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Thesis for the Degree of M.D.

Contributions to the Physiology and Pathology of the Blood

Vol I

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MEMORANDUM.

From the

Dean of the Faculty of Medicine.

To.....

.....

.....

Thesis by Mr Robert Muir

Please circulate as follows:-

Professor Rutherford (read for Report)
" Greenfield
" Simpson
" Swart read for Dr

and return to Dean's Office.

VOL I

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Introduction

The basis of this thesis is an investigation of the changes which the corpuscular elements of the blood undergo in pathological conditions. The subject was proposed to me by Prof. Greenfield as a suitable one for research and my work, under his supervision has extended over two winters. I have investigated the changes not only of the red and white corpuscles of the blood but also of the the blood-plates. My object however was not merely an enumeration of histological changes but a search for facts which would throw some light on the origin and life history of the elements in question. Certain important questions were thus raised, which concerned chiefly the development of the red corpuscles and the possible relation of the blood plates to the same. These questions could only be satisfactorily answered by direct experiment. I accordingly undertook a series of experiments on the changes produced by haemorrhage in the blood and blood-forming organs. These experiments were performed in the Physiological Laboratory of the University under the Superintendence of Prof. Rutherford.

The first part of this thesis is concerned

chiefly with the normal properties of the corpuscular elements of the blood and of the bone marrow, while the second contains the results of clinical observation of and of my experimental work.

Drawings illustrating the subjects in the text are appended at the end of the second part. All these have been done by myself.

Chapter I – Methods

I intend to describe shortly the chief methods which I have employed in examining the blood and the corpuscular elements of the various blood forming organs, viz. Examination of the fresh blood in the juice of the organs without any reagent, examination in various preserving fluids, and the preparation of dried specimens with the methods of staining. All these three methods ought to be employed in concert and the various appearances thereby confirmed in order that error may be avoided. The mode of counting the various elements of the blood especially the blood plates will also be described

In examining the fresh blood everything ought to be done as speedily as possible so as to allow little change in the elements and a magnifying power of 800–1000 diameters is desirable. In this, as in all cases of examination of the blood the skin ought to be thoroughly cleansed, the fat on the surface being removed by a little ether (I find the lobe of the ear to be the most convenient place from which to draw the blood) A small prick having been made, a small drop of blood is caught, as soon as it appears, by a clean cover-glass, is placed on a

Slide

slide and is at once examined. Care must be taken that no undue pressure is exerted on the cover-glass as in that case the white corpuscles become flattened out and their apperent diameter increased. All risk of this may be avoided by drawing on the slide two lines with balsam, which when hardened afford points of support for the cover-glass. The characters of the various elements ~~present~~ may thus be examined, attention being first directed to the blood-plates as they so rapidly undergo change. The sizes of the various elements ought also to be measured, although in the case of the blood-plates this is somewhat unsatisfactory for obvious reasons. The phenomena of coagulation may also be watched and if desired any reagents, such as water, acetic acid &c, may be conveniently applied by a drop of the reagent being placed on the edge of the cover-glass. I believe that after using this method in combination with ~~with~~ actual enumerations for some time, one may be able by examination of the fresh blood to obtain not only a knowledge of the characters of the elements but also an approximate estimation of the relative proportion of the various elements and of the varieties of leucocytes.

A similar method is pursued with regard to the bone marrow, spleen & lymphatic glands. A little of the juice expressed from the tissue is examined in the same way as the blood and with the same precautions. It is sometimes more convenient to dissociate a small portion of the tissue and this is done by placing it on a slide and stirring it perpendicularly with the rounded end of a glass rod.

The following are some of the chief preserving fluids employed – Osmic acid in 1 p.c. watery solution, Hayems fluid, Pfitzner's methyl-violet-salt solution, Ehrlich-Biondi's peptone salt solution. The composition of these is given below. Arnold for examination of the bone-marrow has employed a .6 p.c. Solution of Sod. chlorid. tinted with methyl green to which is added a few drops of a .25 p.c. solution of gold chloride. Litvinoff employs a 1 p.c. Solution of Sod. chlorid with which is mixed a little of Flemmings Chromo-osmic acid Solution. A Solution of Sod. Sulph. (Sg 1023) tinted with methyl violet is also very useful. All these fluids are employed in the same way. In the case of the blood a large drop of the fluid is placed on the skin

[1] Stricker Über die Bildung rother und weisser Blutkörperchen

p. 6

from Sitzungsb. der k. Akad. der Wissensch. zu Wien

Bd 88. 1883

[2] Ehrlich. Archiv f. Anat. u. Physiol. Phys. Abth.

1879. Ss. 166, 571

" Zeitsch. f. klin. Med. Bd I S. 553

and a prick made through the drop, so that the blood at once flows into the fluid. The two are then thoroughly mixed together. In the case of the latter manner a large drop of the fluid is placed on the slide; a little of the juice is added to it and thoroughly mixed with it. For this latter purpose I have made most use of Bujwid's Methyl-violet Salt Solution.

The most satisfactory method of obtaining permanent specimens is to make use of dry films of blood, according to Ehrlich's method, with or without fixing reagents and thereafter to stain. In the case of the blood the method is to take two coverglasses, which must be scrupulously clean, to catch with one a small drop immediately on its appearance after the prick, to lay on the other and spread the drop out ~~flat~~ into a thin layer between the two. The two coverglasses are then separated by sliding the one on the other and the films are dried by a rapid to and fro movement in the air (It is on the whole better not to employ heat in drying the films) Ehrlich recommends that the films ought afterwards to be kept for some hours on a copper plate at a temperature of about 120° C in order that the haemoglobin may

be thoroughly fixed. For most purposes however this is unnecessary especially if the films are rapidly stained in alcoholic solution. Other fixing agents may also be employed eg. osmic acid vapour before or after the film is dried (for half a minute), osmic acid solution, chromic acid Sol. both of 1 p.c. I find a very good method is to float the dried film for about half an hour in Hayem's Solution. For the study of the red corpuscles and the blood-plates as thin a film as possible ought to be made, in order that the red corpuscles may be in one layer and that rapid drying may be ensured. For the leucocytes a thicker layer may be taken as for estimation of the numbers of the different varieties it is desirable to have a good number in the field at once. If the process be properly carried out the red corpuscles retain their shape perfectly and crenation ought not to occur. To obtain the blood plates in as nearly as possible their natural shape a small drop of blood may be allowed to flow into, and mix with, a drop of 1% osmic acid Sol. in the manner described. Dried films are often prepared of the Mixture. It is however

difficult to obtain always the ~~pure~~ proportion of blood to solution which ensures good results A convenient way of making films for the study of the blood-plates is by means of a slide with a hollow cell. A drop of blood is pressed out between the cover-glass and the flat portion of the slide and the cover-glass is then quickly pushed over the hollowed portion and then raised without any sliding. It will be found that the portion of the film which corresponds to the cell is exceedingly thin and contains many blood-plates.

The bone marrow &c may be treated in the same way as the blood. The elements must however be thoroughly dissociated (rubbed) before the film is made and even then the results are often far from satisfactory. I have also tried the method of mixing the marrows with various solutions before making the films but with very indifferent results. I obtained the best specimens by employing the serum of the blood of the same animal as a diluting medium. The juice of lymphatic glands generally forms satisfactory films.

Dried films prepared by any of the above methods may be obtained in a variety of different

ways. The aniline colours are those which, as a rule, give the best results but considerable care is required in their employment as slight variations in the conditions of staining often lead to wide differences in the appearances produced. It is this fact which makes their employment in detecting pathological changes in the elements a problem of great difficulty As a rule more uniform results are obtained with alcohol as a solvent than with water.

The following is a summary of the methods which I have found most convenient and of the results obtained. In all cases the films after being stained are washed in water, dried, and mounted in Canada balsam.

A weak watery solution of methyl or glentian violet is very suitable for staining the characters of the blood plates. The strength which I have employed is about 1:800 and the films are stained for ten to 20 mins. If the films are simply dried and stained in this, the nuclei and the blood plates are stained darkly but the former more of a purplish tint. The protoplasm of the leucocytes is not so deeply stained as the blood plates The red corpuscles are very feebly stained and often only their outlines are faintly visible. If the

V. Pl. 29

film is fixed by floating in Hayem's fluid for half-an-hour the red corpuscles are stained more deeply and all the elements are stained with greater distinctness. If the blood have been mixed with osmic acid before the film is made, the blood plates and red corpuscles are stained darkly and alike. v. Pl 30

2 Stains (Leuc's Saturated) Solution of Gentian or methyl violet in absol. alcohol. Stain dried films for about 20 secs Blood plates are stained deeply like the nuclei of the leucocytes. Red corpuscles are more of a purplish hue.

3 Saturated Solution of methyl blue in absolute alcohol. v. Pl 21
Stain 20-30 secs. Blood plates and nuclei bright blue, red corpuscles pale green - faintly stained. The protoplasm of the leucocytes is stained very faintly of a bluish tint. If osmic acid or Hayem's fluid have been used previous the colour of the red corpuscles is deeper and therefore more distinctly marked

4 Fuchsin (acid) Stain for 20 secs. The nuclei and blood plates are more deeply stained than the red corpuscles 5% alcoholic Sol. v. Pl. 4

5 Amaranth Sat. Sol. in absol. alcohol. Quickly dispenses stained of a yellow colour. This

is very suitable for shewing the forms of the red corpuscles.

The following double stains may also be used

1 Methyl blue and gentian violet — alcoholic solutions as above. Stain first in methyl blue wash and dry; afterwards stain in gentian violet. Stain for half a minute in the former and fifteen seconds in the latter. Nuclei should remain blue whilst the other structures are of a violet colour.

2 Methyl blue and fuchsin (acid). Of the same strengths as in single staining, and staining for the same length of time. The films are stained first in the methyl blue and then in the fuchsin, being washed and dried between the two stainings. The red corpuscles are crimson, the protoplasm of the leucocytes pale pink the blood plates violet and the nuclei of leucocytes blue.

To stain the blood, I most frequently use the watery solution of gentian violet. The red corpuscles stain best in fuchsin or aurantia. For the leucocytes of the blood, for the nucleated red corpuscles and for films of bone marrow &c the alcoholic methyl blue or the double fuchsin - methyl blue stain is by far the most convenient. By the latter stain the nucleated red corpuscles in the bone marrow, can be at once picked out as their perinuclear portion is a deep

2.22.6

[1] Stricker Über die Bildung rothen und weissen Blutkörperchen

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[2] Ehrlich. Archiv f. Anat. u. Physiol. Phys. Abth.

1879. SS. 166, s. 71

" Zeitsch. f. klin. Med. Bd I S. 553

presence of a very large number of large corpuscles
spheroid or oval shape, some of which were also tailed
most of them not. Some of the oval forms = 14μ in long
diameter. The volume of these corpuscles must have
far exceeded that of an ordinary red corpuscle. Also
small forms down to 4μ in diameter though in fewer numbers.
Blood plates were very few, occurs singly in ff the field
and are of small size. The leucocytes are also very few
in number, of small & large varieties.
On Feb 20th fewer irregular forms were present but many
large oval corpuscles.
On March 13th The red corpuscles were very much more regular
in size and shape. A great many of normal shape
had a diameter of 9μ. The blood plates were decidedly
more numerous & the leucocytes slightly also
On April 4th Very few irregular forms were present
most were of perfectly regular shape and well coloured.
They formed roleaux well. Most were 8μ in diameter
but a good number a little more. The blood plates were
rather increased in number. The leucocytes were still
decidedly fewer than normal. At this date there was
comparatively little to be seen to indicate that it had
been a case of pernicious anaemia. I may add that
the haemoglobin was much less diminished p.c. than
the number of the corpuscles

Stained films gave the following facts
On Feb 8. The most noticeable feature was the very small number of multinucleated leucocytes, the number of these being only about a quarter of that of the uninucleated. There were no nucleated red corpuscles to be seen.
On Feb 20, the proportion of uninucleated leucocytes was still very high though less so than before. There were now a considerable number of nucleated red corpuscles. These had not the typical form but were often oval or of slightly irregular shape and possessed one or sometimes more nuclei. The nucleus as a rule did not present the typical reticulated structure v. Plate
On Feb 25th. Nucleated red corpuscles of similar character were seen, though rather fewer on March 13 I could only find one after searching for a long time. On April 9th no nucleated red corpuscles could be found. By this date the proportion of multinucleated forms was quite though still less than normal

Feb 19 Fig 3

Summary. In the general characters of the red corpuscles, blood plates, and leucocytes this case resembled the previous ones. The large number of anisocytic corpuscles, many of them of oval form was a noticeable feature. The presence of nucleated red corpuscles in considerable numbers is of great interest. They were not present at the first examination, but on the two subsequent

occasions they were considerable numbers. They were chiefly of the megaloblastic or pernicious type. They disappeared from the blood during the process of remedy. The increase of the blood plates in the progress of the case is also noteworthy.

Case XIV J. B. aged . Pernicious Anaemia

Had a similar illness before but recovered.

This attack had lasted only a few weeks (according to patient's statement)

This case affords some points of exceptional interest

Mar. 13th Red corpuscles of the utmost irregularity in size and in shape. Megalocytes 9-10 μ in diam. (etc.) were common though not quite so much so as in some of the previous cases. Some of the oval forms measured 16 μ in their long axis. Irregular shapes were of all sizes - large & small. There was no attempt at the formation of rouleaux. The corpuscles were well-coloured. The blood plates were few, occurred singly and few exceeded 2 μ in diameter. The leucocytes were rather few in number - a large proportion were of small size 6-7 μ in diameter.

On Mar. 24 & 27 practically the same characters were seen but on the latter date the blood-plates were more numerous.

On April 8th Fewer irregular red corpuscles were seen and the blood plates were evidently much more numerous

The haemoglobin was less decreased p.c. than the red corpuscles
Dried films on Mar. 9 showed that the mininucleated
& multinucleated corpuscles were well separated from
each other, there being few intermediate forms present.
The latter as a rule contained many nuclei very distinct
from one another. There were no nucleated red corpuscles
On Mar 24th a change was seen (the number of the red
corpuscles had fallen considerably). The mininucleated
leucocytes were in higher proportion than before, multi-
nucleated forms being very few. There were now a
great number of nucleated red corpuscles. There
were almost all of megaloblastic or prikiloblastic type.
In stained specimens the nucleus its typical form. In many
it was single and deeply stained throughout, in some it
was single and of irregular shape and in others single
and stellate or somewhat raniform at its margins. Some
red corpuscles had more than one nucleus and some
contained what appeared to be irregular fragments of
nuclear substance. The peri nuclear portion of these
corpuscles was generally rather more deeply stained than
that the adjoining red corpuscles. Some of the red corpuscles
both of the nucleated and non-nucleated variety con-
tained dark brownish granules. I have not yet
been able to ascertain the exact nature of these but they
did not give the iron reaction with sulphide of

v. Pl 20

Ammonium.

On Mar 29th the same varieties of nucleated red corpuscles were present but they were fewer in number. Corpuscles containing granules were quite as numerous as before

On April 8. A few nucleated red corpuscles could still be found but they were very few in number. All that I saw contained a single nucleus. Corpuscles containing granules were also very few. The proportion of multinucleated leucocytes had increased considerably.

Remarks The most remarkable fact noted was the large number of peculiar nucleated red corpuscles observed on Mar 24 & 29. It is to be noted that these appeared after a marked fall in the number of the red corpuscles. This fall was also associated with a fall in the number of the blood-plates, of the Leucocytes and of the proportion of multinucleated forms. Improvement after so marked a degree of anaemia had been reached is very rare and very striking. With the improvement there was noted a marked increase in the number of the blood plates.

The case is still under observation (April 16th)

A comparison of the preceding four cases will show how closely they agree in the changes both in the red corpuscles in the blood plates and in the Leucocytes.

