A STUDY OF THE CHANGES MET WITH IN THE LEUCOCYTES IN CERTAIN CASES OF MALIGNANT DISEASE.

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The clinical value of the study of the blood by means of the stereotyped bedside hæmatological methods is not very conclusive. It is true that considerable assistance may be rendered in deciding between such conditions as intra-abdominal hæmorrhage and fulminating appendicitis or perforated gastric ulcer, as has been shown more recently by Jules Rey.[1] Nevertheless, the range of data is limited when only total cell-counts and differential counts are utilized.

When Arneth introduced his well-known system of classifying the neutrophile leucocytes, he enabled clinical hæmatology to move one step in the right direction. Up to the present, however, this system has not been extended to the other cell types present in the blood-stream.

The writer has made some investigations in this further direction,[2] but discovered an important fallacy underlying the method of study—namely, that the changes met with in a patient are naturally enough correlated with the clinical diagnosis, whereas they should be correlated with the physiological condition of the patient's tissues. The latter appears to be disregarded because the 'disease' claims first attention; not to mention the circumstance that there are no methods of determining the patient's physiology other than by elaborate 'metabolism experiments.' In other words, the really important thing about a patient remains untouched.

I.—THEORETICAL BASIS OF THE STUDY.

Before describing the findings in the blood in cases of malignant disease, it is necessary to bring the suggestion forward that a mere record of facts will not help diagnosis or prognosis, until they are correctly assessed in the light of every possible conception of 'disease.'

We have pointed out before[3] that a blood-count represents the condition of the blood only at the particular moment when it was collected; that certain features may have changed even within an hour's time; that there is no such thing as a disease of the blood; that the changes one meets with can be nothing more than an index of the relative purity or fouling of a moving stream as tested at any given moment.

The same principle holds good for any section of pathology. A disease is not a fixed picture, but consists in a series of phenomena which steadily pass through a cycle of changes, and only truly terminate when the tissues have become restored to their pristine condition. A disease is therefore not.

really an *entity* at all. The clinical diagnosis of a case is merely a label affixed for convenience upon a certain phase in the development of a number of chemical or metabolic sequences. In producing a picture, various ingredients go to make up the finished canvas—seascape, landscape, genre-painting, and so forth—*plus* a living agent which we may call 'A.' At any moment the colours may be transposed, and convert what was about to become a seascape into a landscape, and so forth. Similarly (the analogy is not altogether fanciful),* a number of factors exist in a given patient, which under one influence give no clinical result, under others give rise to various predisposing causes, until the superaddition of an agent 'A' (pneumococcus, etc.) allows the production of a finished picture labelled 'pneumonia,' 'mastoid abscess,' 'endocarditis,' and so forth.

If this be correct, and if we had the requisite knowledge, it should be possible to step into the stream, divert the morbid processes along different lines, and convert one preliminary picture into another, or at least divert the current into a channel the termination of which is not death. The present study may be regarded as an attempt to discover a track which leads into that stream.

It was found that the use of a classification of blood-cells into nearly fifty varieties introduced a means of investigation of attractive acuity. The problem is now one of interpretation.

II.—METHOD OF STUDY.

A.—**Technique.**—After having tested many of the recognized methods of blood-examination according to, first, the extent of information supplied by each, and secondly, the ease of adoption as a routine method, it was found that the study of smears would alone suffice for the present purpose. The blood is conveniently collected from the lobule of the ear, and smears are made in the usual way. Three slides are used for each case.

The *staining method* selected is that devised by Pappenheim, the 'panoptic stain.' This is used as follows: Three or four drops of Jenner's stain are placed upon the film, and the slide is immediately protected by a Petri-dish lid. After exactly 3½ minutes, the lid is removed, and an equal number of drops of distilled water are added to the stain already present. In exactly 2 minutes this is drained off, and the film is flooded with a freshly made dilution of Giemsa (1 drop to each c.c. of distilled water, with 1 drop of 1 per cent potassium carbonate). This stain may be rinsed off with distilled water in not less than 12 minutes; 15 minutes is a convenient routine time. It is almost impossible to overstain. The slide is now rapidly blotted, left to dry in the air, and examined with the oil-immersion without the intervention of a cover-glass.

B.—**Classification of Cells.**—A very detailed classification was adopted, in order to enable the really important types to be selected more promptly than if individual experiments were tried upon successive series of cases. It

* This is a provisional description of an idea which can be adequately represented only by a mathematical formula.

was found unnecessary to carry out actual *total* counts, because the grades of leucocytosis that really signify can be detected in the smear itself.

The following points proved to be of special interest:—

In the neutrophile leucocytes—

Ratio between juvenile and adult forms
Ratio of living to dead forms
Presence of 'bizarre' forms (see *Plate I*)
Presence of nuclear excrescences ('nuclear pseudopods')
Evidence of active amœboid motion
(Spiral lines in the cytoplasm; tigroid and 'leopard' marking in the nucleus; presence of nucleoli).

In the lymphocytes—

Ratio between juvenile and adult forms
Ratio of living to dead forms

Description of Plate I.—BLOOD-CELLS IN CARCINOMA AND SARCOMA.

The cells figured are selected from different cases of malignant disease.

The main characters of the neutrophile leucocyte, as shown in *Cells* 1–10, 13, lie in an amblychromatism of the nucleus. The outline is clear, but the nuclear substance is pale, and nucleolar bodies frequently occur within (1, 2, 3, 6, 8, 9, 13.)

The nuclear outline tends to tail off into slender processes which give the outline a bizarre form. Pyriform excrescences are well shown in 6. The ring form in 10 is frequent in carcinoma.

The last three rows, as well as *Cell* 11, represent mononuclear cells of different kinds. The most striking feature is the frequency of amitosis in the circulating cells (16, 17, 21, 23); 17 shows a characteristic unequal division of the nucleus. This is seen to a less marked degree in 23.

14 and 15 show two lymphocytes whose cytoplasm is fixed in an amœboid position.

18 is a large mononuclear leucocyte of irregular form; its nuclear outline is blurred from the formation of successive overflow layers. This feature has been seen in cases of sarcoma. 19 is a similar cell except that the overflow effect covers a larger area than the actual nucleus. 20 is a cell whose cytoplasm is continuous with a polymorphous platelet mass, indicating some definite relation between the two. 21 shows three collections of azurophile granules proceeding from the nucleus, and evidently about to be discharged from the cell.

22 shows an amœboid lymphocyte with a spiral body ('spirilloid'), partly placed within a vacuole.

23 is a leucocytoid monocyte undergoing unequal amitotic division.

11 shows a large hyaline cell whose cytoplasm contains many vacuoles, in which are bright azur-red diploid bodies.

Evidence of amœboid activity
Presence of azur granules
Presence of azur rods or spirilloids.

