbits after subcutaneous inoculation with half minimal lethal doses of diphtheria toxin; a fuller account of which I hope soon to publish. In these bone-marrows, pigmented phagocytic cells were found present in considerable numbers within the first twenty-four hours (experiment 26), and in one case (experiment 25), they were distinctly visible as early as six hours after inoculation; and indeed at this early stage, as also in many of the later experiments, they were quite as well marked in the marrow as in the spleen. In this set of experiments in rabbits, the number and size of these cells containing blood pigment gradually

increased until, at seventy-two hours after inoculation with a similar dose of toxin (Experiment 27), they were found crowded in all parts of the marrow in enormous numbers, a similar condition being also found in the splenic pulp, where the evidence of blood destruction, though great in degree, was certainly not more marked than in the marrow itself.

I therefore regard the marrow as one of the principal sites of haemolytic action, scarcely, if at all, of less importance in the carrying out of this function than are the liver and spleen, a fact which is all the more striking when the great extent and wide distribution of the red bone-marrow throughout the body are borne in mind.

4. Ordinary Connective Tissue Cells.

4. Ordinary Connective Tissue Cells:

) Pica ank l.c.

The cells of the adenoid reticulum have already been fully considered, and the ordinary connective tissue cells, such as are found in the outer coats of the blood vessels, etc., call for no special description here, as they present the usual appearances seen in such cells elsewhere. The development and histology of the connective tissue framework of the marrow, both in relation to the contained cells and fibrils, have been recently worked out and very fully described by Professor Jackson⁽⁶⁷⁾ of Missouri in a very able paper in the Anatomical section of the Archiv für Anatomie und Physiologie for 1904, p.33, and it is therefore needless for me to describe these here, as my work throws no new light on the subject.

The connective tissue elements of the marrow may in certain cases undergo proliferative changes, a condition found in the fibroid marrow of old and feeble individuals, and which is usually senile in origin, though it may supervene abnormally early in certain cases of disease. Fibrous tissue overgrowth in the marrow, as in most other tissues, is also found as a characteristic phenomenon in syphilis, the sclerotic condition of this tissue no doubt accounting in large part for the anaemia usually attendant upon this disease.

IV. ENDOTHELIAL CELLS LINING THE WALLS

OF CAPILLARIES, BLOOD SINUSES, etc.

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174IV. ENDOTHELIAL CELLS LINING THE WALLS OF CAPILLARIESBLOOD SINUSES, etc./ Pica ant
cap

1. Found in their normal positions in the vessel walls.

Pica

2. Found proliferating and taking on phagocytic activities, etc.,

ant. l. c.

centre

These cells are merely mentioned again in order to complete the cytological account of the bone-marrow. They have already been fully described in connection with the important part they appear to play in giving rise to ^{one of} the great groups of phagocytic cells in the marrow, and the very important rôle which they play, together with cells of a similar nature, in the spleen pulp and elsewhere, in the haemolytic processes of the organism.

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A P P E N D I X I .

List of Cases.

To printer
in this column
put male or female -
(not the name)

Print the
column

No.	Date	Name	Age	Ward	Disease	N.E. Specif.	Organism	Duration of illness.
1.	1902. Dec. 6	Gibbon, Mrs Mary	49	24	Pneumonia	-	Pneumococcus	
2.	" 22	Campbell, James	27	26	Pneumonia	-	"	6 days.
3.	1903. Jan. 11	Young, David	67	31	Pneumonia	-	"	6 days.
4.	Mar. 14	Paul, Montgomery	45	34	Pneumonia	-	"	1 week.
5.	" 19	Ross, James	48	28	Pneumonia	-	"	6 days.
6.	Apr. 2	Hutchison, Robert	38	23	Pneumonia	-	"	7 days.
7.	May 23	Thomson, William	45	23	Peri- & endocarditis, etc.	-	-	9 days.
8.	" 12	Fleming, William	49	34	Pernicious anaemia	-	-	2 years.
9.	"			34	Pernicious anaemia	-	-	
10.	" 27	Steele, Mrs	24	24	Pernicious anaemia	-	-	5 months.
11.	" 29	Simpson, James	40	23	Pneumonia	-	Pneumococcus	
12.	June 6	White, Mrs Mary	40	24	Pneumococcal pericarditis and pleurisy	-	"	5 days.
13.	" 11	Innes, Robt.	15	28	Pneumonia	-	"	10 days
14.	" 27	Hume, Robert	18	28	Exophthalmic goitre.	-	-	9 months.
14a	" ?	Sangster,	16	1	Acute Rheumatism & pericarditis	-	Diplococcus Rheumaticus	7 days.
15.	July 2	Mitchell	36	31	Pneumonia	-	Pneumococcus	6 days.
16.	" 2	Steven, Robert	53	32	Septic pneumonia (leucopenia)	-	?	1 week.