Table IV Idiopathic Anaemia (c)

	Date	Red Corpuscles	Leucocytes	Blood plates	Corp 1	Corp 2	Corp 3	Poikilocytes
Case 15 J.J. 55 M. Pernicious Anaem	Jan 15	1,206,000	7,000	101,000	1:172	1:12	1:1.8	Numerous
	" 24	1,150,000	3,500	88,000	1:328	1:13	1:1.9	"
Case 16. W.C. aged 6 M. Severe Anaemia with Purpuric Eruption	Dec 25	1,281,000	1,500	33,000	1:805	1:37	1:022	None
Case 17. J.M'K. aged 45 F. Pernicious Anaemia?)	Dec 15	1,720,000	1000	105,000	1:1720	1:16	1:1.5	Numerous
	Jan 21	2,940,000	3500	146000	1:535	1:20	1:1.3	Less numerous
	Jan 23	3,008,000	4,500	144 000	1:668	1:21	—	"
Case XVIII M'P. M aged 36 Pernicious Anaemia (Cured) ?	Jan 17	3,338,000	2,500	402,000	1:1333	1:8.6	1:0.55	Very few
	" 19	3236 000	3,500	380,000	1:925	1:8.5	1:.63	"
Case XIX S.W. aged 55 Pernicious Anaemia (?)	Nov 11	2,623,000	4,500	102,000	1:582	1:25		Very few
	" 27	2,601,000	9,000	212,000	1:289	1:12	1:1.9	"
	Dec 6	2745,000	9500	232,000	1:289	1:11		"
	" 14	2,628,000	7500	152000	1:350	1:17		"
	Jan 16	2,375,000	6000	138000	1:395	1:17	1:2.2	"
Case 20 J.R. aged 18 F. Anaemia (of doubtful nature	Dec 6	1,925,000	2,500	86,000	1:768	1:22		Few
	" 19	1895,000	2,000	74,000	1:947	1:25		"
	" 21	1,954,000	3,000	56,000	1:651	1:35	1:.3	X: " 8
	" 23	2,070,000	3000	104,000	1:690	1:19		"
	" 25	2,122,000	2500	81,000	1:848	1:25		"
	" 29	2,056,000	1500	74,000	1:137	1:27		"
	Jan 16	2,001,000	1000	91,000			1:25	X: 25

Case XV J.J. 55. Simicins Anaemia. The patient was a man who had suffered from the disease for two years and had been in a similar condition for some time before I examined his blood

Jan 15th. First blood examined. The red corpuscles showed the utmost irregularities in shape and varied greatly in size. Many small corpuscles down to 2·5 μ in diameter were found. Most were generally deeply concave on one side, showing a horse-shoe shape on profile record. Many were distinctly over 8 μ in diameter and some of the oval forms measured 13-15 μ in their long axis. This over-size of the corpuscles was a well-marked feature. No intra-red corpuscles were well observed. The blood-plates appeared isolated and were comparatively few. The leucocytes showed the usual varieties; when stained few intermediate forms were seen between the mononuclear & multinuclear corpuscles and the proportion of the latter was slightly decreased. No nucleated red corpuscles were seen.

The chief changes in this case are of the same nature as, but less marked than, in the previous cases.

Case XVI Wm. C. aged 6. This was a case of pernicious anaemia, apparently idiopathic, in a boy. It was associated with general purpuric eruption and had been preceded by symptoms of general weakness and

marked gastric symptoms. He was under observation for only a week and he died shortly after leaving the Hospital. I was able to make only two examinations of the blood which however were entirely conformatory of one another

Dec. 25th. Fresh blood examined. Red corpuscles were practically all of circular shape. They were of normal colour and seemed rather flatter than usual. A few formed aggregations but most lay in small heaps. Their size varied 5-8.5 μ. very many = 6-7 μ. The blood plates were very scanty and of small size; whole fields were seen in which none were present. The leucocytes were very few, ~~two~~ one and most were of small size. Few exceeded 7 μ in diameter and many were less. Fibrin formation was seen to set in very definitely from the groups of blood plates present. When stained the leucocytes were seen to be nearly all of the small mononuclear variety, there being more than four times as numerous as the multinuclear corpuscles. Many of the multinuclear forms were in a transitional state, the typical multinuclear corpuscles being very rare.

Remarks. The condition of the blood plates and of the leucocytes in this case resembled somewhat that in pernicious anaemia, but the characters

of the red corpuscles were different and I may also add that the haemoglobin was rather more diminished than the number of the red corpuscles. There was no other discernable disease present. The nature of the anaemia is accordingly obscure.

Case XVII. J. M'K. aged 45. F. I examined the blood of this patient at two different periods. At the first it had many of the characters of pernicious anaemia, at the second there was marked improvement. She died shortly afterwards of acute obstruction of the bowels due to malignant disease of the colon. Whether this condition was causally connected with the anaemia is very doubtful. The marked anaemia months before and the improvement which took place speak decidedly against such a supposition

Dec. 15th Many of the red corpuscles were of irregular shape, and forms being especially numerous. The variations in size were very great; some were as low as 3 μ in diameter whilst many were 5–10 μ. The blood plates were rather scanty. The leucocytes showed the usual varieties, when stained the multinucleated forms were in excess of the normal proportion

On Jan 21st There were fewer irregular forms. The size of the circular forms = 5–10 μ. Large oval forms

eosinism colours, while their formation from colourless cells of similar structure can be well traced. A great many other combinations of the aniline can be employed. Finally eg. for studying the minute nuclear structure especially of the nucleated red corpuscles, pure dry films by heating according to Ehrlichs method, pure with Flemmings solution and stains with alcoholic solution of Hoffmanns violet and as a double stain afterwards with aurantia. Ehrlichs eosinophile granules are stained by working a glycerine solutions of eosin, pyronin, aurantia indulin &c. In an eosin-indulin glycerine solution the α granules are stained red with eosin the β granules black with the indulin. The basophile granules are stained in the same way with dahlia. The neutrophile or ε-granulations of the ordinary leucocytes are stained in the following solution. To 5 vols of a saturated solution of fuchsin (acid) 1 vol of a watery solution of methyl blue is added and the two are thoroughly mixed together. 5 vols. of distilled water are added, the mixture is allowed to stand for a few days and then filtered.

Method of Enumeration

In counting the different elements of the blood I have employed the Thoma-Zeiss haemacytometer

13-14μ in length were still present. Poikilocytosis imperfectly formed. The blood plates were still scanty though less so than before. The condition of the leucocytes was practically the same. There were no nucleated red corpuscles.

Jan 23. A similar condition was found.
The haemoglobin throughout was less diminished p.c than the number of the red corpuscles.

It is to be noted that here again improvement is again associated with increase in the number of the blood plates

Case XVIII Miss M. aged 36. This was a case of pernicious anaemia in which the number of red corpuscles at one time was considerably under a million per c.mm. When I examined her blood she had almost recovered and she left the Hospital shortly afterwards

Jan. 17th Irregular red corpuscles were still present but they were very few. Most were of perfectly normal shape They varied in size greatly. Few were less than 6μ in diameter but many measured 8-9.5μ. Most were distinctly over-sized but otherwise perfectly normal in appearance. The blood-plates were numerous and many were 3.5μ in diameter. The leucocytes were few, most of them being of the smaller variety

6·5-7μ in diameter. Stained Specimens showed the blood-plates in large numbers and of large size throughout, also that the unnucleated leucocytes considerably exceeded the multinucleated in number.

The haemoglobin was less diminished than the corpuscles.

Jan 19th A similar condition was found.

Remarks. A peculiarity in this case was the presence of a large number of over-sized red corpuscles of normal shape and appearance. A somewhat similar condition, though less marked was seen in Case XIII. The condition of the blood-plates is very different from, while that of the leucocytes remains similar to, the common condition generally found in well marked pernicious anaemia.

Case XIX S. W. aged 55. This was the case of a man who two years ago had shown all the symptoms of pernicious anaemia. I examined his blood on several occasions and found always the red corpuscles about half their usual number. The following are the details

On Nov. 11th a few irregular forms of red corpuscles were seen but they were very few and the irregularities were not well marked. They varied considerably in size - 6·5-9μ. Most were of normal appearance. They formed rouleaux well. The blood plates presented the

usual appearances and were rather scanty. The Leucocytes shewed nothing abnormal

On Nov^{r} 27. The red corpuscles had almost the same characters, though irregular red corpuscles were still fewer in number. The blood plates were more numerous

Subsequent examinations on Dec^{r} 6, 14 & Jan 16 shewed a practically unaltered condition, ~~the characters of the blood~~

On Nov^{r} 24th The characters of the Leucocytes were practically normal, the usual varieties being present in the usual proportions The same state was seen on Jan 16th. I may add that the haemoglobin was proportionately less diminished than the number of the corpuscles.

In this case, with the exception of the persistence of a diminished number of corpuscles and the survival of a very few irregular corpuscles, the characters of the blood had practically returned to normal. The patient did not look at all anaemic and was fairing strong.

Case XX J.R. aged 18. The patient was a girl who had suffered for some time from increasing pallor and weakness. The case was an obscure one. There was very marked uniform enlargement of the Spleen resembling the condition in Leucocythaemia but so far from the number of Leucocytes being increased it was markedly diminished. The haemoglobin was de

decreased in the same proportion as the number of the corpuscles. I examined the blood on several occasions but always with a similar result. The red corpuscles shewed a great number of typically irregular forms but none of these reached the large size met with in pernicious anaemia. A great number of circular corpuscles = 6-7 μ in diameter. They were fairly well coloured and formed nucleus fairly well. The blood plates were always decreased in number but not more so proportionately than the red corpuscles. The leucocytes were very few in number indeed and very few of the large type could be found. In stained films the red corpuscles had a great tendency to appear in the crenated form. The most striking feature was the great paucity of leucocytes and the very marked proportionate decrease in the proportion of multinucleated forms - a decrease equalled only in Case XVI - there being only a quarter as numerous as the mononuclear corpuscles. The multinucleated corpuscles present shewed the usual characters.

The nature of this case was very obscure. The diminution in the number of leucocytes was very marked and very scanty. It might probably be explained by the condition of the spleen but that was quite unknown. Recently the patient has been improving somewhat

From a consideration of the above cases, especially the first four in which the anaemia was most marked, the following would seem to be the chief changes in pernicious anaemia.

(1) Poikilocytes are generally present in large numbers. Their number however is not always in proportion to the degree of the anaemia v. case X. Many of them are in the form of large oval corpuscles reaching 16 μ sometimes in their long diameter. Variations in size are very abundant, the extremes may be said to be 3 μ & 11.5 μ (v.e. of the circular forms. Megalocytes are generally present in large numbers.

The corpuscles are well coloured and the haemoglobin is less diminished proportionately than the number of corpuscles

(2) Nucleated red corpuscles may be present. These, as Hayem says, are often either unusually or irregular shape. The nuclei vary from the characters of the nuclei of typical nucleated red corpuscles (eg. of the adult human mature). The nuclei may be single or they may be fissured. They have generally a homogeneous appearance and sometimes appear to be in the form of fragments.

I have scarcely examined a sufficient number of cases to speak as to their significance. In case X immediately before the death of the patient none were found, while in cases XIII & XIV in which they were most nu-

morons, their appearance was followed by considerable improvement. In the latter of these two cases they appeared after a marked fall in the number of the corpuscles. I am accordingly inclined to think that they indicate an increased formation within the bone marrow with passage of nucleated forms into the blood as in traumatic anaemia. Their alterations from the normal type are probably caused in the same way as those of the ordinary red corpuscles.

(3) The blood plates are invariably markedly diminished in number (This corresponds with the remarks of Rieder, Eichhorst, Osler Hanot & Igny). They are also generally of small size.

(4) The leucocytes are diminished in number and the mononuclear are generally more numerous than the multinuclear. The tall corpuscles of the latter class are generally well developed and often have an increased number of nuclei. The two classes are very distinct from one another and there are few intermediate forms.

If improvement takes place the abnormalities of the red corpuscles disappear. I have always found that the blood plates increase in number often more proportionately than the red corpuscles. The condition the leucocytes may remain (as in case XVIII)

Table F – Chlorosis & Allied Conditions (a)

	Date	Red Corpuscles	Leucocytes	Blood-plates	Prep 1	Prep 2	Prep 3	Poikilocytes
Case 21 C.M.Z aged 31 F. Severe headaches & vomiting	Dec 4	4,160,000	6000	356,000	1:693	1:11.8	1:1	Numerous
	" 17	4,075,000	7000	287,500	1:582	1:14.2	1:1.3	"
	" 29	4,380,000	8000	308,000	1:547	1:21	—	Slightly diminished
Case 22. J.M aged 25. F. Chlorosis (?)	Nov 14	3,810,000	—	—	1:995		1:1.2	Numerous
	" 24	4,480,000	4500	abnormal	1:995		—	"
	Dec 5	4,400,000	5000	326,000	1:880	1:13	1:1.7	Slightly decreasing
	" 17	4,341,000	8000	232,000	1:543	1:19	—	Still numerous
Case 23 J.M. aged 46 F	Nov 8	4,146,000	9000	280,000	1:460	1:15	1:2.4	many
Case 24 E.W aged 24 F Chlorosis	Jan 31	4,233,000	7000	598,000	1:604	1:7		Numerous
	Feb 9	4,600,000	7000	372,000	1:657	1:12	1:.89	"
	" 20	4501000	5500	358,000	1:820	1:13	1:.83	"
	Mar 3	4,440,000	4000	422,000	1:1110	1:10	1:.63	"
	" 13	4,555,000	3500	368,000	1:1587	1:12	1:.6	A few
Case 25 J.C aged 25 F Chlorosis	Mar 1	3,537,000	3500	470,000	1:1010	1:7.5	1:.66	many
	" 8	3,970,000	5000	476,000	1:794	1:8.3	1:.7	"
	" 13	4,070,000	2500	416,000	1:1628	1:9.5	1:.74	"
Case 26 M.C. aged 26. F Chlorosis	Jan 26	2,967,000	4000	328,000	1:742	1:9	1:.75	Few
	" 31	3,200,000	4000	352,000	1:800	1:9.1	—	"

or many disappear. Cases of anaemia of indefinite nature have generally alterations in the blood of less definite nature than the above.

Chlorosis and Allied Conditions

I have included in this class, in addition to cases of ordinary Chlorosis, others of less definite nature, in some of which the chief alteration being marked irregularity in the shape of the red corpuscles with very slight diminution in their number. Some of the first mentioned cases are of this nature

Case XXI C. M^c L. aged 31. The patient was a woman whose chief complaints were severe headaches with attacks of nausea & vomiting. There were no physical signs of organic disease.

Dec 4 The red corpuscles shewed marked irregularities in shape. There were of the usual peculiar forms. The size of the corpuscles = 4·9·5 μ. but most were of normal size & formed Rouleaux well. There was the coexistence of perfectly typical poikilocytes with the mapping of the corpuscles of normal shape & size. The blood plates & leucocytes shewed little alteration.

On Dec. 17 1899 the blood was found to be little altered. On the latter date the number of poikilocytes had diminished a little.

The chief point in this case is the association of

marked poikilocytosis with very slight diminution in the number of the red corpuscles. Its association with gastric symptoms is also noteworthy

Case XXII J. M. 25. ♂. This was a case somewhat similar to the previous, only the features of chlorosis were more marked.

On Nov. 14th the red corpuscles showed a considerable number of irregular forms. There were of the same character as, but less numerous than, in the previous case. A great number of the corpuscles were 6-7μ in diameter.

The number of the blood plates was slightly increased and a good many 3μ in diameter. The leucocytes were few especially those of the lymphocytic.

On Dec. 13th 5 the red corpuscles showed practically the same characters though their number had increased considerably. The proportion of mononuclear leucocytes was a little less than normal

Dec. 17th A similar condition was present.

We have here again the presence of a considerable number of poikilocytes even when the number of red corpuscles was normal. There were also well-marked gastric symptoms.

Case XXIII J. M. aged 46. The patient was a woman admitted with swelling and pallor of face and general dry eczema. The disease was discovered beyond the

eczema

The only point of interest found on examination of the blood was the presence of a considerable number of irregular corpuscles. Many of the ordinary corpuscles were 7μ in diameter but small forms down to 3μ were also found. The number of the corpuscles was little decreased. There were a great number of coarsely granular coloured corpuscles in the blood. Otherwise the leucocytes and also the blood plates showed no alteration.

Case XXIV E. W. aged 24. Patient suffered from great weakness, but her face was ruddy and she appeared very healthy. On admission the haemoglobin was 40% on leaving 65%. It will be seen from the table that the number of red corpuscles was practically normal.

Jan 31st The post blood showed marked alterations. Poikilocytes were not very numerous but those present were of very irregular shape. Great irregularities in size. Small corpuscles down to 4μ numerous but few above 5μ. The corpuscles formed rouleaux fairly well. The blood-plates were greatly increased in number but of normal size. The leucocytes were rather few - most were 7μ in diameter. When stained it was found that the mononuclear corpuscles were the more numerous and that there were few intermediate forms between the two varieties. Subsequent exams. gave similar results. On Mar 3rd 23 the poikilocytes were less numerous. Her general condition was also much better.