In the large mononuclear cells ('monocytes')—

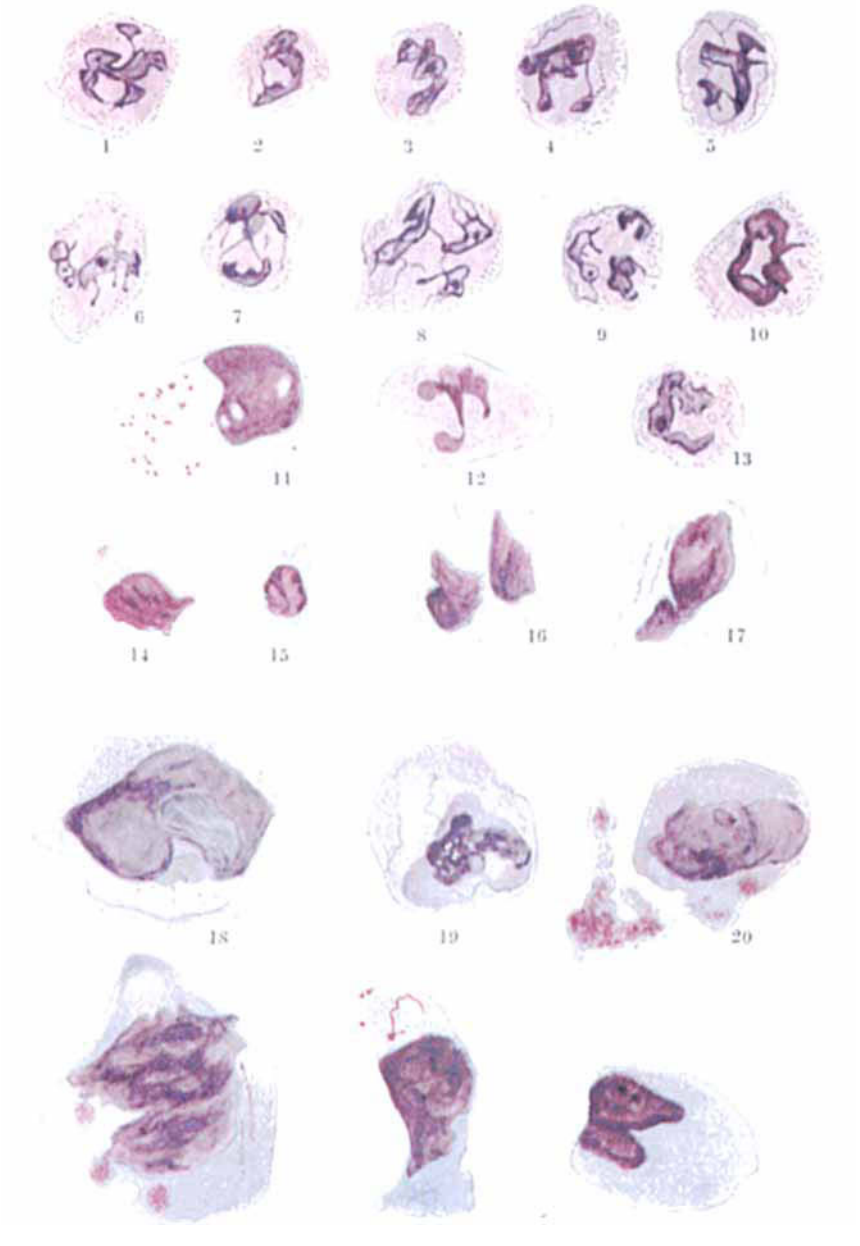
Ratio of amitosing to resting forms
Evidence of amœboid movement in the nucleus (see *Plate II*)
Existence of fatty degeneration
Intranuclear markings, simulating spirilloids
Presence of azur bodies

The so-called hyaline cells, and the micro-monocytes, are included under the heading of monocytes.

New Cells.—A systematic search has been made with a view to discovering points which might distinguish the existence of tumour cells in the films, especially in the case of sarcoma. In one instance a cell was detected which was regarded as a columnar carcinoma cell, and a diagnosis based thereon was substantiated by the subsequent operation. Sarcoma cells are so easily

PLATE I.

BLOOD-CELLS IN CARCINOMA AND SARCOMA



mistaken for lymphocytes in a tissue that it seems possible that they have hitherto been counted among the lymphocytes in the course of a differential count, falsely contributing to the 'lymphocytosis' of some cases of sarcomatosis.

III.—DESCRIPTION OF THE CELLS.

1. **Neutrophile Leucocytes.**—According to the Arneth system, the nuclear forms vary according to the age of the cell. A subdivision of the neutrophiles into five groups according to nuclear form has been extensively used. In the present series, the cells are divided into only two groups. The second group comprises all neutrophiles whose nucleus is actually or almost divided into distinct segments. The first includes Classes I–III as specified by Cooke.[4] The following diagram indicates the nuclear forms:

I.	II.	III.	IV.	V. Completely partite and dead cells
Juvenile			Adult	

i. *Dead Leucocytes.*—All neutrophiles which show defective nuclear staining are regarded as dead cells. A distinction can be drawn into 'dying' and 'dead,' but though recorded in the actual cell counts, it is not referred to specifically in the results appended here. The characteristic form and colouring of these cells is shown on *Plate IV*.

ii. *Ratios.*—These may be expressed in actual counts as percentages of the total white cell-count, or they may be corrected to a percentage of the neutrophiles alone, or they may be given in a ratio where the number of juveniles is taken as unity, or the total of adults is taken as unity. In the tables appended the third method has been adopted. As a matter of fact the ratio does not appear to have very great significance. *Table I* shows that the variation is from 1 : 1·1 up to 1 : 6 in the case of carcinoma. *Table II* shows a variation from 1 : 1·2 to 1 : 8 in the case of sarcoma.

iii. *Bizarre Forms.*—By this term is meant the occurrence of a very irregular contour to the nucleus. Fantastic outlines are often produced, resembling various animal forms—insects (imagined as wingless), caterpillars, rabbits, etc. (*Plate I*). The bizarrerie is partly produced by erratic nuclear excrescences, and is a very common feature of the films of cancerous blood. It is not absent in some other diseases, but is inconspicuous, if not altogether absent, in the fasting, healthy subject.

iv. *Nuclear Excrescences.*—Much attention has been paid to this feature, because of the frequency with which it is noticed in certain diseases. These excrescences are classifiable into three forms: (*a*) pyriform in shape, (*b*) blunt and more or less sessile, (*c*) spiculated. The first-named are the most noticeable, but are not necessarily the most significant. In the tables presented, all three forms are shown collectively. In *Table I* we see that there are many instances in which these pseudopods appear. Where present, they are met with in from 2 to 58·5 out of every 100 neutrophiles. Although they are not so frequent in the simple horseshoe-nucleate forms, they cannot be easily referred to as dependent upon the age of the cell. A comparison of

Table I.—Showing the Frequency of the Changes in the Leucocytes in Cases of Carcinoma.

No. of Case	Region	Neutrophiles			Lymphocytes		Monocytes					Remarks
		Ratio of young to old	Percentage of neutrophiles:—		Excess over 20 per cent	Percentage of cells showing amœboid activity	Excess over 10 per cent.	Percentage of cells showing				
			Dead	With pseudopods				Amitosis	Fatty change	Bizarre nuclei	Death	
370	Rodent	1 : 3·7	25	18	+	3·8	+	0	0	0	13	
555	Jaw	1 : 1.1	9·2	2	++	6·5	+	0	0	0	23	
485	Cheek	1 : 1·5	1·6	16·6	+	4	+	0	0	0	0	Recurrent
681	Cheek	1 : 1·7	7·7	20	+	0	+	0	0	0	54	
293	Tongue	1 : 6	7·7	4	—	46	++	0	11·6	15	31	
420	Palate	1 : 2·1	0	3	—	10	+	12·5	12·5	6·2	0	
454	Palate	1 : 8	16	13	+	0	+++	0	5·7	0	20	
325	Stomach	1 : 5·6	7·8	0	0	0	0	0	0	20	20	Inoperable
328	Stomach	1 : 3·4	0	0	+	11·6	0	0	0	55	0	Inoperable
563	Stomach	1 : 3·3	2·3	24	—	2	—	1	0	10	1	Secondaries in liver
747	Pylorus	1 : 2·3	5·7	22	—	0	—	0	0	0	0	A small growth but inoperable: general condition bad
369	Sigmoid	1 : 1·7	0	23	—	0	—	0	0	0	0	
381	Sigmoid	1 : 2·7	13	35	+	4	0	30	0	0	0	
320	Sigmoid	1 : 1·9	22·6	10	0	50	0	0	0	0	0	
317	Sigmoid (P.M.)	1 : 2·6	39	0	+++	4	+	0	0	0	39	
380	Transverse colon	1 : 2·2	0	0	+	4·3	+	7·1	0	7·1	0	
349	Rectum	1 : 6·1	1·2	0	—	16·6	—	0	0	12·5	0	Columnar cell found
463	Rectum	1 : 1·9	13	0	—	0	—	0	0	0	0	Very fibrous small growth
328	Rectum	1 : 2·3	8	0	++	2	0	0	0	0	22	
487	Generalized	1 : 1·6	7	23	—	0	+	7	7	5	1	
358	Abdominal (peritoneal dissemination)	1 : 3·3	6·5	0	++	33	—	0	0	0	0	Inoperable
300	Breast	1 : 5	73	0	+	58·5	+	8	0	24	32	Inoperable
303	Breast	1 : 4	35	0	—	0	0	0	0	30	10	Recurrent
296	Breast 7 days after excision	1 : 2·1	4·3	5·8	+	25	0	0	0	0	0	
551	Breast 14 days after excision	1 : 3·8	7	17·6	0	12·5	+	7·1	0	0	28	
470	Uterus	1 : 1·8	4·7	3·5	0	0	0	14	0	0	0	
484	Uterus	1 : 1·3	10	15	++	2·9	+	0	7·9	25	44	