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put male & female -
(not the name)

Print the
column

No.	Date	Name	Age	Ward	Disease	N.E. Spec.	Organism	Duration of illness.
1.	1902. Dec. 6	<i>Female</i> Gibbon, Mrs Mary	49	24	Pneumonia	-	Pneumococcus	
2.	" 22	<i>Male</i> Campbell, James	27	26	Pneumonia	-	"	6 days.
3.	1903. Jan. 11	<i>Male</i> Young, David	67	31	Pneumonia	-	"	6 days.
4.	Mar. 14	<i>Male</i> Paul, Montgomery	43	34	Pneumonia	-	"	1 week.
5.	" 19	<i>Male</i> Ross, James	48	28	Pneumonia	-	"	6 days.
6.	Apr. 2	<i>Male</i> Hutchison, Robert	38	23	Pneumonia	-	"	7 days.
7.	May 23	<i>Male</i> Thomson, William	45	23	Peri- & endocarditis, etc.	-	-	9 days.
8.	" 12	<i>Male</i> Fleming, William	49	34	Pernicious anaemia	-	-	2 years.
9.	"			34	Pernicious anaemia	-	-	
10.	" 27	<i>Female</i> Steele, Mrs	24	24	Pernicious anaemia	-	-	5 months.
11.	" 29	<i>Male</i> Simpson, James	40	23	Pneumonia	-	Pneumococcus	
12.	June 6	<i>Female</i> White, Mrs Mary	40	24	Pneumococcal pericarditis and pleurisy	-	"	5 days.
13.	" 11	<i>Male</i> Innes, Robt.	15	28	Pneumonia	-	"	10 days
14.	" 27	<i>Male</i> Hume, Robert	18	28	Exophthalmic goitre.	-	-	9 months.
14a	" ?	<i>Female</i> Sangster,	16	1	Acute Rheumatism & pericarditis	-	Diplococcus Rheumaticus	7 days.
15.	July 2	<i>Male</i> Mitchell	36	31	Pneumonia	-	Pneumococcus	6 days.
16.	" 2	<i>Male</i> Steven, Robert	53	32	Septic pneumonia (leucopenia)	-	?	1 week.

No.	Date	Sex	Name	Age	ward	Disease	N.E. Spec.	Organism	Duration of illness.
17.	1903. July 3.	<i>M</i>	Moses, Thos.	37	29	Addison's disease	-	-	5 months.
18.	Oct. 9.	<i>M</i>	Noble, Robt.	25	23	Diabetes	-	-	4 months.
18a	Nov. 12	<i>F</i>	Combe, Mrs.	46	24	Exophthalmic goitre.	-	-	-
19.	" 16	<i>M</i>	Mackay, James	60	23	Septic embolism, following excision of malignant glands.	-	-	-
20.	" 19	<i>Female.</i>		-	29	Lymphatic leukaemia	-	-	-
21.	" 23	<i>F</i>	Stevenson Mary A.	48	{ ²⁴ 6	Aneurism of aorta with profound secondary anaemia, etc.	-	-	? years.
22.	Dec. 8	<i>M</i>	Hodges, Walter	22	26	Cancer of stomach.	*	-	-
23.	" 8	<i>M.</i>	Gray		23	Pneumonia with leucopenia	-	Pneumococcus	
24.	" 9	<i>M</i>	Paterson, David	59	33	Pneumonia	-	"	
25.	" 12	<i>F</i>	Glover, Elizabeth		25B	Endocarditis	-	Streptococcus etc.	12 months.
26.	" 12	<i>M</i>	Paton, James		6	Meningitis	*	Pneumococcus	3 days.
27.	" 14	<i>M</i>			6	Pneumonia (alcoholic)	-	"	
28.	" 21	<i>F</i>	West, Lizzie	16	{ ²⁴ 14	Pneumococcal empyema.	-	"	
29.	" 22	<i>M</i>	Butter, Bernard	19	23	Septicaemia (supposed typhus)	-	-	14 days
30.	" 22		Grassick, Alex.	41	6	Broncho-pneumonia & meningitis.	-	-	
31.	1904. Jan. 7		Mackay, Robt.	47	35	Sarcoma of intestine.	-	-	3 months.
31a	" 11	<i>M</i>	Smail, David	28	31	Pneumonia	*	-	7 days.
32.	" 14	<i>F</i>	Chalmers, Mrs Jane	39	5a.	Pernicious anaemia with leucocytosis.	*	Pneumococcus?	4 years.
32a.	" 14	<i>M</i>	Collins, Wm.	48	26	Pneumonia	*	"	5 days.