Case XXV J.b. 25 F. Chlorosis. Amenorrhea and weakness for eight months. On admission the Hb. was 45% afterwards it increased to 58%

March 1st Exam. of post blood. There were many irregular forms amongst the red corpuscles and there was great variety. Some of the poikilocytes were very small and delicate. Great variations in size - many = 6-7μ very few were macrocytic. The blood plates were very numerous whilst the leucocytes were few - the greater number being 7μ in diameter. When stained, the multinucleated leucocytes were seen to be fewer than the uninucleated. The latter were as a rule of small size.

March 6th A similar condition was found, Megalocytes were however rather more numerous.

March 13th Still many poikilocytes though there was considerable increase in the number of the red corpuscles.

These two cases had the following points in common

(1) Diminution in Hb. Poikilocytosis well marked. Considerable variations in size but few megalocytes.

(2) Number of blood-plates much increased

(3) Number of leucocytes diminished. Most were of the small uninucleated variety.

In the former case there was practically no diminution in the number of the red corpuscles, in the latter slight.

and have found it extremely convenient. The divided surface contains one a definite number of small squares and is further divided by extra lines into larger squares each of which contains sixteen smaller squares. Including those bisected or quartered by the extra lines, there are altogether 400 small squares (= 100 squares in Gowers's instrument). In the case of the leucocytes all those are counted and unless they are increased in number I generally repeat the counting with another drop of the mixture. The red corpuscles I have counted in the usual way, but if their number fall below two millions per c.mm. a hundred or more squares are counted, as the case may be. In the case of the blood plates I have counted all those in the whole divided area viz. in the 400 small squares. With practice one can do this with considerable rapidity. If they be aggregated into large heaps the enumeration must be repeated. Several points must be carefully attended to in the process. The drop of blood ought to appear on the skin quickly after the prick is made (there the fan offers an advantage) and ought to have nearly a spherical form. The blood ought to be quickly drawn up in the tube to the proper level and the preserving fluid immediately drawn

(over)

Table VI - Chlorosis and Allied Conditions (C)

	Date	Red Corpuscles	Leucocytes	Blood-plates	Prep 1	Prep 2	Prep 3	Poikilocytes
Case 27 M.J. aged 20 ♀ Chlorosis	Mar 22	2,547,000	2,000	412000	1:1273	1:6·2	1:·8	Few
	" 29	3,013,000	2,000	453000	1:1506	1:6·6	1:1·01	"
Case 28 J.C. aged 18 ♀ Chlorosis	Mar 26	2,641,000	5,000	304000	1:508	1:8·6	1:·72	Fairly numerous
	" 31	2,827,000	3,500	324,000	1:877	1:8·7	1:·77	
Case 29. C.M. aged 16 ♀ Chlorosis	Feb 3	3,647,000	3,500	396,000				A few
	" 12	3,900,000	3,500	439,000			1:·9	"
Case 30 J.C aged 20 ♀ Chlorosis	Feb 27	3,520,000	4500	410,000	1:782	1:8·5	1:1·1	Very few
Case 31. M.Y. aged 21 ♀ Chlorosis	" Dec 23	2,188,000	4,000	244,000	1:547	1:8·8		Numerous
	"							
Case 32. J.R. aged 16 ♀ Anaemia with Gastric Symptoms	Mar 11	3,354,000	6500	407,000	1:516	1:8·2	1:1·3	Fairly numerous
	" 22	3,455,000	7000	304000	1:565	1:13	1:1·3	Few
Case 33 J.G aged 17. ♀ Chlorosis	Mar 8	2,470,000	6500	254000	1:380	1:9·7	1:1·3	Numerous
	" 15	3,020,000	6000	332,000	1:503	1:9	1:1·2	"
	" 22	3,510,000	5500	464000	1:638	1:7·5	1:1·4	Fairly numerous
	" 27	4041,000	5500	452000	1:734	1:9	1:1·4	Few
Case 34 H.J. aged 23 ♀. Post-partum Anaemia	Jan 15	3,636,000	10,000	367,200	1:363	1:9·9	1:1·6	Fairly numerous
	" 24	3,841,000	7,000	408,000	1:549	1:9·4	1:2	Few
	Feb 7	4,620,000	9,000	324,000	1:513	1:14·2	1:2·3	Very few

Cases XXVI - XXX All these are cases of ordinary chlorosis, anaemia being present in various degrees In all the red corpuscles were pale and showed variations in size - most being undersized Poikilocytes were always present as indicated by the table.

The blood plates in all were increased in number and in some cases in size also The leucocytes in all were few and in almost all the mononuclear variety was the more numerous

Case XXXI M. G. 21 ♀. Chlorosis

M. G. Anaemia was very marked. Red corpuscles 4.5 - 9 μ. many = 6-7 μ. Numerous poikilocytes of the usual description. The blood plates in this case were not actually increased in number. It would have been interesting to watch if their number increased during recovery (which was a rapid one) Unfortunately I was unable to follow the case

Case XXXII J. H. 16 ♀. Anaemia with gastric symptoms. Red corpuscles 6-9 μ, a good number being of oval shape Poikilocytes were fairly numerous. Blood plates though much increased in number were of usual size. The leucocytes both as regards number and the proportion of the different forms were less altered than in the preceding cases

Case XXXIII J. G. 17. Chlorosis. I was able to watch the progress of this case from the time of admission

till recovery was well advanced

Mar 8. Red corpuscles 4·9·5 M, but those over-sized are oval in shape as a rule. Many corpuscles 5-6 M

~~The leucocyt~~ ~~The blood plates~~ Poikilocytes were fairly common. The blood-plates were very numerous. The leucocytes showed the usual characters.

Specimens on Mar 15, 22, 27 showed that as recovery took place there were fewer irregular corpuscles present in the blood and they were of more uniform size. A very noticeable feature is that the number of blood-plates which at first was nearly normal, lately became much increased - nearly doubled.

Case XXXIV H. J. 23. ♀. Post-partum anaemia.

The anaemia occurred apparently spontaneously. There was no haemorrhage at the time of delivery.

Jan 15. Red corpuscles showed great inequality in size and in shape Size 3-9 M. Poikilocytes of ordinary and of small size. Blood plates were numerous but of usual size. Leucocytes were of normal number and of the usual varieties.

On Jan 25 poikilocytes were fewer, and on Feb 7 they could only be seen after careful search.

Through the process of recovery was noted no intermediate forms between the blood plates and the smallest red corpuscles could be found

Summary of cases of Chlorosis.

(1) Red corpuscles. Irregularities of shape appear very frequently. They were present in all my cases and were sometimes very numerous. They appear much earlier and more constantly than in secondary anaemia. (Sahli) The poikilocytosis does not vary in degree with the diminution of the number of the red corpuscles; it is sometimes very well marked when the number of the red corpuscles is little reduced. Variations in size are always present and the smaller forms are specially abundant. Megalocytes also occur but are not nearly so numerous as in pernicious anaemia.

(These results - both with regard to the poikilocytosis and the size of the corpuscles - agree closely with those of Grawitz)

(2) The number of the blood-plates is generally markedly increased though not in proportion to the degree of anaemia. In one case they were little increased when the anaemia was greatest but increased greatly during the process of recovery. I cannot however state this to be the rule in such cases.

Their size is sometimes increased, so that forms are found 3·5 μ in diameter, sometimes not.

(3) The leucocytes are generally deficient in number, sometimes markedly so. Even more striking is the marked diminution in the proportion of multi- to mu-

(nuclear

Table VII Secondary Anaemia

	Date	Red Corpuscles	Leucocytes	Blood-plates	Prep 1	Prep 2	Prep 3	Poikilocytes
Case 35 J.B. aged 50. M. Malignant Disease(?)	Aug 10	1,048,000	33,000	346000			1:4·3	Few
Case 36 J.H aged 52. M. Pyloric Cancer	Feb 2	1,332,500	8,500	408000	1:457	1:3·2	1:3·3	Practically none
Case 37 A.M. aged 55 F Cancer of body of stomach	Feb 29	1,775,000	7,500	388,000	1:236	1:5·2	1:3·5	Numerous
	Mar 3	1,765,000	6,000	332,000	1:290	1:5·3	1:3·5	"
	" 14	1,680000	8,000	282,000	1:210	1:5·9	1:4·1	"
Case 38 J Cancer of Stomach & Kidney	Jan 23	2,610,000	18,000	528,000			1:5	Numerous
	Feb 20	1,960,000	13000	550000			1:5·2	"
Case 39. Mrs H Aged 35	Mar 12	2,775,000	11,000	442000	1:231	1:6·2	1:3·3	A few
	" 22	2,514,000	11,500	448,000	1:218	1:5·6	1:3·1	Numerous
Case 40 J.B. M.H. aged 49 Cancer of Liver	Feb 4	3,301,000	21,000	416000	1:157	1:7·6	1:3·5	None
	" 13	3,158,000	17,000	341,000	1:185	1:9·2	1:3·7	"
	" 18	3,040,000	15000	383,000	1:184	1:7·9	1:4	Very few
Case 41 J.B. 62 M. Malignant Disease	Dec 6	3245000	9000	312000			1:3	A few
Case 42 J.B. 487 Cancer of Liver	Mai 21	3,525,000	10,000	322,000	1:352	1:11	1:4	None
	28	2,553,000	9500	426,000	1:310	1:6·9	1:3·9	"
Case 43 R.W. 50 M. Cancer of Omentum	Feb 28	4,170,000	7000	212000	1:564	1:19	1:3·1	None

corpuscles. In about 50 p.c. of my cases this proportion is below 1:1 whilst in almost all it is below 1·5:1.

4 In none of my cases did I see any nucleated red corpuscles.

4 Secondary Anaemia

This class includes cases of anaemia associated with & caused by some other disease such as cancer, lead poisoning &c. Strictly the list ought strictly to contain several cases of cachexia due to phthisis &c given below. The following however suffice to shew the main features of this variety of anaemia. Most of the cases given are cases of cancer.

June XXV J. P. aged 50 This was a somewhat obscure case. There was profound anaemia with marked cachexia. Later there was haemorrhage from the bowel. Probably there was some abdominal malignant disease. There was no section.

Aug. 10th The red corpuscles varied greatly in size 4-8·5 μ. They were pale and formed rouleaux imperfectly. Many showed slight irregularities but true poikilocytes were rare. The blood-plates were in enormous numbers being almost half as numerous as the red corpuscles. They quickly broke down & formed heaps. The fibrin formation took place early and was dense. The leucocytes were very numerous especially those of the large finely-granular variety. When stained the multinuclear leucocytes were

formed to be in much larger proportion than usual. The ~~nuclear~~ nuclear arrangement showed the most varied forms but in few were the nuclei separate and distinct from one another. The number of small unnucleated corpuscles was small.

The blood plates formed large flocks, some of which are figured in Plate

Case XXXVI Cancer of Pylorus. J. H. 52. M. In this case cachexia and anaemia were extreme

Feb 2. Blood corpuscles were of various sizes 3-9.5 μ but the extremes were rare. Very many = 6-7.5 μ. A few slightly irregular forms were present. The corpuscles were very pale, many of them were folded and they showed a tendency to early crenation. A few formed rouleaux but most lay irregularly in heaps. The blood-plates were in very large numbers, occupying a considerable proportion of the field. The leucocytes were rather numerous and the large forms were more abundant than usual. In stained specimens the usual two varieties were seen. The multinucleated were rather increased in proportion, their nuclei had a somewhat granular appearance.

Along with the usual changes in cachexia, it is to be noted in this case that poikilocytes were practically absent though the number of red corpuscles exceeded

but slightly a million per c.m.m.

Case XXXVII A.M. 55. ♂. Cancer of the body of stomach.

Cachexia in this case was extreme

Feb 29. Red corpuscles were greatly altered in size and appearance. Most = 6·7 μ in diameter, also small forms down to 3 μ in diameter were seen. Some as large as 9 μ were seen but these were rare. Poikilocytes were numerous and of smaller size than they generally are. The corpuscles were pale and transparent. The blood-plates were very numerous and some as large as 4 μ in diameter were seen. The white corpuscles were increased in number especially those 8–9·5 μ in diameter. When stained the proportion of the multi-nucleated corpuscles was seen to be increased. They showed the usual varieties but the nuclei had a somewhat granular appearance as in the previous case. On Mar 3, 18 similar conditions were found the poikilocytes being even more numerous.

This case shows the changes of the blood in cachexia in a marked degree. Though poikilocytes were numerous the red corpuscles both in size and colour differed markedly from those found in pernicious anaemia.

Case XXXVIII ♂. aged Cancer of stomach & kidney (?)

Anaemia was well marked but there was comparatively little cachexia.

Jan 23. Red corpuscles were pale and did not form
rouleaux well. A good number were undersized 6-7 μ.
A considerable number of poikilocytes were present.
The blood-plates were exceedingly numerous and many
were 3 μ in diameter. The leucocytes were increased
in number especially those of the larger size. When stained
it was found that the proportion of multinucleated
forms was very high and many of the mononucleated cor-
puscles were of the larger size; also intermediate forms
were fairly numerous.
On Feb. 20 The anaemia was more marked and the changes
in the red corpuscles were more pronounced. Other con-
ditions were practically the same.
In many all its features this case corresponds with the
type of secondary anaemia.
Case ~~XXIX~~ Mrs H. 35. Cancer of liver, secondary
to cancer of stomach. Marked primordial cachexia
Most of the red corpuscles = 5-6 μ. A few up to 9 μ but
these were very rare. Poikilocytes were present but
not very numerous. The blood plates were numerous, of
small size and rapidly broke down. Leucocytes were
increased in number but beyond an increase in the pro-
portion of multinucleated forms there was no other change.
Jan 22. Condition the same. Poikilocytes rather more numerous.
This case shows the features of the blood when cachexia is

well-marked though not extreme.

Case XLI J.W. M.H. aged 49 Cancer of Liver.

Feb 4th Red corpuscles looke pale. Many = 6·75 μ in diameter. Very few exceeded 8 μ. A very few slight irregularities in shape were seen. The corpuscles formed rouleaux well but there was a tendency to early crenation. The blood-plates were very numerous and shewed great variation in size. The leucocytes were young all of the large finely granular variety. Correspondingly in stained specimens the proportion of multinucleated forms was very high.

Feb. 18 Red corpuscles shewed even more variation in size some as small as 4 μ in diameter were present.

The blood was evidently at that stage in advancing Cachexia where there are variations in the size of the corpuscles and where irregular forms are beginning to appear.

Case XLII A.C. Aged 62 F. Malignant abdominal disease (?)

There was present slight Cachexia with pallor of the face.

Dec 6th The red corpuscles were pale and shewed slight variations in size with a few irregularities in form.

The blood plates were increased in number. The leucocytes were little altered. There was in stained specimens scarcely any of the small uninucleated corpuscles to be seen.

Case XLII J.B. 46 F. Cancer of Liver

Shewing the same changes as in the previous Case

Table VIII Secondary Anaemia cont'd.

Malarial Anaemia

Miscellaneous

	Date	Red Corpuscles	Leucocytes	Blood-plate	Corp 1	Corp 2	Corp 3	Corpuscles
Case 44 J.D. Anaemia due to lead poisoning	Feb 1	2,500,000	15,000	240,000	1 : 166	1 : 8·6	1 : 3·6	None
	7	2,560,000	15,000	254,000	1 : 170	1 : 10	1 : 4·6	None
Case 45 J.J. 51M Chronic Bright's Disease	Feb 5	3,341,000	15,000	267,000	1 : 176	1 : 12	1 : 4·1	None
Case 46 J.F. 45M Malarial anaemia	Feb 7	1,165,000	5500	125200	1 : 211	1 : 7	1 : 2·5	Practically None v. descriptus.
	14	1,213,000	7500	123,000	1 : 161	1 : 9·8	1 : 2·8	
	21	1,223,000	6000	127,000	1 : 204	1 : 9·6	1 : 1·5	
	28	2,135,000	7000	224,000	1 : 305	1 : 9·5	1 : 2	
	Mar 7	2,215,000	8500	159,000	1 : 260	1 : 13	1 : 1·9	
	19	2,825,000	10,000	348,000	1 : 282	1 : 8	1 : 2·1	
Case 47 J.G. 27M Malarial Anaemia (?)	Jan 10	3585 000	11,000	332000	1 : 3			
	23	3634,000	11,000	312,000	1 : 2·7			
Case 48 A.J. 46M. Antic Disease	Mar 7	3,325,000	5500	288,000	1 : 604	1 : 11	1 : 2·5	None
Case 49 A.B. 60M Antic Disease	Mar 7	4,218,000	8000	316,000	1 : 529	1 : 13	1 : 1·6	None
Case 50 A.H. 39M Syphilitic Disease (?)	Jan 8	1,765,000	7500	152,000	[illegible]		1 : 1	Fairly numerous
	14	1,735,000	7000	226,000	[illegible]		1 : 1·4	
	25	1,970,000	7000	201,000	[illegible]			
	Feb 8	1,937,000	5000	242,000				

The red corpuscles were of quite regular shape. A good few = 6-7 μ in diameter. They formed rouleaux well. The blood plates were very numerous and of the usual characters The leucocytes were little altered.