+ means an excess up to 5; ++ means an excess up to 10; +++ means an excess up to 15.

the first and third column shows that there is no numerical relation between the excess of old neutrophiles over young, and the number of pseudopods per hundred neutrophiles.

Regarding these appearances as definitely due to nitrogenous metabolism, one would anticipate that they would be either absent, or at any rate less marked, in those cases of carcinoma where the tumour was so circumscribed by fibrous tissue that cancerous excreta might not enter the blood-stream. An objection to this line of reasoning might lie in the possibility that a tight stricture of the intestine, for instance, leading to chronic obstruction, might be associated with the absorption from the stagnant intestinal contents of toxic bodies of a similar group to the cancerous toxins; such bodies circulating in the blood would equally influence the neutrophile morphology. Two instances in which the neoplasm was circumscribed in the manner indicated are found in *Cases* 747 and 463. The former tumour was, however, not scirrhoid.

Description of Plate II.—Mononuclear Leucocytes in Carcinoma and Sarcoma.

The central portion of the figure shows a collection of cells all taken from the same blood-film—a case of epithelioma. They are grouped together to show how conspicuous the amœboid outline of the small cells is, and the peculiar bizarre character of the other cells.

1–12 present different forms of large mononuclear leucocytes with bizarre forms of nuclei; 1, 3, 5, 6, 7 are from breast cancer. 8 is a dying cell (change of reaction of cell-body as well as of nucleus). 13 has a trifid nucleus. 14 shows a closely coiled nucleus. 15 shows overflow of substance from the nucleus into the cell-substance. An azurophile diploid lies in a vacuole within it. 17 is a micromonocyte with overflow of material in a similar way. Nucleoli are conspicuous. 18 is an aberrant amitosis from a case of mediastinal sarcoma. 19 is a cell taken to be a columnar carcinoma cell. 25, 26, 34, 35 are from one case of sigmoid cancer, showing peculiar forms of lymphocyte-like cell. Note the azur bodies within. 27 is a leucocytoid hyaline cell with azur diploids from a case of gastric cancer. 28 shows a blood-platelet with included spirilloid.

36 shows beaded linear azur lines; 37, 42, 43 show abnormal nuclear outlines. 38, 45, 52 show various forms of amitotic division. 44, 46, 47, 48, 49, 50, 51 show marked lobation of the nucleus giving rise to the appearance of amœboid movement in the nucleus, or irregular budding.

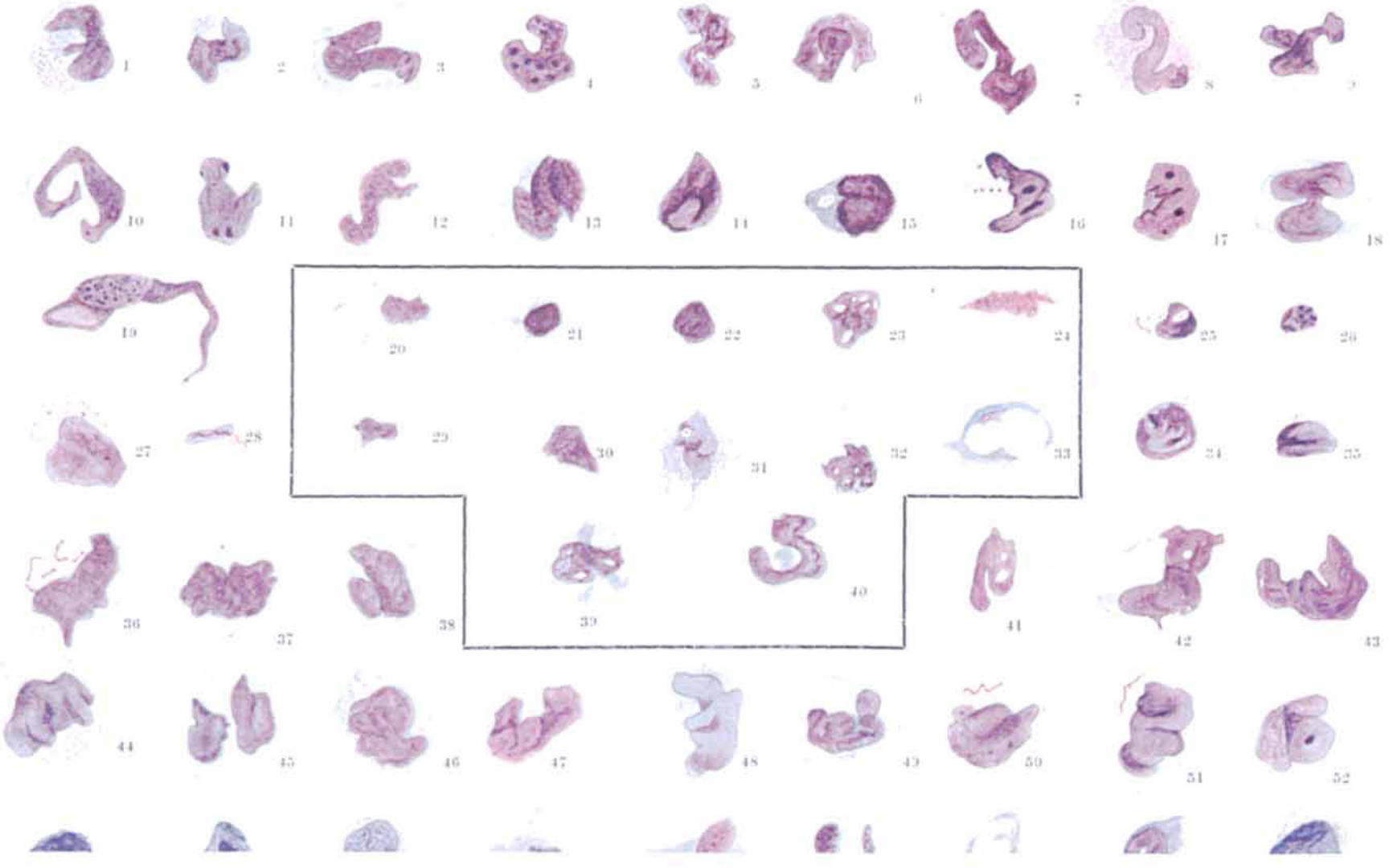
38, 41 show fatty granules in the leucocytes. Small fat-droplets are also seen in 42, 43, 45.