No.	Date	Name	Age	Ward	Disease	N. E. Spec.	Organism	Duration of illness.
34.	1904 Jan. 19	Dobson, Harry	14	5	Strangulated hernia (early pneumonia)	*	B. Coli.	56½ hours.
35.	" 20	Fairbairn, Jas.	61	29	Cerebral softening, intestinal carcinoma.		-	12 years.
36.	" 21	Robertson, Annie	17	32	Ulcerative endocarditis	*		2 weeks.
37.	" 22 23rd-03.	McCall	44	31	Pneumonia with leucopenia		Pneumococcus	10 days.
38.	" 26	Adamson, Chris.	24	30	Ruptured gastric ulcer	*		3 days.
39.	" 28	Kerr, Elizabeth	7	24	Tubercular meningitis		B. Tuberculosis	16 days.
40.	Feb. 2	Baillie, John	59	5	Sarcoma		-	
41.	" 5	Fowler, Wm.	14	6	Tubercular meningitis	*	B. Tuberculosis	19 days.
42.	" 6	Finnerty, Jas.	15	34	Pneumococcal meningitis	*	Pneumococcus	12 days.
43.	" 7	Girvan, Thos.	16	28	Exophthalmic goitre	*	-	6 months.
44.	" 8	Anderson, Robt.	9	3	Chronic Pyaemia (staphylococcus aureus.)	*	Staphylococcus aureus)	6 "
45.	" 9	Wood, William	46	6	Septicaemia (streptococcus)		Streptococcus	2 days † ?
46.	" 13	Fleming, Isabel.	38	24W.	Myxoedema	*	-	4 years. ?
47.	" 15	Scott, Isabella	22	27	Septicaemia (nephritis, cardiac etc.)	*	Streptococcus	8 days ?
48.	" 16	Docherty, Mary	7	25	Tubercular peritonitis.		B. Tuberculosis	11 days +
49.	" 19	Mitchell, George.	14	33	Acute rheumatism with pericarditis.	*	Diplococcus rheumaticus	14 days.
50.	" 22	Paton, William	32	29	Pernicious anaemia	*	-	2 years, 7 months.
51.	" 25	Miller, Mrs.	56	24	Lymphocythemia	*	-	2 years.
52.	" 26	Nicol, Isabella	35	33	Puerperal Septicaemia	*	Streptococcus	7 weeks.
53.	" 29	Mackay, Harriet	9	27	Leucocythemia + ?	*	-	3 weeks.

No.	Date	Name	Age	Ward	Disease	N. B. Specif.	Organism	Duration of illness
54.	1904. Mar. 5	Elphinson, Susan	65	24	Pneumonia with leucopenia	*	Pneumococcus	14 months.
55.	" 7	Rews, John <i>m</i>	40	23	Chronic Venous Congestion, (aortic etc.)	*	-	8 days.
56.	" 11	Walker, James <i>m</i>	17	28	Pneumonia	-	Pneumococcus	5 years.
57.	" 14	Townsley, Agnes	23	30	Exophthalmic goitre	-	-	5 months.
58.	" 14	Naysmith, Mrs.	58	33	Scirrhus of breast with wide secondary growths.	*	-	3 weeks
59.	" 14	Wood, Thomas <i>m</i>	19	23	Ulcerative endocarditis	-	-	-
60.	May 12	Calderhead <i>m</i>	59	23	Leukaemia (blood only)	-	-	-
61.	" 30	Collins, Wm. <i>m</i>	13	23	1st Transient case (pneumococcal?)	*	Pneumococcus, etc.	38½ hrs.
62.	June 2	Alexander, Robt <i>m</i>	11	23	2nd " "	*	do.	21¼ hrs.
63.	" 26	Infant <i>m</i>	3m.	Pilton	Diphtheria	*	B. Diphtheriae	-
64.	July 5	Child <i>m</i>	3	do.	Diphtheria	*	B. Diphtheriae	-
65.	" 16	Forbes, Margaret	7	24	Exophthalmic goitre	*	-	-
66.	Aug. 9	Wilson, Wm. <i>m</i>	11	Pilton	Typhoid, 3rd week, no perforation.	*	B. Typhosus	3 weeks.
67.	Oct. 28	Texeira, Muriel	7	priv.	Streptococcal septicaemia	*	B. Coli	-
68.	Dec. 5	Paxton, Annie	7	24	Ulcerative endocarditis with septic emboli.	*	" ?	6 mos.
69.	" 5	Little <i>m</i>		34	Sarcoma of kidney	*	-	8 mos.
Dec. 21. (see 78.)	1905							
70.	Jan. 27	Mitchell, Chas. <i>m</i>	20	23	Cardiac Rheumatism	*	-	-
71.	"	Prof. Muir's Case <i>m</i>	12	Muir	Fracture of Skull	-	-	-
72.	"	" " <i>m</i>	13	"	Accident	-	-	-