XLIV

Case R. 10 50 M. Cancer of Ileo-Colon. Through the growth was of large size there was practically no change in the blood except a slight diminution in the number of red corpuscles

Case XLIV J. D. 50. M. Chronic Bright's disease due to lead poisoning. Death from uraemia. No Section.

Feb 1. Most of the Red corpuscles were of normal size and all were of fairly normal shape. A few small forms but no true poikilocytes Many fair sized 8 μ in diameter. A good number were undersized and had a tendency to become folded The blood plates and leucocytes were increased in number & the proportion of multinuclear leucocytes was also increased

XLIV J. J. 51 M. Chronic Bright's disease due (?) to lead poisoning.

Feb. 5 The condition closely resembled that of the previous case Red corpuscles were of regular form - many 6-7.5 μ. The increase in the Leucocytes was more marked than in the blood-plates.

Summary of Changes in Secondary Anaemia

(1) The reduction in the number of red Corpuscles is associated with variations in the size; 3 μ & 9 μ may be given as extremes. Most are 6-7.5 μ. Megalocytes are rarer than the small forms. Poikilocytes may be present and be sometimes

very numerous. The number of corpuscles may be greatly reduced without any being present 5.(1332,500 cmm 36) On the other hand they may appear when the number is about 2,500,000. I believe that they rarely appear till the number has fallen below 2,000,000 (In this respect there is a contrast to the condition in chlorosis). They are of smaller size than in pernicious anaemia. Determination of the corpuscles is also seen in their paleness, increased softness sometimes a tendency to fatty degeneration

In a well marked case of secondary anaemia the red corpuscles are generally pale and somewhat delicate looking, and assigned with many pinkish cells

(2) In only one case did I see any nucleated red corpuscles and in that case there was hemorrhage as a complication.

(3.) The blood plates are increased in number. Sometimes forms of larger size are seen, generally not

(4.) The leucocytes are generally more or less increased in number. Sometimes they shew a tendency to break down and when stained their nuclei often have a granular appearance. Sometimes however this is not formed.

The proportion of multinucleated corpuscles is generally decidedly increased. This statement is in opposition to the results of Ehrlich who found in such cases a diminution in the number of multinucleated forms which he explained by a failure on the part of the leuc-

Nuclei

after it. (The method of mixing the blood with the solution employed with Gowers's instrument is unsuited for the enumeration of the blood plates) The first drop of blood which appears ought always to be used, for, as Hayem says in the subsequent drops the blood plates have a much greater tendency to be aggregated together. Any of the fluids mentioned above may be used but I think A[mmonium] S[ulphate] solution or the Sulphate of Soda solution tinted with methyl violet the most convenient, as in them the blood-plates and the leucocytes are distinctly tinted. The blood-plates take a long time to settle to the bottom of the cell than the other elements, and for this reason about ten minutes ought to elapse before their enumeration is begun.

A[mmonium] S[ulphate] in his tables always gives the number of poikilocytes. I have found it however extremely difficult to draw the line (and say what is poikilocyte and what is not. I also believe that when there is poikilocytosis irregularities are produced in addition by the fluid employed. I have accordingly simply said whether the irregular forms were few, many &c.

In any one acquainted with the great adhesiveness of the blood-plates (v. Sub) the above method of counting will appear to possess the fallacy that some of them may adhere to the tube. This I do not

corpuscles to develope into higher forms due to impaired nutrition. All my specimens however show the alteration as I have stated it and in some the change is very marked.

Malarial Anaemia

Case XLVI J. F. Malarial anaemia aged 45. The blood suffered frequently from ague but about four months had elapsed since his last attack

Feb. 7th Red corpuscles showed great variation in size 4-9.5 μ Some were slightly oval and some were large & flat-looking The most striking feature was the large number of corpuscles folded into various forms. Many of these were cup-shaped with a diameter of 6-7 μ. No true pinkish cycles could be found, all the ring-like bodies being apparently due to foldings. The blood plates were rather scanty - of usual size. The leucocytes showed 6-9.5 μ in diameter - There were a great many coarsely granular forms present. When stained the usual varieties were seen in their usual proportion. There were also large corpuscles with broad nuclei and stained of a purplish tint. Also pale bluish forms corresponding with dark purple granules, corresponding to the coarsely granular corpuscles. The protoplasm of some of the leucocytes had a greenish tinge when stained with methyl-blue. I do not know the significance of this

On Feb. 14, 21 practically the same conditions were found

on Jul 26. There was considerable increase in the number of the red corpuscles and also of the blood plates. Red corpuscles were nearly all circular but some were of a diameter of a little more. Many were folded. Coarsely granular corpuscles were present as before, not quite so numerous (erms)

Mon 19th. Great improvement. Red corpuscles still showed variations in size 6.8-5μ & with a tendency to be folded. The blood plates were much increased in number and now exceeded the normal number

The chief changes were

(1) In the red corpuscles. Considerable variation in size but no true poikilocytes were present. The corpuscles had a peculiar tendency to become folded.

(2) The blood plates were diminished at first but during the process of recovery increased more rapidly than the red corpuscles.

(3) The leucocytes were slightly increased in number. There were many coarsely granular corpuscles present but few granules of pigment were seen.

I have had the opportunity of examining blood films from other cases of severe malarial anaemia (sent from America) and in them the number of the blood plates has always been exceedingly small. In some also the leucocytes were exceedingly scanty & some contained pigment granules. The statement holds regarding

4

On the Homeostasis of Melanin p. 7. Reprint from the Brit Med Journ. Mar. 13th 1867

& the blood-plates is confirmatory of what Osler has found.

Case XLVII J. G. aged 27 M. The patient had had malarial fever and his spleen was much enlarged. There was also some thickening in the abdomen below the spleen. It was doubtful if it were a simple case of malarial anaemia.

Jan 10th Red corpuscles were pale but formed Ambeans fairly well. A good few poikilocytes were present. Many of the corpuscles = 6-7 μ in diameter. The blood-plates were increased in number The leucocytes were slightly increased The proportion of multinuclear leucocytes was somewhat high and there were many intermediate forms. Small mononuclear corpuscles were few. The characters of the blood accordingly resemble that the condition in slight Cachexia.

Cases XLVIII + XLIX Acute disease The number of the red corpuscles was considerably decreased. There were a good number of diameter 6·5-7·5 μ. No poikilocytes. The blood plates were slightly increased, the leucocytes were practically unaltered

Case * L. A. H. aged 39. The case was somewhat obscure. There were enlargement of the Spleen and enlargement of the liver somewhat nodular in character. There was marked anaemia. The case was probably of Syphilitic origin

Table IX Leucocythaemia

Case

Case 51 Mrs R. 35
Leucocythaemia

Date	Red corpuscles	Leucocytes	Plaques	Prep 1	Prep 2	Prep 3	Poikilocytes
Dec 12	3,190,000	161000	1,116,000	1 : 19	1 : 2·8	1 : 1·12	a Few
20	3,280,000	152 000	1,210,000	1 : 21	1 : 2·9	v. decrepl-lum	do
Jan 20	2,774,000	137,000	—	1 : 20	—		humerous
Case 52 Leucocythaemia G.R. aged 10 M.							
Dec 30	2564,000	93,000	194,000	1 : 27	1 : 13	v decrepl	Very few
Jan 3	2,245,000	112,000	252,000	1 : 20	1 : 9·0		"
7	2,260,000	229,000	230,000	1 : 9·8	1 : 98		"
12	2,145,000	221,000	241,000	1 : 9·5	1 : 9·0		"
14	2,170,000	226000	222,000	1 : 9·6	1 : 9·8		"
17	2,085,000	209,000	192,000	1 : 9·9	1 : 10		"
23	2,031,000	219 000	~~72,000~~	1 : 9·3	1 :		"
27	1,992,000	24,000	72,000	1 : 9·1	1 : 27		Q few
30	1,686,000	214,000	19,000	1 : 7·8	1 : 88		"
Feb 3	1,181,000	222,000	7,000	1 : 5·31	1 : 168		"
6	1,150,000	342,000	19,000	1 : 3·3	1 : 68		"
9	902,500	379,000	13,000	1 : 2·4	1 : 69		"
12	~~721,250~~	283,000	7000	1 : 2·5	1 : 103		"

Jan 1. The blood was very pale & watery – a quick bleeding for some time. On microscop. exam the red corpuscles were very pale and adhered in irregular masses. There was great variation in their size but most were undersized. There were many irregularities of shape some of these being very well marked. The blood plates were fairly numerous & some retained their shape better than usual. The Leucocytes were proportionately as the norm they shewed all varieties. – Coarse granular & fine granular corpuscles &c. When steam'd the two varieties were seen to be in equal proportions – the multinuclear as a rule had few and large nuclei and a distinct nucleolus was generally visible, from which often fine threads could be seen radiating to the margin of the nucleus.

Exams on Jan. 8, 14, 25 & Feb 8 shewed similar characters. The red corpuscles increased in number a little under treatment but afterwards diminished slightly.

Leucocythaemia & Lymphadenoma

Of the former disease I have had two cases, which present peculiar features. In the changes observed these two differ considerably from one another.

Case I. Wm R. aged 55 Leucocythaemia

The increase in the number of the Leucocytes was well marked

Lent was not excessive. There was great enlargement of the Spleen. No affection of lymphatic glands.

Dec 21. The red corpuscles showed great variations in size = 3-9 μ most = 6-8 μ. Also a few irregular & oval forms were seen. The blood-plates formed enormous flocks but changed their shape very rapidly. The leucocytes under a great number. They could be roughly divided into the following three classes (1) Those about 7μ and a little above. These were not very numerous Both finely & coarsely granular, the latter being the more numerous. (2) About 8 μ These were the most abundant variety; both finely and coarsely granular forms, the latter being proportionately fewer than in class (1) The coarse granules sometimes entirely covered the Corpuscle sometimes areas on the surface were free from granules (3) Large corpuscles up to 15 μ in diameter, few with coarse granules, most were finely granular.

All the leucocytes were circular in form and had no tendency to break down. When preserved in osmic acid very few fat granules were seen.

When stained (Dec 17) the following varieties were seen. The uninucleated & multinucleated forms were in nearly equal numbers. Of the former (1.) uniformly darkly stained throughout, some were much larger than the largest multinucleated corpuscles (being

fact the largest variety present) These formed about a third of all the uninucleated corpuscles. Sometimes they showed a lighter spot or area at one part of their surface. Some again were about the size of an ordinary multinucleated corpuscle and others were much smaller. In all these varieties especially around the edges of the cells a considerable number of all these varieties contained deeply stained granules arranged chiefly round the edge of the cell. The multinucleated forms were of various sizes yet the largest were smaller than the largest uninucleated corpuscles. A good few intermediate forms between the two classes could be seen. The nuclei of the multinucleated form were rarely distant from one another, generally they were connected by bands. There were also large corpuscles or fragments of corpuscles of a purplish colour. These were of irregular shape often and had one or more large nuclei stained scarcely more darkly than the protoplasm.

Enormous flocks of blood-plates were seen

The results of subsequent examinations were confirmatory.

Remarks. It is specially to be noted that though all varieties were increased in number, the uninucleated were more so than the multinucleated and some of these were the largest corpuscles present. These

V. Pl 14

are not normally formed in the blood and are practically nucleus.

The number of blood plates is the highest I have seen in any case.

The changes in the red corpuscles were like those formed in a moderate degree of cachexia. There was a decided fall in their number at the last examination but no corresponding increase in the number of white corpuscles

Case 44 G.R. aged 10. This was a case of Leucocythaemia in a boy. There was enlargement of the Spleen and general enlargement of the lymphatic glands throughout the body - most marked in the neck. The bone marrow also seemed on post-mortem exam. to be affected to some extent v. page.

Dec. 30th The red corpuscles, as in the previous case, were pale and of various sizes 6-8.5μ - few very small forms or megalocytes. Poikilocytes were present but were not numerous. They formed irregular heaps & nucleaux but appeared to be of normal consistence. The blood plates were in moderate number and general appearance. The Leucocytes were in great numbers. They varied in size from 5μ to 12μ - a very large number = 6.5 - 7.5μ. In most in the fresh condition no nucleus was visible, but in a few of the

Largest corpuscles one or more rarely two large nuclei were visible, with protoplasm containing somewhat coarse granules though not nearly so coarse as in the coarsely granular corpuscles. These latter were exceedingly few in this case

When stained the most striking feature was the great scarcity of multinucleated forms; they could not have exceeded 5 p.c. of the corpuscles present. The mininucleated corpuscles were of various sizes, a very large proportion being of very small size. Some of the larger showed the outline of a large nucleus & a few showed an intermediate stage to the multinucleated condition. As a rule however the mininucleated corpuscles were uniformly darkly stained. The multinucleated corpuscles ~~were~~ showed the nuclei having distinct and separate form one another. Pl. Pls. 15 & 16

I can only give an abstract of the subsequent changes The red corpuscles gradually diminished in number, more rapidly ~~for~~ before the death of the patient, when their number was less than a million per c. m. m. Poikilocytes became rather more numerous, and most of the corpuscles were small and pale.

The blood plates, at first of normal number, gradually diminished in number till before the death of the patient only a few could be seen by searching for them

This decrease in the number of the blood plates was very marked indeed.

The Leucocytes increased greatly in number and Noticed on Feb 6th that a much larger proportion of large corpuscles was present than before, many reaching 1/25 in diameter and often containing somewhat coarse granules especially towards the periphery. In stained specimens these corpuscles could be recognised in large numbers some were uniformly stained throughout but most showed a large nucleus somewhat ill defined and stained little more deeply than the surrounding protoplasm. The number of multinucleated leucocytes was even smaller than before. I saw only two nucleated red corpuscles

This case agrees with the previous in the presence of large corpuscles (Monsters) uniformly stained throughout, these not being present in normal blood The changes in the red corpuscles in the two cases are practically similar.

The blood plates in this case, at first in normal number afterwards greatly diminished, whilst in the previous case their number was vastly increased. In connection with this, perhaps is to be noted the fact that the Multinucleated Leucocytes in the former case were as numerous as the Uninucleated, while in the latter they formed but a small proportion

Table X Lymphadenoma 1C

Case	Date	Red Corpuscles	Leucocytes	Plaques	Rep. 1	Rep. 2.	Rep. 3	Polkilocytes
Case 53 J. K 11m. Lymphadenoma	Nov 6	4,130,000	20,000	501,000	1:206	1:8	1:1·2	None
	" 15	4,180,000	15,000		1:280		1:1·1	"
	" 23	4,320,000		480,000		1:9	1:1·4	"
	Jan 9	4,200,000	15000	402,000	1:280	1:10	1:1·1	"
Case 54 J.Y. 9m Lymphadenoma	Mar 13	4,127,000	23,000	502,000	1:178	1:8·2	1:1·3	Few slightly marked
	" 16 ★	4,117,000	19,000	456,000	1:216	1:9	1:1·5	"
	Mar 22	4,190,000	13,000	490,000	1:322	1:8·5	1:1·2	"
	Mar 30	4,256,000	19,000	452,000	1:224	1:9·4	1:1·6	"
Case 55 J.D 48m Lymphadenoma	Jan 12	4,276,000	22,300	512,000	1:191	1:8·3	1:2·1	None
	23	3,954,000	25,500	501,000	1:151	1:7·9	1:2·5	"
	31	3,950,000	21,700	378,000	1:182	1:7·6	1:2·4	"
	Feb 7	3,801,000	21,000	458,000	1:181	1:8·3	1:3·8	Very few
Case 56 J.S. 21m Lymphadenoma	Jan 17	4,560,000	35,000	488,000	1:130	1:9·3	1:2·6	None
	Mar 19	4,209,000	26,000	392,000	1:161	1:11	1:1·9	"
Case 57 J.D 19 F Lymphadenoma(?)	Feb 2	4,250,000	6000	252,000	1:708	1:16	1:·9	None
	13	4,230,000	6500	268,000	1:650	1:15	1:1·1	None
Case 58 A.Y 21 F Lymphadenoma	Mar 19	4,181,000	10,500	308,000	1:3	1:13		None
Case 59 J.S. 29M	Dec 9	4740,000	7 000	272,000	1:690	1:18	1:1·5	None

of the corpuscles present.