The last row shows various forms of cell regarded as sarcomatous. Their different staining power, and the nuclear markings, indicate a distinct difference from natural blood-cells. 55, 56, 58 show finely granular nuclei.

56 shows a platelet which appears to have fused with the cytoplasm (not an infrequent occurrence). 57 is amœboid in outline.

Influence of Diet upon the Neutrophile Leucocyte.—The fact that the nuclear pseudopods stain like nuclear matter, suggests that they represent an overflow of nuclein bodies into the cell substance. If so, they should be more numerous after certain articles of food have been taken in. In this case the appearance of these structures might be due to the patient's diet, and not to his neoplasm. The matter was therefore tested as follows: The writer commenced using a strictly purin-free diet, allowed some weeks to elapse before regarding the blood as standard, and then partook of isolated and sufficiently heavy purin-containing meals, immediately relapsing to the purin-free dietary for a further period of time. The blood was examined hourly, full differential counts were made on the lines of this study, and control analyses of the urine were made in order to ascertain when the nitrogenous material had actually passed round the circulation. The results were of considerable interest, showing as they do that *it is possible to trace this food material in the blood-stream.* It was found, in other words, that one can reproduce the blood

PLATE II.—MONONUCLEAR LEUCOCYTES IN CARCINOMA AND SARCOMA



picture of carcinoma, in respect to the nuclear pseudopods, by partaking of certain articles of food—notably pork, and, to a less extent, other red meats. The change did not appear in the urine until the altered morphology had already been established.

In *Fig.* 172 we see the effect of (*a*) animal fat, (*b*) pork, upon the blood-cells. In each case the 'meal' consisted solely of the article of diet in question. The rise in the number of the juvenile leucocytes is well shown in (*b*), and the increased numbers of pseudopods is clearly shown. After the diet (*a*), on the other hand, there is a fall of both juvenile leucocytes and of the number of pseudopods. Fat requires the interaction of lymphocytes for its disposal, nitrogenous matter requires neutrophiles. Similarly, following a 'meal' of nothing but boiled cabbage (*c*), we find the pseudopods steadily diminish.

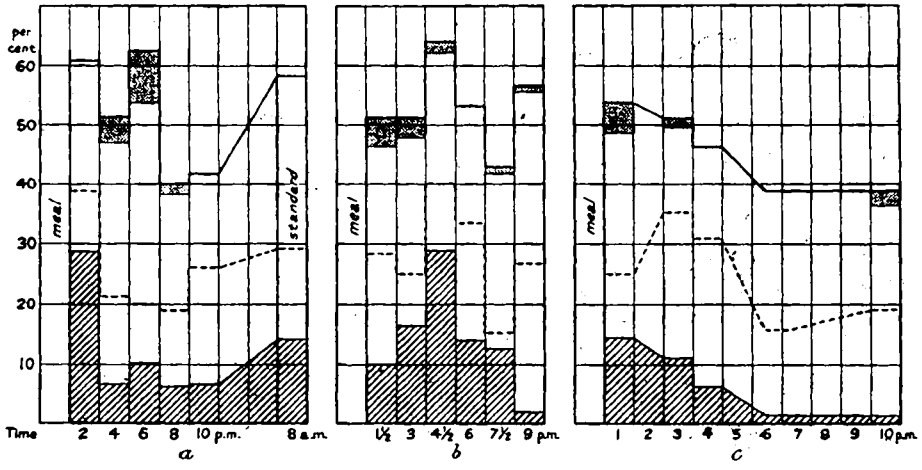


FIG. 172.—CHARTS REPRESENTING THE HOURLY CHANGES IN THE LEUCOCYTE FORMS DURING THREE EXPERIMENTS WITH DIETARY. The standard value is that found in the early morning, the diet being continuously of the strict purin-free material mentioned in the text.

In each chart the complete columns represent the total percentage of neutrophiles; the middle broken line indicates the percentage of juvenile neutrophiles, and the shaded columns the percentage of neutrophiles which contain pseudopods. The dotted areas at the top represent the number of dead or dying neutrophiles per cent of total leucocytes.

a. Changes subsequent upon a meal composed entirely of suet (pudding); *b*. Ditto, composed of pork (with gravy); *c*. Ditto, composed solely of boiled cabbage.

In all the cell-counts made in cases of cancer, the mid-day hour was chosen for the collection of the sample, so that the patient had not partaken of animal food since at least mid-day of the previous day.

Strictly speaking, one would put a case upon purin-free diet for several days before collecting the blood; but as we shall see, there are more important points to study—points which are not interfered with or masked by the use of ordinary diet. On the other hand, a pure vegetarian diet will do the patient no harm, and makes the interpretation of the blood-findings more precise. (By a 'pure' vegetarian diet is signified the use of vegetables cooked in the simplest possible way; the introduction of 'fancy-work' into the vegetarian dietary—sauces, spices, etc.—appears to deprave its value to the level of

purin-containing food as far as the blood-cells are concerned.) Tea and coffee must be discarded. The diet per diem consists roughly of three pints of milk, one pint of pure water, one pound of bread, half an ounce of butter, jam *ad libitum*; this is sufficient, with the addition of prunes or a little cabbage for lubricating purposes.*

Table II.—Showing the Frequency of the Changes in the Leucocytes in Cases of Sarcoma.

No. of Case	Region	Neutrophiles			Lymphocytes		Monocytes				
		Ratio of young to old	No. of dead cells in every 100	No. of cells with pseudopoda in every 100	Excess over 20 per cent	Percentage of cells showing amœboid activity	Excess over 10 per cent	Percentage of cells showing			
								Amitosis	Fatty change	Bizarre nuclei	Death
655	Retroperitoneal lymphosarcoma	1 : 1·4	0	44	++	5·7	4	7·1	7·1	14·2	28·4
708	Mediastinum	1 : 2·2	0	45	—	6·7	7	5·8	0	11·7	5·8
434	Lung	1 : 1·6	3	47	—	0	1	9	0	0	0
872	Axilla	1 : 1·2	1·1	31	—	0	1	0	0	27	0
710	Ankle	1 : 1·3	3	39	++	0	20	10	3·3	13·2	28·5
451	Kidney	1 : 2	0	20	+	0	—	0	0	0	0
693	Lymphogranuloma (Hodgkin's disease)	1 : 1·26	0	3·1	—	6·2	8	16	0	5·5	0
743	Glioma (gliosarcoma?)	1 : 1·7	5	40	—	0	5	6·6	0	26·4	0
336	Myoma uteri (myosarcoma ?)	1 : 8	20	0	+	—	3	0	0	0	23

Incidentally, these observations suggest the physiological reason for the dietary imposed upon the Hebrew race (Gen. ix. 4; Lev. xi.).

v. *Evidence of Active Amœboid Action.* This is a noticeable feature, but as it is difficult to estimate its degree with anything like mathematical precision, it is desirable at this point to dismiss it with mere mention.

When we come to consider cases of *sarcoma* in the manner described, we find very striking results. *Table II* shows that from 20 to 47 of every 100 neutrophiles present nuclear excrescences. One case (lymphogranuloma) shows only 3 per cent, and the only case which reveals none was one of myoma, clinically believed to be becoming malignant. Doubtless it is reasonable to regard this case as of quite a different type of malignancy, even if the microscope verified the clinical diagnosis, inasmuch as the sarcomatous change is an incident, and is not associated with dissemination—certainly in this particular case (see also *Fig.* 173).