No.	Date	Name	Age	Ward	Disease	N.E. Specn	Organism	Duration of illness.
73	1905. Feb. 4	Male, aet.	25	18	Amputation of leg for accident	*	-	2 hrs.
74.	"	Male, aet.	20	Chalmers	Ununited fracture of femur.	*	-	
75.	" 9	Male, aet.	23	7	Sarcoma of thigh	Tibia *	-	From Shepherd.
76.	"	Male, aet.	22	Muir	Accident	*	-	6 hours.
77.	" 10	Thomson, Mrs. 7	47	24	Chronic Bright's Disease, granular contracted kidneys.	*	-	2 mos + +
78.	1904 Dec. 21	Male		23	Chronic Bright's disease	*	-	-
79.	1905 Mar. 7				Ulcerative endocarditis	bad *	-	-

leave room for 30 or 40 more cases at end of this table. I shall forward these in a few days.

A P P E N D I X II.

T A B L E

Shewing the Comparative Numbers of the

E O S I N O P H I L C E L L S

in the Bone-Marrow of thirty Cases of
Pneumococcal and Other Acute Septicaemic Diseases, etc.

T A B L E

Shewing the Comparative Numbers of the
EOSINOPHIL CELLS
 in the Bone Marrow of thirty Cases of
 Pneumococcal and Other Acute Septicaemic Diseases, etc.

Proportion of Eosinophils in marrow	Case No.	Age	Disease	Period in days.	Remarks.
Entirely absent. (Total 2)	67	16	Streptococcal Septicaemia	3 - 4	Both very acute streptococcal cases
	45	46	Streptococcal Septicaemia	2 +	
Very scanty (Total 7)	5	48	Pneumonia	6	The majority are pneumonia cases with leucopenia. No special age or period.
	6	38	Pneumonia	7	
	23	—	Pneumonia	—	
	33	48	Pneumonia	5	
	37	44	Pneumonia	10	
	54	65	Pneumonia	—	
68	28	Ulcerative Endocarditis	6 mos.		
Diminished (total 3)	24	59	Pneumonia	—	No special remarks.
	30	41	Broncho-pneumonia.	—	
	56	17	Pneumonia.	8	
Normal (total 5)	7	45	Peri- & Endo-carditis	9	Includes both very acute and less acute cases.
	12	40	Pneumococcal Pleurisy & Pericarditis.	5	
	27	—	Pneumonia	—	
	36	17	Ulcerative Endocarditis	14	
	38	24	Peritonitis.	3	
Increased (Total 8)	3	67	Pneumonia	6	Cases vary much in age and period.
	4	43	Pneumonia	7	
	11	40	Pneumonia	—	
	13	15	Pneumonia	10+	
	25	—	Endocarditis	—	
	23	—	Meningitis	3	
	28	16	Empyema (pneumococcal)	—	
34	14	Strangulated hernia.	56½ hrs.		
Greatly increased. (total 4)	16	53	Septic Broncho-pneumonia, with leucopenia.	7	With exception of Case 16*, cases in this group are young.
	42	15	Pneumococcal Meningitis.	12	
	61	13	Acute Septicaemia (pneumococcal?)	38½ hrs.	
	62	11	Acute Septicaemia (pneumococcal?)	21¼ "	

Note In this case the pneumonia supervened while the patient was in hospital, having been admitted for some obscure abdominal disease characterised by ascites. The condition of the marrow may therefore be due to this and not to the pneumonic condition.

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Correct the numbers when
the deleted references are
removed - 199

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