Lymphadenoma & Enlargement of Lymphatic Glands.

Case 4 III J. K. aged 11. M. Lymphadenoma. Glands enlarged on both sides of the neck and in left axilla. Largest = size of pigeon's egg. Irregularities of temperature to 102°

March 6th Red corpuscles normal in size & shape

Blood plates very numerous, some = 3μ in diameter.

Leucocytes were numerous but those 6-7μ were specially abundant. In stained specimens it was found that the unnucleated corpuscles, nearly equalled the others in number. Many of the former were of small size.

Jan 9th (two months after) a practically similar condition was found, the anaemia not having increased.

There was here an increase in the number of leucocytes which was chiefly on the part of the small unnucleated corpuscles i.e. an increased number probably passed into the blood from the lymphatic glands. The increase in the number of the blood plates was well marked

Case 4 IV J. Mg. 9. Lymphadenoma. The enlarged glands in this case were almost confined to the right side of the neck.

March The red corpuscles showed slight irregularities in shape

saw and the following method occurred to me which seemed to be theoretically free from objection. A drop of the preserving fluid is placed on the cleansed skin and the blood is allowed to flow into it and is mixed with it in the usual way. A small quantity of the mixture is placed in the haemacytometer cell and both the red corpuscles and blood plates are counted in a given area. The proportion of the two elements to one another can thus be ascertained, and thus from the absolute number of the red corpuscles obtained by a separate enumeration in the usual way, the absolute number of blood-plates can easily be calculated. (I learned afterwards that this method had been proposed by Zeiker some time before.) In trial of it, however, I found that the regulation of the proper amount of blood was exceedingly difficult and the recorded dental trials I also found that the red corpuscles and blood plates were not uniformly mixed and therefore the result was not accurate. For clinical purposes it was inapplicable and I accordingly abandoned it. My experience is that so by acting with speed and attention to the various precautions necessary one can get very good results with the other method. In fact I believe that very few, if any, of the blood plates will be left in the capillary tube if the fluid be drawn through with

but otherwise were unaltered. The case resembles the previous in the increased number of leucocytes and blood plates, & also in the increase in the proportion of uninucleated corpuscles

On Mar 17 the greater number of the glands were removed by operation. This was followed by a slight decrease in the number of leucocytes but the number speedily rose again. In the glands excised I found a great number of small lymphoid bodies about 2-3μ in diameter similar to what I have described below in lymphatic glands. Some of these were homogeneous some were granular round their edge. These bodies will be referred to later.

Case LV J. D. 48. M. Glands enlarged on both sides of neck. Cachexia was well marked.

Jan 12. The red corpuscles were of normal shape. They varied a little in size 6-8·5μ. The blood-plates were in great numbers & rapidly broke down. The leucocytes were very numerous, but all sizes up to 30. In Stain's specimens large and small uninucleated corpuscles were seen & many intermediate to the multinucleated stage. The multinucleated corpuscles were not diminished in proportion — in fact rather the reverse. In this respect it differs from the two preceding cases

Case LVI J. S. M. 21 Lymphadenoma. The chief enlargement was a large mass at the root of the neck. The red corpuscles were normal. The number of the blood plates and leucocytes greatly increased. The talla showed the usual varieties in ordinary proportion.

This case resembles the previous in the condition of the blood plates and leucocytes.

Cases LVII & LVIII were cases of enlarged glands in females. The glands were of large size and were very hard. There was practically no change in the blood.

Case LIX Enlarged glands due to Syphilis. The corpuscles of the blood were unaltered.

Summary of Cases of Enlargement of Lymphatic Glands.

In Lymphadenoma the red corpuscles may become diminished in number and then alterations are found as in other cases of anaemia viz. the appearance of small forms and some irregularities of shape. In four cases there was a considerable increase in the number of leucocytes in the others not. In all these four cases the number of the blood-plates was increased also and these found the blood plates increased in number without the leucocytes being similarly affected. In two of the these four cases in which the leucocytes were increased, the uninucleated forms were more abundant, causing a relative diminution of the multinucleated proportion

Table XI Cases with high Temperature (a)

Case	Date	Red Corpuscles	Leucocytes	Plaques	Rup 1	Rup 2	Rup 3	Notes
Case 60 E. 27 M Suppuration in Liver	Feb 1	4,640,000	16,000	181,000	1 : 290	1 : 25·5	1 : 4·4	Feb 2–10 Temp varied between normal & 104°F
	11	3,510,000	25,500	398,000	1 : 141	1 : 8·8	1 : 6·2	Temp 104·8
	12	3,352,000	29,000	502,000	1 : 115	1 : 6·7	1 : 7·6	Temp. irreg but grad. fell on Feb 22 99·2°F
	14	2,800,000	25,000	355,000	1 : 112	1 : 7·9	1 : 9	
	18	2,983,000	25,000	411,000	1 : 119	1 : 7·2	1 : 6·5	
	23	2,947,000	18,000	413,200	1 : 163	1 : 7·1	1 : 6·3	Temp normal
Mar 1		2,933,000	21,000	538,000	1 : 139	1 : 5·4	1 : 6	Normal to 101·5 Mar 15. 104·2
	8	3,130,000	17,000	494,000	1 : 114	1 : 6·3	1 : 5	
	19	2,954,000	10,000	448,000	1 : 295	1 : 6·6	1 : 8	varied slightly abve normal rarely high
Apr 1		2,801,000	14,000	416,000	1 : 200	1 : 6·7	1 : 3·9	
Case 61 G. R. 5m Broncho-pneumonia with nephritis	Jan 15	3,983,000	31,000	284,000	1 : 128	1 : 14	1 : 6	Temp 100°–103°
	16	3,930,000	49,000	422,000	1 : 80	1 : 9·3		
	17	3,908,000	36,000	542,000	1 : 185	1 : 7·2		
	18	3,840,000	47,000	463,000	1 : 81	1 : 8·3	1 : 4·6	Jan 18 fell at midnight
	19	3,790,000	27,000	428,000	1 : 140	1 : 8·8		Normal
	21	3,836,000	17,000	—	1 : 225			Temp was normal or subnormal
	23	4,002,000	10,000	418,000	1 : 400	1 : 9·5		
	24	3,963,000	9,000	411,000	1 : 440	1 : 9·6	1 : 2	
	26	3,958,000	10,000	362,000	1 : 395	1 : 10·9		
Mar 1		3,900,000	8,000	157,000	1 : 487	1 : 25	1 : 1·5	
	10	4,037,000	6,800	311,000	1 : 593	1 : 13	1 : 1·2	
	19	4,130,000	6,000	242,000	1 : 688	1 : 17	1 : 1·9	

of multinucleated forms. In the other two all varieties were increased proportionately. In the other cases the condition of the blood was practically unaltered.

Cases with high Temperature

Case LX H. E. aged 27. M. This was a case of severe biliary colic + jaundice followed by a considerable degree of cachexia. There was for a long time a high temperature which was either due to hepatic suppuration caused by the perforation of the gall-stone or caused reflexly by the continued irritation.

The case will be best followed from the table and the chart. At first (Feb 1) the blood was practically normal save except a slight increase of the Leucocytes. As the temperature rose the number of Leucocytes rose also and the blood-plates also became much more numerous (Cells) The number of red corpuscles on the other hand rapidly fell. The increase of the Leucocytes was attended by a great excess of the Multinucleated forms so that on Feb 14 they were nine times as numerous as the uninucleated. They generally contained many nuclei which however were rarely quite separate, being generally joined by threads of nuclear substance. In many a nucleus network was seen, others a Nucleolus.

As the temperature fell the Number of Leucocytes and the proportion of multinucleated corpuscles fell somewhat (also

with great rapidity. The problem is one where selection lies in mechanical contrivance and I doubt not an instrument could be constructed which would obviate all the difficulties.

The method of finding the proportion of mononucleated to multinucleated leucocytes is a simple one. A dried film of medium thickness and stained with methyl blue in the usual way is examined. The best power of the microscope for the purpose is that the lowest which enables the different forms to be clearly distinguished. (Zeiss Oc. 4 obj. D with the tube out is a good combination) The number of mononucleated and multinucleated corpuscles in a given field is counted and the process is repeated till 200-300 leucocytes have been counted. The proportion of the two forms is then easily ascertained.

During the further progress of the case, the blood showed the characters usually found in cachexia viz diminution in the number of the red corpuscles, with variations in their size most being 5-7 μ and very slight irregularities in shape, marked increase in the number of the leucocytes and blood plates and increase in the proportion of multinucleated leucocytes

Case 6 XI This was a case of bronchopneumonia and acute nephritis in a boy aged 8 7.

During the acute illness the red corpuscles were practically unaltered. The leucocytes were greatly increased in number and the proportion of multinucleated forms was very high. The nuclei in these were generally very distinct but connected by thin threads. There were also many leucocytes with broad somewhat ill defined nuclei and with the protoplasm stained rather more darkly than usual, the whole corpuscle having besides a somewhat purplish tint. The blood plates were also markedly increased in number.

With the fall in temperature the leucocytes fell in number so rapidly as almost to constitute a crisis, the proportion of multinucleated forms falling at the same time. The blood-plates

Table XII Cases with high Temperature (b)

Case	Date	Red corpuscles	Leucocytes	Plaques	Prop 1	Prop 2	Prop 3	Notes
Case 62 J.J. 50 M. Parotid abscess following pneumonia	Feb 18	3,491,000	25,500	438,000	1:136	1:7·9	1:7·3	Temp irreg reaching a little over 103° F
	21	3,519,000	16,500	424,000	1:213	1:8·2	1:5·9	
	25	3,460,000	19,500	308,000	1:177	1:11	1:6·5	
	Mar 4	3,327,000	23,000	321,000	1:144	1:10	1:6·1	
Case 63 J.E. 28(?) Acute phthisis with minute abscesses in lung	Nov 30	4,984,000	42,000	680,000	1:118	1:7·3		Temp high
Case 64 J.McW. 9m Tubercular peritonitis	Feb	3,370,000	13,500	344,000	1:250	1:9·8	1:3·1	Temp irreg up to 103°8
	4	3,170,000	11,300	378,000	1:280	1:8·4	1:3·5	
	Mar 2	3,162,000	12,500	314,000	1:253	1:10·1	1:5·4	
~~Tubercular peritonitis with incipient phthisis~~ Case 66 J.G. 17 M. bronchial pneumonia	Mar 8	4,354,000	20,500	96,000	1:217	1:4·5	1:3·8	Temp. 104°-105°7
	9	4,072,000	13,000	130,000	1:313	1:31	1:4·3	
Case 65 Tubercular peritonitis with incipient phthisis	Mar 20	3,280,000	17,000	376,000	1:193	1:8·7	1:3·5	Temp occasionally to above 103°
	29	2,909,000	14,000	368,000	1:207	1:7·9	1:3·5	
Case 67 J.b. 50 M Phthisis with acute pneumonic condition	Feb 14	4,080,000	10,000	172,000	1:408	1:23	1:4·2	Temp: irreg: up to 103°
	15	3,890,000	9,000	185,200	1:436	1:21	"	
	18	3,801,000	9,000	284,000	1:422	1:13	1:6·9	
Case 68 J.N. Transverse myelitis with cystitis bedsores	Feb 14	4,300,000	10,000	156,000	1:430	1:27	1:3·5	Temp irreg: sometimes reaching 104°7
	Mar 25	3,754,000	9000	322,000	1:417	1:11·6	1:4·2	
	Mar 2	3,230,000	9500	278,000	1:340	1:11·6	1:3·9	
Case 69 J.M. Acute rheumatism	Mar 5	4,909,000	12000	142,000	1:409	1:34	1:2·3	Temp about 102°

diminished also but more gradually. Late there was a slight rise. This shews that during high temperature the number of blood-plates may be greatly increased and fall with the defervescence.

Case LXIV Carried suppuration following pneumonia J. T 50? F. The abscess had been opened when I examined the blood but accumulation of pus took place from time to time.

Oct. 18. Red corpuscles soft & sticky but otherwise unaltered in appearance. The blood plates were very numerous The leucocytes were markedly increased & this was chiefly in the part of the multinucleated forms. Many had upto many nuclei – in one corpuscle I counted nine nuclei – arranged in a variety of ways but always distinct from one another.

Subsequent examinations gave similar results.

Case LXIII Acute phthisis, with small abscesses in the lung. The blood plates & leucocytes were greatly increased in number. The red corpuscles were of normal appearance Fresh specimens were not obtained

Cases ~~LXIV~~ LXIV & LXV Tubercular peritonitis. The one patient was a boy the other a girl. They were about the same age In both there was considerable anæmia, the blood plates and leucocytes were

increased in number and the proportion of multi-
nucleated forms was high. In the latter case there
were many corpuscles of purplish hue but with broad
nuclei - mentioned below
Case LXVI J.G. 17. M. A severe case of croupous
pneumonia. Blood examined on 5th & 6th day. Dec 1 & 8th
The red corpuscles ~~of~~ were of normal colour and shape
but showed a remarkable adhesiveness in that when
they had formed rouleaux they could not be separated.
The blood plates were rather scanty. The leucocytes
were increased in number and the increase was
found to be chiefly on the part of the multinucleated
variety. Fibrin formation took place early and
was very dense.
Case LXVII J.C. aged 50 M. This was a case of
phthisis followed by an acute pneumonic exudation
Feb 14. The red corpuscles & leucocytes in fresh blood
appeared quite normal. Blood-plates were somewhat
few. When stained the multinucleated leucocytes
showed their proportion to be increased.
Feb 18 Blood plates more numerous also the proportion
of multinucleated corpuscles was higher.
Case LXVIII J.M. Myelitis with Cystitis & bedsores
bed-sores Feb 14. Blood appeared normal. Multi-
nucleated leucocytes rather ~~more~~ than increased

Mar 2

Feb 18 The blood plates had increased somewhat whilst the number of red corpuscles had fallen. The white corpuscles had altered very little.

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Case 53 J. M. Acute Rheumatism.

Red corpuscles normal, Blood-plates rather few.

Leucocytes slightly increased as also the proportion of multinucleated corpuscles. In stained specimens a considerable number of purplish corpuscles with broad nuclei were seen.

Summary of cases with high temperature

(1) The red corpuscles showed a slight decrease in number during the period of high temperature. This was especially marked in Case 43. If cachexia followed the usual changes were seen. In the case of pneumonia they showed a peculiar thickness.

(2) With regard to the blood-plates all the cases do not agree and I find the statements of others on this point also somewhat contradictory. Mosny (Soc Hospl Paris, Oct.) found that there is no increase during the first stage of the fever but an increase is found during convalescence. Fusari also finds that as the temperature rises the number of blood-plates falls. Afanassiew makes a similar statement but finds that pneumonia & tuberculosis form exceptions. Most of these statements are founded

on observations made on fevers such as typhoid &c.
which I have not been able to examine Hall
says that in some fevers the number of blood-plates
and of leucocytes is increased, in others not and that
the increase is not proportional to the strength of the
fever. Osler also says that in wasting diseases with
fever the number of blood-plates is increased. If we
examine the above cases we find that in all the
cases of acute suppuration (3) the blood plates were increased.
In case [illegible] the increase actually took place during the
period of high temperature So also in the case of broncho-
pneumonia in which there was no wasting there was a
marked increase which fell some time after the tem-
perature fell. In the cases of pneumonia & acute rheum-
atism there was a decrease in the number of the blood-
plates. The other cases were attended with wasting &
in them the blood plates were increased in number.
(3) The leucocytes were increased in number in all
the cases where there was suppuration and in most of
the others. Almost invariably associated with the
increase of leucocytes was an increase of the proportion
of multinucleated leucocytes, and this was seen some-
times where there was no leucocytosis. Also often
in these cases leucocytes were found with large round
nuclei and protoplasm stained somewhat darkly and
of a purplish tint.