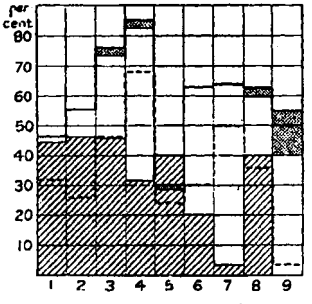


Fig. 173.—Diagram showing the Character of the Neutrophile Leucocytes in Cases of Sarcoma. The successive values (1–9) are in the same order and refer to the same cases as in *Table II*. The meaning of the different lines is explained under *Fig.* 172.

*I am indebted to Dr. Raper (Bio-chemical Department, Leeds Medical School) for guidance in this matter.

2. **Lymphocytes.**—The two tables presented take cognizance of only two points about the lymphocytes. For the sake of simplicity the bulk of the data obtained are omitted, inasmuch as no very significant conclusions have been drawn from them as yet.

The outstanding feature was in relation to *amœboid movement*. It is usually considered that lymphocytes exhibit no amœboid power. Experience shows that this statement or opinion must be modified. In the blood of both carcinoma and sarcoma there is some agent which brings out amœboid motion in lymphocytes, sometimes to a very marked degree. From this feature alone it has been possible to venture on an opinion that a case is malignant, even though the clinical evidence has been doubtful. The tables show that this phenomenon is commoner in cases of carcinoma.

Description of Plate III.—Cell-forms in Pernicious Anæmia.

The four upper rows present various forms of neutrophile leucocytes. The two lower rows show mononuclear cells. The examples are selected from different cases.

Rows 1 and 2 show marked polymorphism of the nucleus, the effect being produced by marked beading, by the extrusion of pyriform and rod-like excrescences of nuclear matter, and by coiling of the nucleus.

Rows 3 and 4 show juvenile and dying forms. Cells 11 and 18 are juvenile leucocytes (thick, rather pale, massive nuclei). Cells 13 and 14 show less bulky nuclei, with tortuosity or coil formation.

Cells 15–18 are dying cells, as shown by different staining reaction, less evident cell-outline, and less conspicuous cell-granulation. Cell 19 is introduced to show two kinds of pseudopod excrescence from the nucleus: (*a*) spiculated, (*b*) blunt and sessile. The linear arrangement of the ferment granules is also shown.

The last two rows illustrate cell-types not infrequently met with in this kind of blood. Cells 20 and 21 are large mononuclear leucocytes with bright azurophile-ex-nuclear lines. In the one case a rounded cluster of azurophile granules such as are seen in platelets is being formed. Cells 22 and 23 are typical small lymphocytes, the latter being typical of the 'naked' form so common in pernicious anæmia. 24 is a large leucocytoid mononuclear, with a fat drop in the nucleus, and another in the cell-substance. 25 and 26 are dead and dying mononuclear cells. 27 is a hyaline leucocyte (leucocytoid micro-monocyte) with a few minute azurophile granules.

Summing up the hæmatological characters of this blood, we have: (*a*) Bizarre nuclear forms, with abundant ex-nuclear pseudopods; (*b*) Preponderance of multifid forms; (*c*) A number of dead and dying leucocytes, giving the phenomenon of leucocytolysis, and indicating the existence of leucocytotoxins in the circulating blood; (*d*) Evidence of lymphocytolysis; (*e*) Presence of very juvenile lymphocytes.

The last-named point rules out carcinoma, almost with precision. Some of the other characters, which also occur in the blood of patients with cancer, merely indicate that there is something in common between the two diseases as regards metabolic processes, or as regards the configuration of the hæmolytic substances produced in each.

The microscopic appearance upon which this opinion is based has been portrayed on *Plates I, Cells* 14 and 15; and *II, Cells* 20, 21, 22, 23, 25, 26, 29, 30.

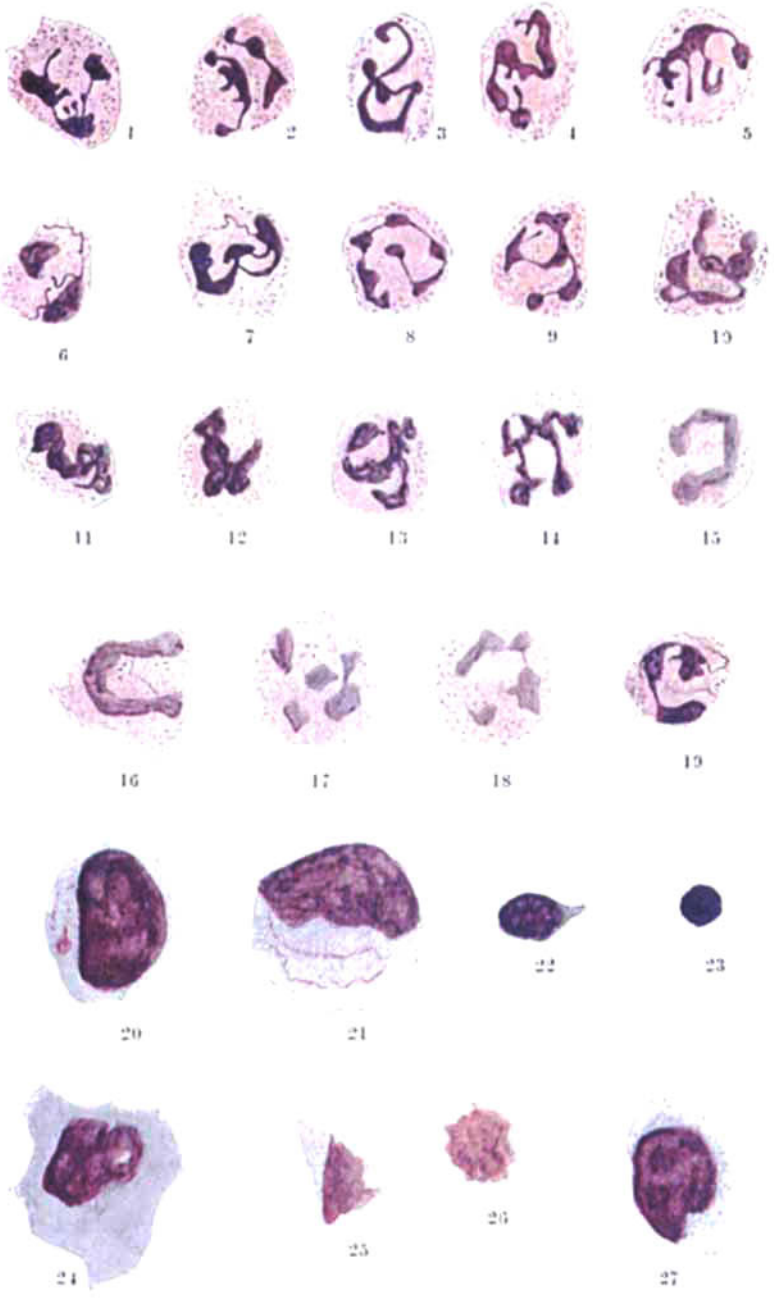
As regards the percentage of lymphocytes in the differential count, the relative neutrophilia is found to be so constant that the percentage of lymphocytes is almost invariably subnormal.

3. **Monocytes.**—For the sake of convenience we consider here the various forms of large mononuclear leucocytes, as well as what are known as 'transitional leucocytes.

The various features which were studied, as set forth on the preliminary list, do not yield results of special interest as far as the present study goes. We therefore confine our attention to: (i) The percentage number; (ii) The occurrence of amitosis; (iii) The appearance of fatty change; (iv) The occur-

PLATE III.

CELL FORMS IN PERNICIOUS ANÆMIA



rence of bizarre forms (see *Tables I* and *II*); (v) Intranuclear markings; and (vi) Azur bodies.

i. *The Percentage Number.*—It is well known that polynuclear cells are usually increased in cases of malignant disease. Consequently, the one or other group of mononuclear must suffer in the differential count. It is found, however, that there is a greater tendency for a decrease in the lymphocytes than in that of the large mononuclears. Indeed the latter often show a relative (and therefore absolute) increase. *Table I* shows that among the carcinoma group, as many as 14 out of 24 cases give no relative increase; *Table II* shows that all cases of sarcoma but one give an increase, which in some cases is considerable.

ii. *Amitosis.*—Ross[5] has spoken of the occurrence of division of mononuclear cells while circulating in the blood-stream, and other observers have verified a fact which does not yet appear to have attracted attention on the part of the clinical pathologist. This oversight may be attributed to the inadequate staining and optical methods employed. While the condition is observable in any blood, yet it is found to be quite conspicuous in some cases of carcinoma, and in very many cases of sarcoma. In some instances the proportion between amitosing and resting forms has been estimated by the actual count of a hundred monocytes, thus verifying the results obtained by calculation from a smaller count. The appearances of the cells are quite characteristic. It is rare for the two portions of nucleus to be equal (*Plate II, Cell* 45); the commoner finding is where the portions are decidedly unequal (*Plate I, No.* 17, *Plate II, Cell* 38). Partially divided nuclei produce a bizarre apearance, as shown in several of the cells of the fifth and sixth rows of *Plate II.*

iii. *Fatty Degeneration.*—The occurrence of fine round vacuoles within the cell substance, and even within the nucleus, has been a feature of certain cases (7 times in 57 cases). The appearance is portrayed on *Plate II, Cells* 23, 32, 38, 39, 41.

iv. *Bizarre Forms.*—As stated, bizarrerie is produced by irregular and incomplete amitotic change. The nuclear contour is, however, rendered very irregular by the formation of buds (*Plate II, Cells* 46, 47, 48). The effect produced is that of "amœboid movement in the nucleus" itself. This constitutes a convenient method of description of a phenomenon which is very common in sarcomas. In carcinomas, the bizarrerie is more often produced by the in- and ex-curvation of an elongated nucleus, thus producing C and S forms (*Plate II.* first row, and the first three cells of the second row). Reference to the tables will show how frequent this feature is in both forms of malignant disease. Some of the cells may be classifiable as transitional cells whose normal horseshoe nucleus has become bent upon itself.

v. *Intranuclear Markings.*—These are not so conspicuous, but call for mention. In some instances fine spiral lines have been noted, having ten or more turns, apparently staining a purple blue, or sometimes an azur colour. They may be described as spirilloids, although they are by no means necessarily actual entities; a creasing of the cell-substance by smearing may be the explanation. In *Fig.* 174 is shown one of these cells compared with a cell from an actual tumour scraping, where a similar intranuclear marking is

present. At first sight these phenomena suggested superadded bodies, but at the present time it seems correct to regard them as belonging to the category of artefacts.

Other nuclear markings are shown in *Plate I, Cells* 18 and 19, and in *Plate II, Cells* 15 and 17, where an 'overflow' is taking place from the nucleus, producing bizarre effects. The significance of this appearance is not understood.

vi. *Presence of Azur Bodies.*—Azur granules of different kinds are frequent in both lymphocytes and in monocytes. They occur in health and in many forms of disease. The majority of observers regard them as secretory in character. Some consider them to be parasitic. There are one or two argu-

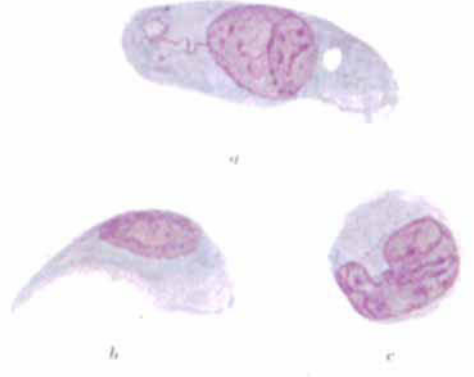


FIG. 174.—The upper of the three cells shown (*a*) is a drawing of a cell obtained by scraping a spheroidal-celled carcinoma of the stomach. A delicate spiral body is shown extending from the cytoplasm into the nucleus. This kind of body is frequent in the cells of the scraping.

The left lower cell (*b*) is very similar in staining reaction to the above; the nucleus is entirely different from an ordinary monocyte, but similar to a degenerating carcinoma cell as stained by this method. The cell is therefore diagnosed as a carcinoma cell. It is taken from a blood-film made from a case of generalized epithelioma of the pharynx, etc. (Tracheotomy necessary: patient still living.) [I am indebted to Mr. Constable Hayes for this case.]

The right lower cell (*c*) is a monocyte from the same film, showing a similar spiral body (spirilloid), partly in the cell-substance, partly in the nucleus.

All are drawn to the same scale. Panoptic stain. Oil-immersion lens, draw-tube out, Zeiss binocular eyepiece (the spiral bodies are hardly, if at all, visible with a single eyepiece).

ments in favour of the latter view, in connection with malignant disease. *Plate I, Cell* 11, and *Plate II, Cells* 27, 36, 50, 51, show special forms of azur bodies. *Cell* 27 shows a common form of diploid, the pair resembling the gonococcus. *Cell* 36 shows the azur granulation in the form of coccal chains. *Cell* 50 shows the azur body in the form of a geniculate spirillum; a similar body is shown in *Cell* 22 of *Plate I.* The occurrence of secretory granules in the form of dot lines seems unusual, although it must be admitted that similar dot lines occur in neutrophile leucocytes, being there formed of neutrophilic granules as they wind their way from nucleus to periphery of cell, and thence into the surrounding fluid medium.

4. **New Cells.**—One of these is depicted in *Plate II, Cell* 19. An

elongated cell-structure, with an oval nucleus near one end, strongly suggested the possibility of its being a columnar carcinoma cell which had become dislodged into some radicle of the inferior vena cava. The last row of cells on *Plate II* presents different forms of 'lymphocyte' which the writer has concluded must really be sarcomatous cells, owing to the marked difference in their morphology from an ordinary large mononuclear. Collectively, the characters of a circulating 'sarcoma' cell would be :—

Size : Much larger than a small lymphocyte, smaller than a large mononuclear leucocyte. *Shape :* Angular, tending to ovoid. *Contour :* Not very sharply defined. *Cell-body :* Relatively small, distinctly basophile, dusky, but showing no spongioplasmic network. No granules. *Nucleus :* Relatively large. Shape irregular, often budded, sometimes trifid. Outline sometimes indistinct, at other times clearly marked owing to a difference of staining reaction from the basophile cell-body. Staining power variable. Structure

Description of Plate IV.—Dead and Dying Leucocytes.

The illustration includes forms derived not only from the neutrophile, but also from the lymphocyte and monocyte series. The signs of death consist in (1) The defective staining power of the nucleus; (2) Alteration in tint; (3) Ground-glass appearance; (4) Loss of definition of outline; (5) Increase of size of the whole cell. The granules are preserved to quite a late stage in the process of death. The type of change has some relation to the condition from which the blood is derived. Thus the case showing 13 will not show 1 and 2.

The commonest form of necrobiosis met with in neutrophiles is represented by 1 and 2. Here the nucleus is tripartite and merely shows an altered tint (metachromatism, subchromatism).

Various degrees of disappearance of nuclear structure are shown in 5, 6, 7. 8 shows granular change at the edges of the nucleus, and a delicate fibrillation is well shown in 13 and 18. Complete loss of granules is shown in 30 and 11, almost complete in 12. 5 and 14 are abnormal cells. 15 and 16 well show the appearance of the so-called Klein-Gumprecht shadows.

Commoner shadow forms appear in 17–22, which are probably necrosed large mononuclear leucocytes (monocytes). 22 shows a monocyte with a curiously lobated nucleus, and distinct colour change in the cytoplasm (basophilic tendency).