Table XIII - Miscellaneous

	Date	Red Corpuscles	Leucocytes	Blood-plates	Prop 1	Prop 2	Prop 3	Poikilocytes
Case 70. J.J. aged 10 Y. Carcinoma Cric	Aug 10	3027000	28000	286000			1:7.5	None.
Case 71 B.M. 127 Emphysema	Nov 17	3,381,000	36,500	Abnormal	1:91.2	—	1:13.5	"
Case 72 M. aged 29 Emphysema	Mar 25	3,880,000	14,000	402000			1:3.5	"
	Apr. 8	3773000	10500	432000			1:2.1	
Case 73 J. Gaged 35 M. Double Psoas abscess	Mar 18	3,700,000	15,500	300,000			1:6	"
	Apr 1	3,040,300	18,800	402000			1:5.7	"
Case 74 M.C. 257 Sinus of the urinary organs	Jan 11	4,670,000	30,000	301000	1:155	1:15.5	1:2.5	None
	22	4,560,000	19,000	340000	1:240	1:13.4		"
	Feb 12	4,330,000	21000	342,000	1:211	1:12.6	1:2.7	None
Case 75 J.P. 38 M. Bronchitis	Jan 30	4033,000	14000	384000	1:288	1:10.5	1:3.4	None
	Feb 14	4110000	10,000	226000	1:411	1:18.1	1:2.5	"
Case 76 213.507 Pulmonary Tuberculosis	Dec 10	3,730,000	9,000	513,300	1:414	1:7.2	1:5	"
Case 78 Chron. Gastro-Duodenal J.S. aged 55	Feb 27	2,760,000	5000	414000	1:552	1:6.6	1:2.4	None
	Mar 6	2723,000	3500	406000	1:777	1:6.7	1:2.4	"
Case 79 J.M.S. 29 M. Sarcoma (Abdominal)	Feb 27	3,158,000	5000	168,000	1:631	1:17	1:3.7	None
	Mar 3	2801,000	6000	90,000	1:466	1:31	1:3.1	"
	" 28	2664000	2500	68,000	1:1060	1:39	1:2.9	"

Case LXX Cancrum Oris. J. J aged 10 yrs. This was a severe case in a girl, it proved fatal about two days after I examined the blood.

Aug. 10th The red corpuscles shewed no irregular forms but a good few were undersized 6-7·5 μ. The blood plates were of normal appearance and considerably increased in number. The leucocytes were very numerous especially the large finely granular variety. When stained the multinucleated corpuscles were markedly increased in proportion. The nuclear figures shewed great variety of arrangement and the nuclei were easily separated from one another. In many a nuclear network was well seen. There were also many intermediate forms - chiefly of large size.

Case LXXI C. M. 12. F. Emphysema with extensive suppuration. The red corpuscles shewed the usual alterations. The blood plates were fairly numerous. The leucocytosis was well marked and the great increase in the proportion of multinucleated forms was very striking - it being nearly seven times the normal proportion. The multinucleated corpuscles shewed the usual great variety in the nuclear arrangement.

Mch 25

Case LXXII M. aged 29 Emphysema. The number of red corpuscles was diminished and their characters altered

slightly, as usual. The blood plates were markedly increased in number. The leucocytes were increased also and so also was the proportion of multinuclear forms. On April 8th after the emphysema had been opened and drained for some time the condition of the leucocytes was practically normal.

Case LXXIII J.G. aged 35 M. Double psoas abscess Mar. 18 The number of red corpuscles was markedly decreased and their average size diminished. The blood plates also were very numerous. The leucocytosis was well marked and multinuclear forms were very numerous. These shewed the usual fantastic arrangement of nuclei - few being quite separate from one another. In some of the nuclei a nucleolus was visible but in most a network could be made out with tolerable distinctness.

On April 1. A similar condition was found.

Case LXXIV M.C. 25 F. Sinus of hip-discharging General waxy disease

Jan 11 Many of the red corpuscles were undersized. The most striking feature was their exceeding softness. They did not form rouleaux but seemed semi-fluid and blended into irregular masses in which the outlines of the individual elements could not be recognised

they also circulated with great readiness. The blood plates were increased in number The Leucocytes were increased in number especially those of larger size 8-10 μ.

Case LXXV J. P 38 M. Tubercular disease of Sternum Jan 30 Red corpuscles had the usual appearances. The blood plates were numerous The Leucocytes were slightly increased in number and the proportion of multinucleated forms was high.

Case LXXVI Z.B. 507. Laryngeal Phthisis.

Red corpuscles for the most part regular in shape, though a few oval forms were seen. A good many = 6-7 μ

The blood plates were increased in number and and were very prominent in the field, many = 2·5-3 μ

Leucocytes of usual varieties, the proportion of multinucleated corpuscles being high viz four times as numerous as the red corpuscles.

Case LXXVII J. D. 14. M Phthisis.

Jan 12. There were the usual changes in the red corpuscles & in the Leucocytes. The blood plates, which are generally very numerous in cases of phthisis, were in this case not increased in number.

Case LXXVIII J.S. 50. M. Chronic gastric disease

There was considerable emaciation and the case was at one time supposed to be one of malignant disease. The progress however, showed this not to be the case

Feb 27. Red corpuscles pale and many were small – no megalocytes. The blood plates were greatly increased in number and some of them kept their form well. The leucocytes were scanty but showed the usual characters.

The association of an increased number of blood-plates with a decrease in the number of leucocytes is somewhat unusual.

Case LXXIX J. M'L. 22. M. A huge sarcoma filled nearly the whole of the lower half of the abdomen. There was marked and increasing cachexia

Feb 27. Red corpuscles were of regular shape but many = 6-7.5 μ in diameter. The blood plates and leucocytes were somewhat ~~less~~ fewer and this was more marked on March 28.

In the condition of the blood-plates & leucocytes this case differs from most cases of cachexia.

Cases ~~LXXVII~~ & LXXX & LXXXI Carcinoma of liver. In both the leucocytes & red corpuscles were practically unaltered in ~~character~~ appearance. In both the number of the blood plates was low.

Case LXXXII–IV Diabetes. There was in all latter anaemia and the red corpuscles were of normal appearance. The blood-plates were generally increased in number. The number of leucocytes was

diminished in all

Cases LXXXV–VII Simple Chlorosis. These cases showed that this condition may be present without any anaemia – without any alteration in the element of the blood. In the third case there were seen some of the changes in the red corpuscles & leucocytes generally found in chlorosis.

Cases LXXXVIII–IX Myxoedema.

In the first of these cases the characters of the red corpuscles were practically unaltered. In the second a good number were undersized and a few slight irregularities were seen. In short in this case all the features of slight cachexia were present.

I have note of another case in which there were practically no changes of the characters of the elements In myxoedema there is then no change in the blood necessarily associated with the condition. If then be cachexia the usual changes are found.

I may add a few facts about some other cases. In a case of malignant jaundice the red corpuscles were of abnormally soft consistence and ran together in string-like masses so that a few minutes after the blood was shed their outlines were not recognisable. The leucocytes were considerably increased in number and the proportion of multi-

Chapter II The Blood-plates (a) Descriptive

In the following description of the histological and general physical & characters of the blood plates I shall endeavoured to shew, that they have properties peculiar to themselves, which distinguish them from the red and white corpuscles and make them worthy of the name of distinct corpuscular elements of the blood. The description is in the order in which their properties would naturally be studied.

It will be convenient first of all to consider their appearances in normal blood, without the employment of any diluting medium, and the changes which they undergo after the blood is shed. To make such an examination satisfactorily it is necessary to use a sufficiently high magnifying power (800 - 1000 diams) and to act with great quickness, so that only a few seconds intervene between the time when the blood is shed and the time of its examination. A prick being made in the finger, which has been previously cleansed, the first drop of blood which appears is caught on a cover-glass, dried lightly on a slide, and brought under the microscope. Care must be taken that the layer is of sufficient thinness to allow the separate elements to be well seen. In the blood thus examined, there may be seen by careful looking around the red and

nucleated forms was very high. Two days before the patients death the proportion of the two varieties = 1:7.5 and on the day of his death 1:9. The nuclear substance formed fantastic figures showing often an intra nuclear network — the nuclei were rarely distinct from one another. The blood plates were decreased in number.

In a case of oesophageal cancer with profound emaciation there was practically no anaemia & no change in any of the elements of the blood.

In several cases of intestinal tuberculosis I have found no regular alteration in the blood.

Also in cases of pleurisy, acute Bright's disease subacute rheumatism, venous thrombosis &c the alterations in the blood were very slight indeed

General Summary

The following is a short summary of the chief changes in the corpuscular elements of the blood observed in the cases which I have described

I Red Corpuscles (a) Variations in Size. Undersized corpuscles, of which there is always a small number in normal blood, become more numerous when the number of the corpuscles is diminished. They are seen well in the anaemia due to cachexia but also in chlorosis and in pernicious anaemia

Date	Red Corpuscles	Leucocytes	Blood-Plates	Prop. of Reticulocytes	Nucleated Red Corpuscles
Feb 30	5,818,000	24500	588000		
Mar 1	5,863,000	21,000	610000		
2	5,338,000	21000			
2 3	3,364,000	23500	682000		
3 4	2722000	30,000	696000		
4 5	3,080,000	25000	660,000		
5 6	3,045,000	13000	530,000		
7 8	3,571,000 ~~4,215,000~~	11500	836000		
8 10	4,218,000 ~~4,445,000~~	11000	847000		
10	4,445,000	10250	768000		

In Haemolytic anaemia they are most numerous when the anaemia is chronic. In Leucocythaemia also they are generally abundant. If the anaemia be intense some of these corpuscles may be very small - sometimes 3-4 μ. Some are concave others convex surfaces and a very common condition to be seen is a deep concavity on one side with a corresponding convexity on the other apparently formed by a folding of the corpuscle. These are seen in all varieties of severe anaemia. Megalocytes were found especially in pernicious anaemia, the presence of many corpuscles 9-13 μ in diameter being a very striking feature in that disease. Also there were found along with these, oval corpuscles of very large size whose long diameter reached sometimes nearly 16 μ. Megalocytes were also seen though in small numbers in the anaemia due to haemorrhage; they were rather more numerous in cases of chlorosis, whilst in severe secondary anaemia they were comparatively rare. A good number were seen in the case of severe malarial anaemia. In Leucocythaemia they were very scanty

(b) Irregularities in Shape. Poikilocytes were found to be of much more frequent occurrence than is generally supposed (In this respect my results confirm those of Gram). They were formed

found in all varieties of severe anaemia. Their appearance, though more common in severer cases, cannot be said to have any definite relation to the number of corpuscles. This will be seen at once on an examination of the tables of anaemia due to haemorrhage and to cachexia. In case 1 eg. they were practically absent though the number of the corpuscles was below a million per c.m.m. whereas in case 4 where the number of red corpuscles was much higher they were in great numbers. In cases where there was little anaemia their association with symptoms of gastric disorder may be noted. In pernicious anaemia their presence in large numbers is one of the chief features and they are then associated with great variations in the size of the corpuscles 3-4-5μ. In children's the presence of poikilocytes is very constant; indeed I have never found them absent, though they were few in some cases. In children very marked irregularities are sometimes seen where there is comparatively little variation in the size of the corpuscles? Poikilocytes of typical appearance are formed mixed with corpuscles many of which are of normal size and shape. In secondary anaemia their appearance takes place only after much greater reduction of the corpuscles than in children. My experience is that they are

Scarcely ever seen till the number of red corpuscles has fallen below 3,000,000 per c.mm. In such cases they are generally pale and small and are associated with a great number of under-sized corpuscles. The same statement applies to traumatic anaemia. I may here mention again those minute poikilocytes (minicatines) formed in large numbers in Case 4. which I considered could not be detached fragments of large corpuscles. Poikilocytes were also formed in considerable numbers in leucocythaemia (c) Nucleated red corpuscles occurred in no fewer than Eight cases — four cases of pernicious anaemia, two of traumatic anaemia, one of secondary anaemia (complicated by haemorrhage) and one of leucocythaemia. In six of these cases the number of red corpuscles was under a million per c.mm. whilst in the other two only slightly above that figure. In three cases they were in considerable numbers viz two cases of pernicious anaemia and one of traumatic anaemia, in the others only one or two could be found after a careful search. In traumatic anaemia they were of regular shape and about 8-8.5 μ in diameter with a single large nucleus of reticulated appearance (Normoblasts of Hayem), in pernicious anaemia they were

often of large size or of irregular shape – "megaloblasts" & "prikiloblasts" – and contained sometimes more than one nucleus. In these latter the nuclei were generally homogeneous and small, sometimes also presenting the appearance of fragments. In the other cases they were of the "normoblast" type.

My results are therefore entirely confirmatory of Ehrlich's.

(d) Minor varieties. A tendency to easy crenation was found in many cases of anaemia – especially those due to cachexia, and a peculiar soft, almost semi-fluid, condition of the red corpuscles (as Hayem describes) was seen in a case of suppuration and cancer disease and to a less extent in other cases of suppuration. This softness was seen in an exquisite degree in a case of malignant jaundice. A remark-able adhesiveness (without softness) of the corpuscles was seen in a case of pneumonia and in malarial anaemia the corpuscles had a striking tendency to become folded in a variety of ways.

I saw none of Richard's corpuscles in any of the cases of pernicious anaemia.

II. Leucocytes. Their number varied from 1600 (pernicious anaemia) to 379,000 per c. m. m. (Leucocythaemia)

Their number was increased in the following conditions

1 Leucocythaemia

2 Sometimes of lymphadenoma

3 Suppuration - acute & chronic

4 In certain septic conditions eg. Cancrum oris and malignant jaundice

5 In some other cases of high temperature unattended with suppuration eg. pneumonia & broncho-pneumonia

6 In certain cases of secondary anaemia, especially in those due to Cancer and to chronic lead poisoning with Bright's disease

7 In traumatic anaemia - especially shortly after the haemorrhage

They were decreased in number

(1) In severe idiopathic anaemia - pernicious anaemia

(2) In many cases of chlorosis and allied conditions in females

(3) Of three cases of diabetes there number was decreased in all.

The following general statements may be made regarding their characters in these conditions

(1) In Leucocythaemia (two cases) the increase is chiefly on the part of the uninucleated (or non-differentiated corpuscles) and large corpuscles appear in the blood (15-16 μ in diameter) which are

practically normocytes. There is also a much greater variety in the size and appearance of the corpuscles than in the healthy condition.

(2) In some cases of Leucocy lymphadenoma with Leucocytosis the increase is chiefly on the part of the small uninucleated forms. In others again all varieties are increased alike.

(3) With regard to other conditions it may be stated as a general rule, that wherever there is Leucocytosis there is increase in the proportion of multinucleated forms and wherever the number of leucocytes is diminished the above proportion is also diminished. In other words, the number of uninucleated corpuscles though not remaining absolutely fixed, is a much more stable quantity than that of the multinucleated corpuscles. Also when there is Leucocytosis especially if it be acute there are formed more intermediate corpuscles between the two varieties. In pernicious anaemia, on the other hand, where the number of Leucocytes is diminished scarcely any intermediate forms are to be found. In such conditions as acute suppuration, the nuclear substance forms a variety of fantastic figures while the nuclei are very distinct and separate. Also a distinct intranuclear network is often seen. The nuclei

are somewhat irregular in outline. In pernicious anæmia on the other hand, the corpuscles often contain many nuclei which are generally of rounded form and distinct from one another, often shewing a fairly distinct nucleolus. Corpuscles of this description I consider to be the oldest & most fully developed leucocytes, so that in cases of suppuration & a great ~~number~~ proportion of the multinucleated corpuscles are of younger age & more recent formation. With regard to pus corpuscles I have found that their nuclei have practically the same structure as those of leucocytes but they are ~~rarely~~ rarely joined by threads of nuclear substance as is the common condition in the leucocytes in blood in cases of suppuration. This would seem to shew that the leucocytosis in suppuration is due rather to an increased formation of leucocytes in the blood, than to an absorption from the pus-forming area.