23 shows a leucocytoid lymphocyte which has undergone marked alteration of staining reaction, the nucleus being of a much more blue colour, and the cytoplasm somewhat oxyphilic.

24 is a shadow form bearing little resemblance to a known cell. The nucleus alone remains, though in an advanced degree of dissolution.

The colouring of the cells depicted is that shown by the use of the panoptic stain described in the text. The magnification is approximately 700.

The colouring and drawing in *Plate II* is partly diagrammatic.

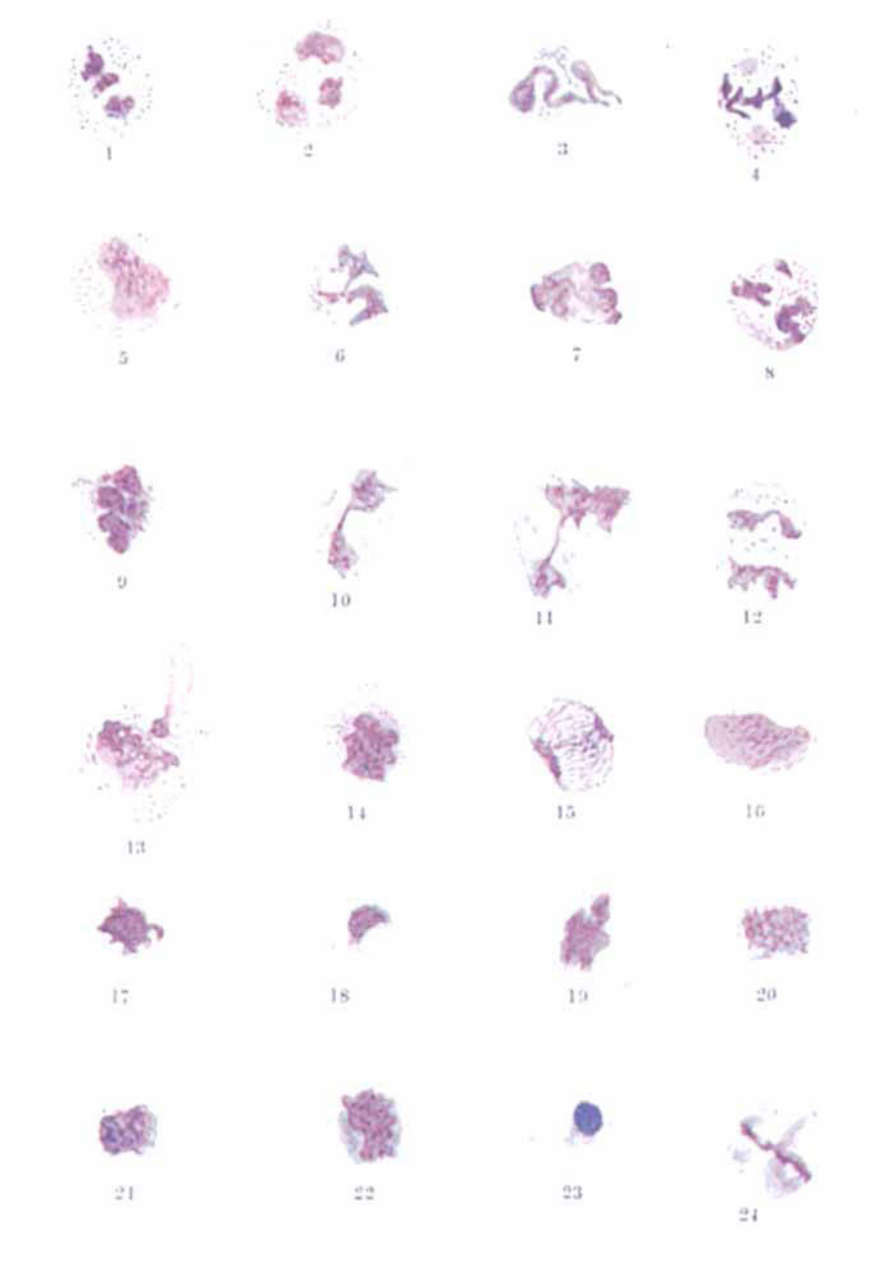
very finely granular rather than hyaline or striate (as in monocytes). Amitosis common. *Nucleolus :* Present in some cases.

This description does not cover all the possibilities, because a larger number of cell-forms in a larger number of cases must be studied.

5. **Platelets.**—The character of the platelets in the blood-smear of cases of malignant disease is sufficiently striking to merit careful investigation. The frequency with which these bodies are actually adherent to the leucocytes, and the frequency with which they appear to be manufactured within the nuclear substance, and then within the cell-body (*Plate I, Cells* 20, 21, *Plate III, Cells* 20, 21), suggest some close relationship between the two series of structures. A cell like that on *Plate II, Cell* 56, shows apparent fusion between the platelet and the tumour cell. This appearance is by no means isolated. It has reminded one of Ross's theory of autofertilization of body-cells by the functioning of other body-cell outpourings as male gametocytes.

PLATE IV.

DYING AND DEAD LEUCOCYTES



The interest attaching to these bodies is not so much one of diagnosis as of etiological relationship. If these phenomena indicate the existence in the serum of special forms of agglutinins, a combination of the present method of study with other laboratory methods might be productive.

IV.—RELATION BETWEEN BLOOD-CELL FINDINGS AND CLINICAL PHENOMENA.

A study was made on several occasions in a case of Hodgkin's disease (lymphogranuloma), where the blood-smear showed evidence of sarcomatous processes having begun. (I am indebted to Dr. Telling for permission to use this case.) The case passed through several pyrexic periods running in cycles. The temperature was intermittent, and the rises were accompanied by rigors. *Figs.* 175 and 176 represent the results of the investigation on two occasions. A twenty-four hour cycle is recorded, the differential count having been carried out on hourly specimens. The position of the rigor, with the temperature rise, is shown. It is seen that there is a decided increase in the proportion of dead neutrophiles just before each rigor. It is also seen that 'sarcoma' cells appear in the blood-stream, as the temperature rises to its acme. The smaller chart (*Fig.* 175) demonstrates the relation between the monocytes and the temperature change. It is seen that these cells fall very markedly at the time of the rigor, to re-ascend as the temperature declines. The 'sarcoma' cells have been increasing until the time when the temperature ascends. Both charts, from the same patient, show that the cycles did not uniformly correspond. An explanation may lie in the fact that the first experiment was carried out in the middle of a three-weekly pyrexic period, while the latter one was towards its decline.

The finding is reported partly for its interest, and partly to demonstrate that it is possible to obtain more intimate knowledge regarding the inner processes of disease by a comparatively simple procedure, the only appliance needed being the microscope.

V.—GENERAL SUGGESTIONS.

Evidence has been brought to show that a fine morphological study of the blood-cells may convey further information about the processes of disease. A morbific agent may stimulate the leucocytes to greater activity—be it phagocytic (amœboid movement) or reproductive; it may damage the leucocytes, and cause the proportion of 'dead and dying' leucocytes to increase; or it may bring about the appearance of new structures within the cells.

In assessing the various findings, it is necessary to remember that the cellular composition of the blood depends upon—(1) The actual composition of the serum; (2) The degree of vitality of the cells; (3) Individual idiosyncrasies.

The blood-cell formula, as studied by more detailed methods, furnishes a clue to the chemical composition of the serum. The same principle must hold for the blood (whose cellular composition is a reflection—more or less precise—of the cellular composition of the floating population of the tissue spaces) as holds good for the infiltrating cells of morbid tissues. Whether the

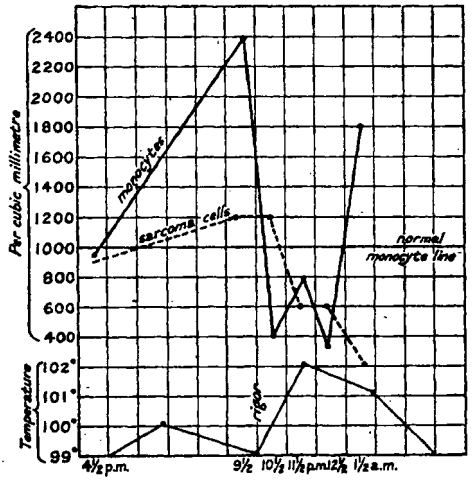


FIG. 175.—CASE OF LYMPHOGRANULOMA. Hourly variations in the numbers of certain circulating cells during a febrile period.

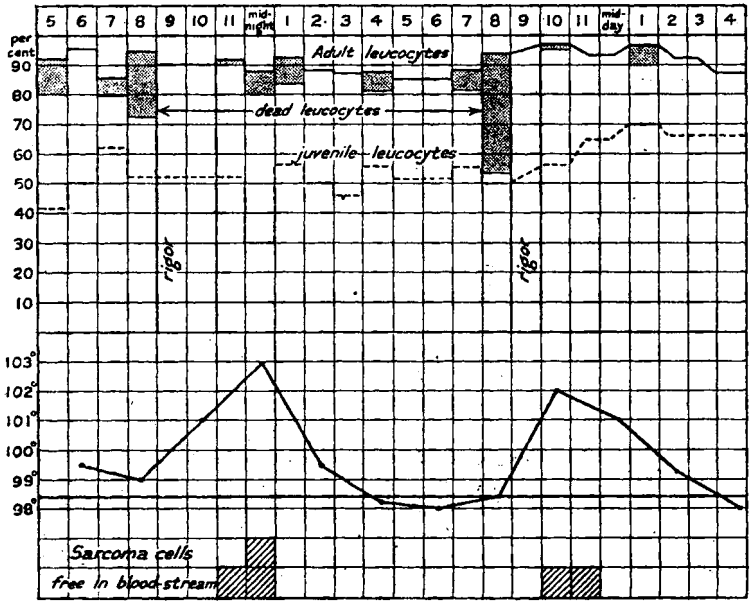


FIG. 176.—FROM THE SAME PATIENT AS FIG. 175.—A complete twenty-four hour cycle, showing the proportions of certain blood-cell types from hour to hour. Note the increased mortality of the leucocytes just before a rigor, and the appearance of 'sarcoma' cells after a rigor.

inflammatory infiltration is polynuclear, lymphocytic, eosinophilic, fibroblastic, and so forth, depends on the nature of the chemical substances which are stagnating in that given area. These substances are in turn dependent upon the type of organism present. If we divide the organisms into (1) cocci

and exotoxic bacilli, (2) endotoxic bacilli and acid-fast bacilli, and (3) protozoa, we have three types which are responded to by three types of inflammatory cell exudate; and we have the same types of blood-cell 'leucocytosis.' But it is not the organisms (as organisms) that evoke the infiltration, it is the kind of substance which constitutes their respective excrement. The neutrophile can deal with the one, the lymphocyte with the other, the monocyte deals with solid residues. Exactly what sort of formula appears, depends on a number of factors, which are well enough known. The only point of importance is to realize that *the same factors hold good in the determination of the blood-cell formula*, the 'differential count.'

It is unscientific to expect a certain cell-count in a certain *disease*; therefore it is necessary to emphasize the fact that *a cell-count merely means that such and such chemical substances are circulating* in the blood at that given time. If these substances are of such a nature as to cause unusual changes in the morphology of individual cells, then they can be identified. The blood-cell picture is a pattern corresponding to the type of abnormal substance circulating, and is not concerned with the clinical diagnosis, excepting at the crest of a cycle where special symptoms come into view.

With these reservations, it may be regarded as possible to make a diagnosis of malignant disease, conclusively, from a careful study of a single blood-smear. It is possible to distinguish between carcinoma and sarcoma.

The object of the blood examination, therefore, is to determine whether the blood contains: (*a*) Leucocytotoxins—shown by various grades of 'death' of the leucocyte. (*b*) Leucocytotic substances, i.e., bodies which either stimulate leucocytic proliferation, or attract leucocytes into a more confined area than is customary, or actually irritate the individual leucocyte; the latter is shown by abnormal nuclear outlines, and superadded structures of various kinds. (*c*) Products of metabolism; these may be natural, or peculiar to certain pathological processes. Cases of malignant disease are characterized in many instances by the discharge into the blood of substances belonging to each of these groups.

A further point is that the student of the blood-smear is in search of certain aberrant cell forms, and not a percentage valuation. The figures which are here presented are solely for the purpose of fixing certain findings before the reader. To attempt to force the value of a percentage, in its bearing on a clinical diagnosis, implies neglect of certain fundamental principles in biology. If we find a certain type of cell or certain intracellular changes with frequency, we are enabled to conclude that some abnormal bodies are circulating in the patient's blood, and that these may or may not (probably do) account for his symptoms.

A class of case where this argument becomes of value occurs in pernicious anæmia, which notoriously simulates gastric cancer. This is presumably because the carcinoma has damaged the same portion of gastric mucosa as is diseased in pernicious anæmia. The blood picture is therefore similar. *Plate III* illustrates some of the important differences. The main feature is the abundance of small ('naked') lymphocytes in the anæmia, and the marked diminution of the total white-cell count. Dying cells are much more

common in pernicious anæmia, and bizarrerie of the neutrophiles is rather less frequent.* The changes described in carcinoma under the heading of monocytes are almost peculiar to this disease.

SUMMARY.

1. The cell-findings in a blood-smear vary in health, under the influence of diet.

2. The findings vary in disease according to the type of product of the causative agent, and not according to the agent itself.

3. The findings in malignant disease vary according as the mass is actively growing or not, according to its limitation by fibrous tissue or not (no discharge of specific excretion into the juices), and according to the gross mechanical effects of the neoplasm on the body (starvation, hæmorrhage).†

4. Monocytosis is a frequent accompaniment of protozoan infections. It is also decided in some cases of carcinoma and in many cases of sarcoma.

5. Since one phase of the blood-picture produced by circulating toxins of malignant disease can be imitated by ingestion of highly nitrogenous food, it suggests that long-continued over-use of the same may form an advantageous substratum for the subsequent development of the disease.‡ While this statement has only speculative value, there is a practical value in the fact that blood-smears should be taken before a meat meal, or preferably after instituting a purin-free diet for one or two days before.

Investigations upon blood-cells up to the present enable the following rules to be formulated :—

If the smear shows neutrophilia, and fat drops in many of the neutrophiles ; if the nuclei of the neutrophiles are largely multifid : the case is one of a coccal infection of great or very great severity.

If the smear shows a relative abundance of lymphocytes (especially of the very small variety) ; if there is no leucocytosis ; if multifid nuclei preponderate : the case is almost certainly *not* one of malignant disease.

If there is neutrophilia, with bizarre forms, or pseudopods in number ; if the lymphocytes are in many instances showing amœboid outlines ; if the monocytes show amœboid nuclei (see central portion of *Plate II*, which contains cells from a single smear) : the case is almost certainly one of malignant disease.

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[3] *Biology of the Blood Cells.* J. Wright & Sons Ltd., Bristol, 1913.
[4] *Jour. Path.*, 1915, April, 494.
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*The subject of differential diagnosis is being reserved in connection with a joint study upon pernicious anæmia with Dr. Telling.

† And according to co-existent bacterial infections.

‡ It may also mean that the cleavage products of the food resemble those of carcinomatous excretion.