I have found the proportion of multinucleated leucocytes to be highest in cases of suppuration -acute and chronic- and in certain 'septic' conditions such as Roma & malignant jaundice. The proportion of the uninucleated to the multinucleated corpuscles varied greatly viz from 1:22 in a case of pernicious anæmia to 1:13·5 in

a case of myelaema i.e. the proportion of multi-nucleated forms in the latter case was about sixty times greater than in the former.

I have in several cases noted the presence of leucocytes with broad nuclei and surrounding protoplasm stained more deeply than usual — these corpuscles moreover having a somewhat purplish tint when stained with methyl blue. I do not know their significance but they were generally numerous in cases of high temperature with leucocytosis — notably in a case of broncho-pneumonia. In a case of malarial anaemia they were also abundant

Coarsely granular leucocytes were most numerous in a case of leucocythaemia and in one of malarial anaemia.

III Blood-plates

Their number varied from 7000 per c.mm in a case of pernicious anaemia to 1,210,000 in a case of leucocythaemia. Their number was increased

(1) In traumatic anaemia, especially if the anaemia were chronic.

(2) In secondary anaemia due to malignant disease and poisoning, phthisis, myxoedema with cachexia &c

(3) In chlorosis.

(4) In one case of leucocythaemia to an enormous extent

(5) In cases of lymphadenoma where there was an increase also in the number of Leucocytes

(6) In some cases of high temperature where there was Leucocytosis – notably in acute Suppuration They were diminished in number

(1) In severe Spontaneous anaemia – notably in typical cases of pernicious anaemia

(2) In severe malarial anaemia of persistent nature

(3) In a case of Leucocythaemia specially during the period immediately before death

(4) In some cases of high temperature. In one of these there was Leucocytosis

(5) In some miscellaneous conditions eg. in two cases of cirhosis of the liver, and in a case of large abdominal Sarcoma

The following alterations in their number during the progress of cases were observed

In traumatic anaemia their number diminished as the red Corpuscles were restored to their normal number.

In cases of pernicious anaemia, of Chlorosis and of malarial anaemia their number invariably increased as improvement took place, Sometimes more so proportionately than the number of the red

Corpuscles

white corpuscles fairly numerous disc-like bodies having a diameter about a third of that of a red corpuscle. Their outline is faintly marked, they are faintly granular and at first their refractive index differs little from that of the serum. They have a rounded shape but whether they are really round or oval cannot be exactly determined by this method of examination. When seen edge-wise they present the appearance of little rods or little grains of corn. They are perfectly devoid of any yellow colour so far as I have been able to determine. They resemble small seeds of corn.

After the red corpuscles have formed rouleaux the leucocytes and the blood-plates are formed in the spaces between them. It will be found that the last two elements have become adherent to the coverglass, for if a drop of water be placed at the edge of the coverglass the red corpuscles are carried away with great rapidity while the white corpuscles and the blood plates remain fixed in position. It will also be seen that the blood plates have arranged themselves in small groups and have changed their appearance and form. A few remain isolated but most by their aggregation have formed clusters or flocks which may contain a hundred or more blood plates of these

In a case of Leucocythaemia where their number was normal at the first time of examination, they diminished greatly in number till ~~the time~~ death of the patient. Immediately before death they were in exceedingly scant numbers

In some cases of high temperature with Leucocytosis they increased in number with the Leucocytes and in one case (broncho-pneumonia) their number fell with that of the Leucocytes though rather more slowly.

In some cases they were of larger size than usual, notably in some cases of chlorosis.

Chapter VIII - Experimental

The object of the following experiments, which were in plan of a simple nature, was to determine, the changes produced in the blood and in the so-called blood forming organs of healthy animals as a result of haemorrhage, or in other words, what signs of blood formation could be seen in the blood and those organs when regeneration of the blood was going on. I employed dogs and rabbits as offering types of mammals very different in habit and structure. In the latter however only single haemorrhages were practised, as the animals are so apt to shew impairment of general nutrition and even to die of secondary disease. The blood was drawn in some cases from an artery, in others from a vein. The femoral vessels are very convenient for the purpose, but it is difficult to protect the wound in that situation and to obtain primary union. The jugular vein is on the whole the most suitable vessel, but I always afterwards avoided examining the blood from the ear on that side, lest any objection might be raised regarding stasis &c. I made daily estimations enumerations of the corpuscular elements - red corpuscles leucocytes and blood-plates

Experiment I Dog 1. wt.= 6·3 kilos

Jan	Red corpuscles	Leucocytes	Blood-plates	Prop. 1	Prop. 2	Prop. 3	Nucleated Red Corpuscles
~~15~~							
16	7,275,000	19,500	452,000	1:373	1:16	1:2·2	Absent
~~16~~	7,366,000	14000	438,000	1:526	1:16		—
17	7,460,000	13000	381,000	1:574	1:19	1:2·1	—
18	7,296,000	12000	394,000	1:608	1:18		—
20	7,558,000	10500	382,000	1:719	1:19		—
21	7,355,000	14,500	426,400	1:639	1:17	1:2	—
	Cd 12·45 p.m. 120cc blood withdrawn allowed to drink						
2-45 p.m.	6508,000	26,500	313,000	1:245	1:20	1:4·1	—
22	5,797,000	21,500	364,000	1:269	1:16	1:4·6	—
23	6,176,000	23,000	347,200	1:268	1:15	1:4·1	—
24	6,686,000	24,500	386,000	1:273	1:17	1:3·5	—
25	6,902,000	25,500	380000(?)	1:270	1:18		—
27	6,944,000	18,000		1:386		1:3·3	—
28	7,361,000	14,500	384000	1:377	1:19		—
29	7,563,000	10,500	461000	1:720	1:16		—
Feb 1	7,532,000	10,000	450400	1:753	1:16	1:2·9	

Prop 1 = Prop. of leucocytes to red corpuscles

" 2 " blood plates "

" " unnucleated to multinucleated corpuscles

and from time to time examined the blood in its fresh condition and in various preserving fluids, chiefly, in Hayem's solution, in the Sod. sulpho. solution and in the methyl-violet-salt-solution. Dried films were made and stained almost daily. In most cases, often marked anaemia was produced and the regeneration of the blood had clearly begun, the animal was killed and the organs examined in various ways to be described below.

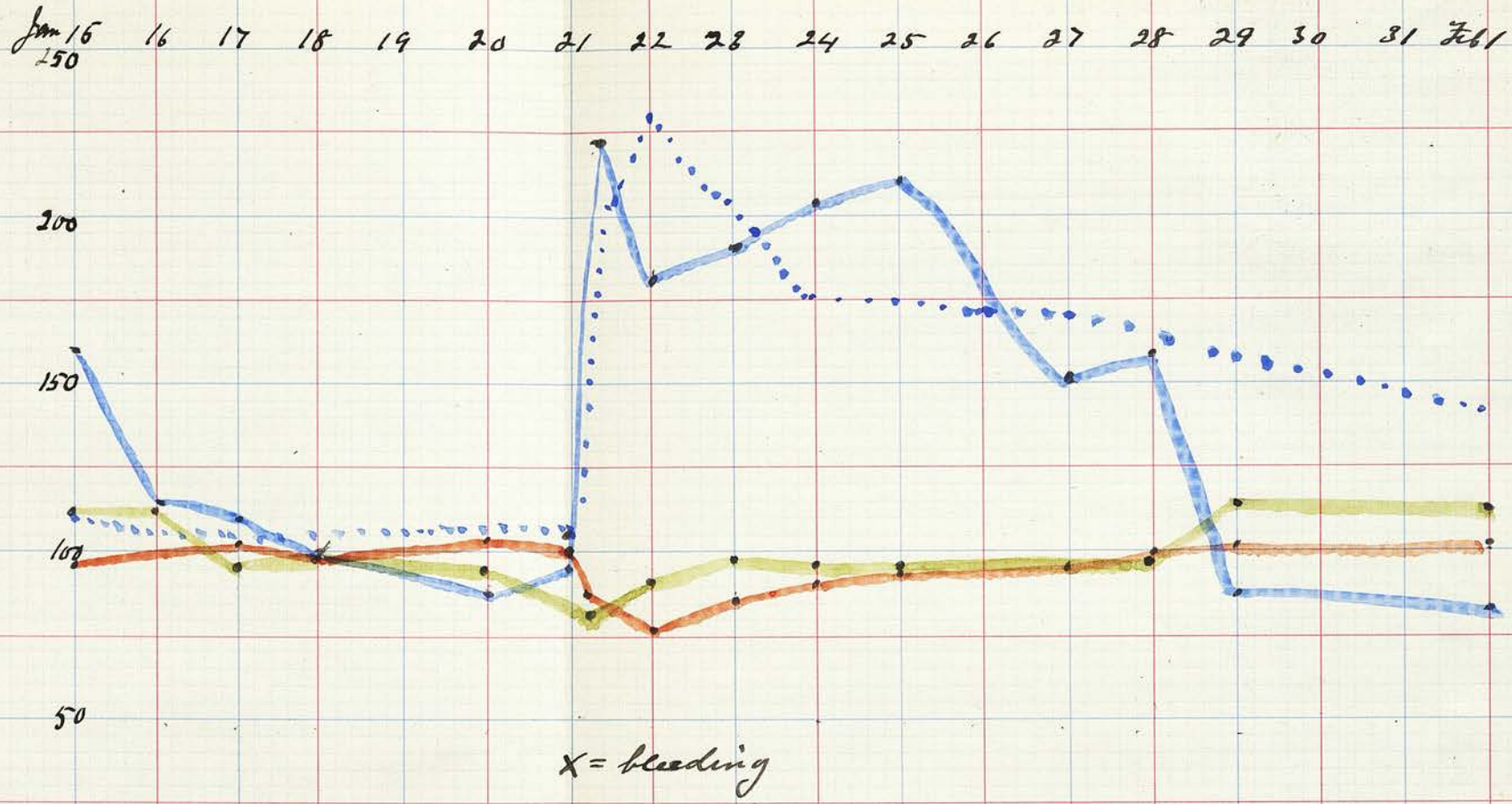
The description of the changes observed is for the most part arranged in the following order, viz the chief numerical alterations observed, the characters of the corpuscular elements in the fresh blood and in preserving fluids, and the appearances seen by means of dried films.

Experiment I Dog I

The animal was a healthy bitch of the fox terrier breed about a year old and weighing 6·3 kilog. It was fed with porridge and milk.

The blood examined for several days before the bleeding showed a very constant condition. The difference between the two extremes of the number of the red corpuscles represents a variation of about 3%. Their size was very uniform all being about 7·8 μ in diameter, and their shape was regular

Dog I Infer I



X = bleeding

The blood plates varied from 382,000 to 452,000 per c.m.m. and presented the usual appearance, most being about 2μ in diameter. The leucocytes shewed more variation in the four enumerations before the operation did not exceed 14000 per c.m.m. In the fresh blood preparations the leucocytes shewed the usual two chief varieties, the large and the small, and in stained specimens the uni- & multi- nucleated varieties were seen with a few intermediate forms. The proportion of these two forms was approximately 1:2. The uninucleated corpuscles as in man were of a smaller and a larger size - the latter being about the size of a multinucleated leucocyte.

On Jan. 21st at 12-45 p.m. 120 c.c. of blood or 2 p.c. of the body weight was taken from the left femoral vein, the animal being under anaesthetics. After the operation the animal was a little giddy but could walk about fairly well.

At 2-45 p.m. on the same day the red corpuscles shewed a decrease of about 60% while the leucocytes shewed a marked increase their numbers being rather more than doubled. The blood-plates were decreased in number rather more than proportionately to the red corpuscles.

In this case the animal was allowed to drink freely after the operation.

* No irregular corpuscles were present

At the same hour on the day after, the red corpuscles shewed another fall of about 10 p.c. and this was the lowest number reached. On the following days a rapid increase was seen and in a week after the haemorrhage the normal number was reached. The Leucocytosis which occurred within two hours after the haemorrhage continued for several days, gradually diminished and disappeared about the time the red corpuscles had regained their normal number. The blood plates, so far from being increased, as I expected were actually diminished in number. Of this fact there can be no doubt, as their number was fairly uniform before the haemorrhage and shewed a constant decrease afterwards They did not reach their normal number till all signs of Anaemia had gone.

As regards the character of the red corpuscles after the haemorrhage, there was practically nothing to be seen in the fresh blood which would suggest that young corpuscles were present. Almost all the corpuscles were of normal size — 7·8 μ One or two about smaller were seen but there were scarcely more numerous than in the normal condition.* The blood plates appeared much as usual, the

largest not exceeding 3μ in diameter.

In specimens stained with methyl blue, the red corpuscles before the haemorrhage were almost of the same depth of colour. After the haemorrhage a few were stained of a darker and bluer tint, and were of somewhat softer consistence as was shewn by their tendency to become folded in an irregular manner. This however was not nearly so marked as in subsequent experiments. No nucleated red corpuscles were seen.

An interesting point is whether or not, during the Leucocytosis all varieties of the white corpuscles were increased in the same proportion. Now the proportion of uni- to multinucleated Leucocytes before the haemorrhage was 1:2, whilst after it, it was 1:4 and remained diminished during the anaemia i.e. the Leucocytes added to the blood were nearly all of the multinucleated variety. The multinucleated Leucocytes were of typical appearance, the nuclei exhibiting the most various arrangements. In many a nucleolus could be seen within the nucleus. Besides these, there were many intermediate forms between the uninucleated and multinucleated corpuscles. The intermediate forms were most

Experiment II Dog II

Wt = 6·8 Kilos.

Date	Red Corpuscles	Leucocytes	Blood-plates	Prop 1	Prop 2	Prop 3	Nucleated Red Corpuscles
16	6698000	7000	128000	1:957	1:52	1:2	
18	6375000	7500	185600	1:850	1:34		
20	6425000	10000	206000	1:642	1:31		
25	6350000	15000	164000	1:423	1:39		
27	6309000	7500	146000	1:852	1:43	1:1·8	
	12·15 p.m. 145 C.C. blood with drawn						
Immediately after	6,400,000	8,000	132000	1:800	1:49	1:1·7	
28	5,461,000	26,500	129,200	1:206	1:42	1:4·6	
29	3,975,000	21,000	138000	1:189	1:29	1:4·6	
30	3,840,000	21,000	200,000	1:182	1:19	1:2·9	
31	3,938,000	11,500	189000	1:342	1:21	1:1·9	Present
	1 p.m. 120 C.C. blood with drawn						
Jul 1	2,447,000	29,500	285000	1:83	1:8·6	1:4·9	"
2	2,480,000	27500	—	1:90	1:—		"
3	2451000	27000	345000	1:90	1:7·1	1:4·8	"
4	2664000	27000	411,000	1:98	1:6·4	1:4·1	"
5	2513000	27000	403000	1:93	1:6·2	1:3·6	"
6	2762000	26000	489000	1:106	1:5·6		"
7	2922500	19000	401000	1:154	1:7·2		"
8	2975000	15000	400000	1:198	1:7·4	1:2·8	"
10	3311000	17000	400000	1:195	1:8·2	1:2·9	"

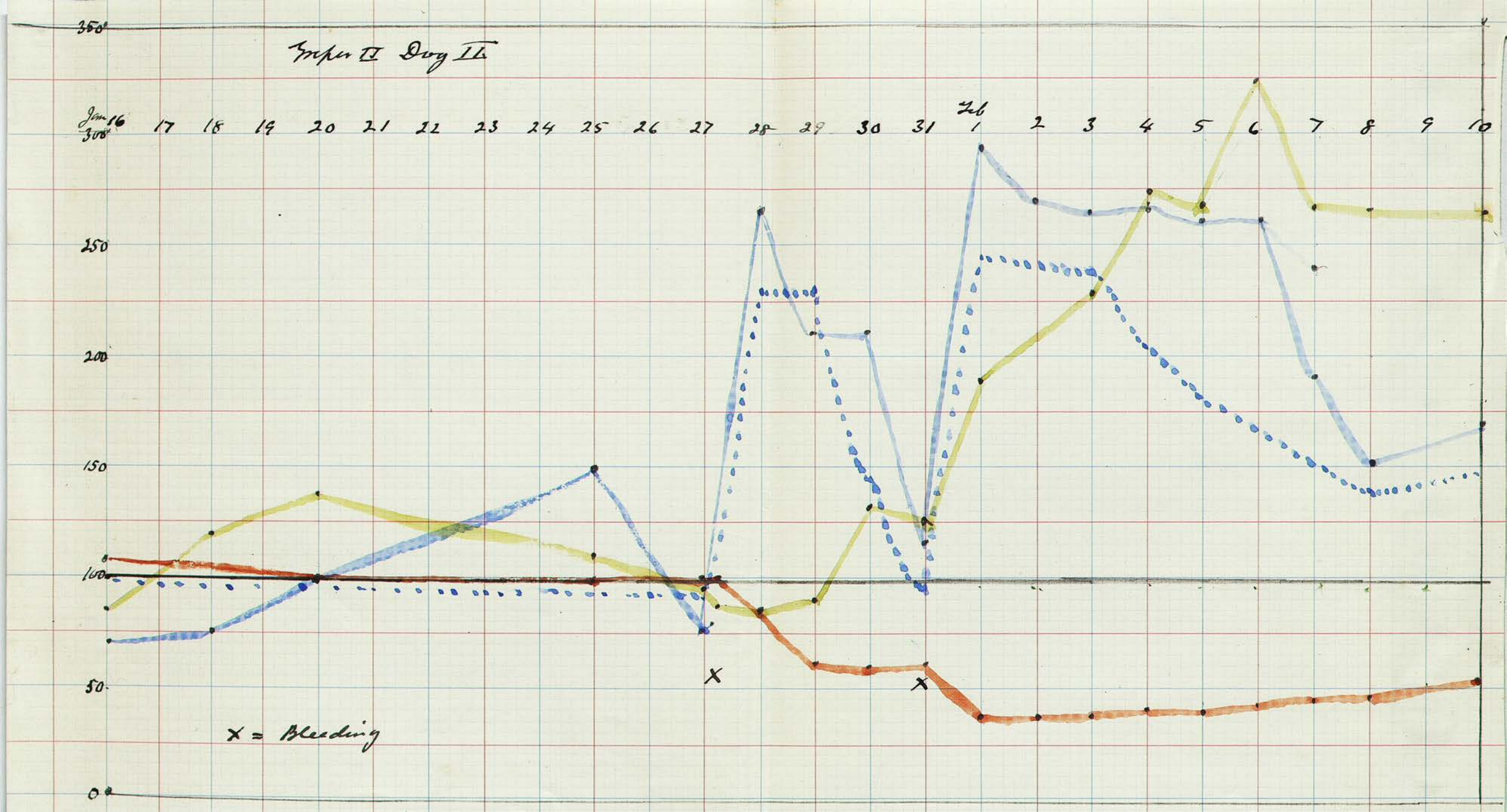
numerous during the first two days

This experiment proves that a rapid regeneration of the blood can take place without the size of the red corpuscles being diminished, and without the blood-plates being more increased in size or in number. The leucocytosis was a very marked feature and was seen to be present at a very early period. The question arose – Is the number of leucocytes immediately after the hemorrhage. The question is answered in the next experiment

The rapidity of regeneration of the red corpuscles was really remarkable. The same animal showed the same recuperative power in a later experiment (No III).

Experiment II Dog II

The animal was a healthy dog of the fox terrier breed and weighed 6·8 kilos. Bleeding was practised on two occasions, the total reduction of corpuscles per c.mm. being about 60% of the original number. The animal was killed after definite signs of blood-regeneration were visible. From the Jan 31st its diet was porridge and milk afterwards animal diet was added as it did not seem to be receiving satisfactorily.


Exper II Dog IL
Jan 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 Feb 1 2 3 4 5 6 7 8 9 10
350
300
250
200
150
100
50
0
X
X
X = Bleeding

The variations in the number of the corpuscular elements before the bleeding were comparatively slight, the number of the leucocytes never exceeding 15000 per c.m.m. and that of the blood plates 206,000.

On Jan. 27th at 12·15 p.m. 145 c.c. of blood were taken from the left femoral vein. After the operation the animal was quite able to walk

Immediately after the haemorrhage the blood was examined and shewed practically no alterations in the numbers or qualities of the corpuscular elements.

On the day after, the number of the red corpuscles had fallen considerably, and continued to fall till the minimum was reached on the third day after the operation, when it shewed a loss per c.m.m. of 39% of the original number of red corpuscles. A marked Leucocytosis was present on the day after the haemorrhage and continued for three days. For some days the blood plates shewed little or no increase in number.

On Jan 29th the fresh blood examined undiluted shewed the following appearances. The red corpuscles were little altered beyond shewing a tendency to run together in heaps rather than in

Corpuscles. They were softer in consistence than usual and retained their shape less perfectly. A few were undersized but there was no marked increase of small forms. No irregular corpuscles were seen. The leucocytes appeared markedly increased in number especially those of the large sort about 9 μ in diameter. They were seen in groups of two or three scattered over the field. The blood plates seemed to be in moderate number (blues)

On Jan 31st a practically similar condition was seen, the red corpuscles showing softness and stickiness in even a more marked degree. Stained films showed that the increase in the number of the leucocytes was almost entirely on the part of the multinucleated forms, the proportion the two varieties having altered from 1:1·7 to 1:4·6. There were also seen many forms intermediate between the two varieties. The multinucleated forms had as a rule many nuclei and a nucleolus was generally visible in each nucleus. The small uninucleated corpuscles were scanty. With the fall in the number of the leucocytes which followed afterwards, the above proportion again approached its normal condition.

number is great. In normal blood in such clusters there are generally about three to twenty blood plates whose outline at first can be clearly seen. They have lost their rounded form and smooth outline and have become stellate or prickly ("jacky"). It will also be seen that they are now more granular and that they refract the light more strongly. Indeed one of the best ways for seeing them is to rapidly move the adjustment screw to and fro, when they will be seen to flash in the light as they pass into and out of focus. The whole surface of a blood-plate in this condition comes into focus at once thus showing clearly to be quite flat. The aggregation into flocks is thus associated with change of form and increased granularity and reproducibility. If individual elements be watched after they have assumed the stellate form some may be seen to differentiate into a granular part more or less central, and a peripheral more homogeneous portion. In short the same change takes place in the elements in the heaps but the change is more easily watched. In it, in great part, is due the granular appearance which the heaps present. Sometimes a heap is pretty uniformly granular throughout; in other cases

On Jan 31st the animal was again bled, 120 c.c. of blood being withdrawn from the right femoral vein. On the day after, the red corpuscles shewed a loss of about 38% per c.m.m. The number remained at that level for about three days, and then gradually began to increase till a week later, when the animal was killed, the corpuscles shewing during that time an addition of nearly 1,000,000 per c.mm. A marked Leucocytosis was present on the day after the haemorrhage and lasted for several days, thereafter gradually disappearing. The blood plates after this haemorrhage began to increase in number, reaching a maximum on Feb 6th when their number was more than doubled. The blood examined at the height of the anaemia, on Feb 1st, shewed the following features. The red corpuscles were soft and easily crenated and adhered to one another in heaps — as before. A few were a little undersized but scarcely more so than usually are. The blood plates were not only present in increased numbers, but were larger than they usually are, some when measured in the dodecasulph. Solution reaching 4·5 μ in diameter. Nevertheless they shewed the usual changes in form, had no increased resistance, and exhibited

no approach to the characters of the red corpuscles. The Leucocytes also were much increased in number and shewed the usual characters, there being however few of the smallest size present

On Feb 4th The red corpuscles shewed the same characters in even a higher degree, appearing in fact almost semi-fluid. A few were 6-7 μ in diameter but none were smaller. No true forms were present. The Leucocytes occurred in small groups and some were fused together and broken down, blood plates often being mingled with and adhering to them. The blood plates also had an increased vulnerability which made their enumeration a matter of the greatest difficulty as in the Sod. Sulph. Solution many were fused together, and even broken down. They were however much more numerous than usual.

On Feb 7th The same features but less marked.

Dried films during this period shewed that a marked increase in the proportion of the multinucleated Leucocytes followed the hemorrhage as before. There were also many intermediate forms to be seen from a film of blood on Feb 5th plate II is taken which shews the development of the multi-nucleated Leucocytes from the mininucleated. Many of the mininucleated forms were large and some

V. Pl. II

nuclei apparently in process of division. I stained specimens in various ways to determine whether intermediate forms could be found between the blood plates and the red corpuscles. Though the former were seen to be increased in size, no alterations in their characters and staining reactions were found and the two elements were as distinct as in normal conditions.

v. Pl. 29 & Pl 8.

Nucleated red corpuscles were found in the blood first on Jan 31st (two days immediately before the second bleeding) and were seen to be present till the death of the animal. They were most numerous about Feb 5th. They showed the usual characters. They were generally about 8 μ in diameter with a single circular nucleus about 4 μ in diameter, showing in some cases a well marked reticulated structure. The perinuclear portion was generally stained rather more deeply than the ordinary red corpuscles and showed greater liability to be deformed in the making of the film, though some were seen in perfect condition. Their characters were observed both in specimens stained simply with methyl blue and in those doubly stained with methyl blue and fuchsin; the latter combination is exceedingly convenient for this purpose. I found

none in which the nucleus was reduced to a collection of granules. I noticed also that many of the red corpuscles during the anaemia stained more darkly than in normal conditions and were more apt to have their shape altered — exactly corresponding in fact with the permuclear portion of the nucleated red corpuscles. I accordingly believed that these were the corpuscles which had most recently lost their nucleus. Such corpuscles were most numerous also about Feb. 5th. They were first seen in considerable numbers on Jan 29th

The changes may be summarised as follows.

After both bleedings the red corpuscles showed a somewhat corresponding decrease in number, and ~~so that~~ their number at the time of greatest anaemia was only 37% of the normal number.

The red corpuscles during the anaemia became softer in consistence and showed a tendency to adhere irregularly in heaps. The average size was scarcely decreased, those of 6-7 μ in diameter being very few in number. There were no true poikilocytes.

Nucleated red corpuscles appeared in the blood and were present during the anaemia, and

Some of the ordinary red corpuscles corresponded in character and staining reactions with their permuclear portion

After both such haemorrhage there was a marked Leucocytosis, chiefly due to a great increase in the number of mononuclear Leucocytes. This Leucocytosis was not present immediately after the haemorrhage, but appeared when the number of red corpuscles had begun to diminish.

The number of the blood-plates increased after the second haemorrhage, very markedly and some were of larger size than usual. They however did not not alter in character beyond shewing a greater tendency to adhere together.

A diminished power of resistance was shewn on the part of all the corpuscular elements probably due to an impoverished condition of the blood serum.

The animal was killed under anaesthetics on Feb 10th.

Before its death, a small puncture was made in the capsule of the spleen. The blood thus obtained, which was really the blood circulating in the spleen with some elements of the splenic pulp added to it, was examined both fresh in methylviolet-salt solution and also in dried films. In the former solution the red corpuscles were of normal appearance. The colourless cells were of various sizes. Most were abt 18-9μ in diameter and evidently ordinary Leucocytes, no

nucleoli

being visible. Some were smaller being as low as 4 μ in diameter, whilst some reached 12 μ. In most of them one large nucleus was visible. There were also small lymphoid elements of about 3 μ in diameter with definite outline and faintly granular appearance and feebly stained. (The colourless elements evidently included the leucocytes of the blood and elements added from the splenic pulp.) The blood plates were in enormous numbers, their proportion to the red corpuscles formed on counting them in the haemocytometer being 1:4. The largest were about 3 μ in diameter. They changed their appearance and formed lumps in the usual way. A few nucleated red corpuscles were seen but the nucleus in many was somewhat indistinct and feebly stained.

In films dried and stained with methyl blue the following elements were seen (v. plate) Pl. 27 Pl. 31 fig 2 Pl 32 fig 1

(a) Multinucleated leucocytes of usual type - not so numerous as the mononucleated

(b) Mononucleated corpuscles uniformly deeply stained of three sizes roughly, about 11 μ, 8 μ, and 6 μ. The latter small corpuscles showed no structure whatever. Between the large mononucleated corpuscles and the multinucleated a few intermediate forms could be seen.

(c) Ordinary non-nucleated red corpuscles regular in shape and size.

(d) Nucleated red corpuscles in considerable numbers though few in comparison to the leucocytes. They were of typical appearance, perhaps exceeding 8μ in diameter and most having a reticulated nucleus. In some the nucleus was smaller and of more homogeneous appearance. In one I saw two nuclei a larger and a smaller — an appearance which I have also seen in the embryonic condition (v plate .) Such Pl.
a condition is never found in leucocytes. In some of the nucleated red corpuscles the peripheral portion seemed slightly irregularity, as if it had been folded and in such cases it was stained more darkly and of a somewhat bluish tint. I also saw corpuscles with single nuclei of the same character, but with the surrounding protoplasm stained of a blue colour (viz. free from haemoglobin) and I saw others in which the nucleus had scarcely any protoplasm around it at all. In fact one could trace all stages from the last-mentioned to the fully developed nucleated red corpuscle. I saw also ordinary red corpuscles presenting the same characters as regards staining as the peripheral portion of the nucleated variety viz. more darkly stained. v. Pl 31 fig 2

(e) Blood plates were also seen in enormous numbers and had the usual characters. There were

also bodies (3-4 μ) faintly stained, some of which had slightly irregular outline, I was doubtful regarding their nature but they might have been large blood-plates v. plate 27 Pl. 27

In Specimens stained with methyl green Jenk. and L. corresponding appearances were seen. The difference between the staining of the nuclei of the red corpuscles and of the blood plates was very marked, the former being of a dark rich crimson, the latter a pale and much duller crimson. There were also round bodies of about 4 μ in diameter, stained faintly - these were probably the lymphoid bodies described as seen in the methyl violet salt solution

In films of the blood taken from the spleen vein during life, many red corpuscles presenting the same appearances viz. Some of the usual pale green colour and others of a dull bluish tint, some of the latter being of perfect shape others variously folded. Nucleated red corpuscles were also seen but in smaller numbers than in the fresh blood from the pulp. The mononuclear leucocytes showed the same characters and some of the largest seemed to be in process of division. They were rather more numerous than the multinucleated whilst in the blood the proportion was 1 : 2

~~Condition of the Nerves~~. ~~Description of the Engravings~~

The spleen was of rather softer consistence and redder than usual. When examined post mortem it contained nucleated red corpuscles of typical appearance but not in large numbers. Their origin immediately could be traced from columnar cells. Very few blood plates could be seen i.e. they had nearly all passed out with the blood at the time of death. Condition of the red marrow. The shafts of all the long bones were filled with red marrow (This immediately I have found to be the case in a normal dog of this class, and neglect of this fact has led some writers often to speak of a transformation of yellow into red marrow, where the latter is really present in the normal condition.) To the naked eye it presented no unusual appearances.

On microscopic examination it was found to contain numerous nucleated red corpuscles of typical appearance. Most were about 8 μ in diameter and the nucleus about 4 μ, though the size of the latter varied somewhat. The nucleus usually presented the reticulated appearance, but was sometimes homogeneous and less deeply stained. Their origin from columnar cells of the same structure could be clearly traced. Exceedingly few were in process of division. I saw none with a small granular nucleus. In one I saw the

the nucleus half protruded and as the examination was within a few minutes of the death of the animal I could scarcely regard it as a post-mortem phenomenon. The ordinary small lymphoid elements were seen as usual. There were more large round cells, with single & round nucleus and very finely granular protoplasm, these were such cells as are common in the marrow of the rabbit. The ordinary marrow cells were of usual appearance. Also there were a good few cells containing pigment, in some it was in fragments in others in a diffuse form. These cells were in considerable numbers and I cannot explain their presence in this case — I found them in none of my subsequent experiments. The nucleated red corpuscles were also seen in stained specimens and their origin could be traced in the same way. v. Pl 32 fig 2

The nucleated red corpuscles were more numerous in the marrow of the shaft of the femur than of the ribs or vertebrae.

The mesenteric glands were slightly red, swollen and juicy. They contained a large number of small lymphoid elements 2-3 μ in diameter as a rule, some however reaching 4 μ. There were perfectly circular homogeneous slightly granular and stained faintly. Somewhat similar bodies were seen within the

the blood plates may be seen united by their projecting prickles, without being actually fused, and sometimes I have been able to make out a substance filling the interstices between them. This arrangement is probably merely a stage towards complete fusion and is often well seen in dried specimens (v. plate 5). The extent to which differentiation takes place in the blood-plates varies greatly but it is generally less extensive than many writers would appear to imply.

Shortly after the formation of the heaps (generally about two minutes after both removal of the blood) the formation of fibrin commences. Delicate needles may be seen to shoot out in various parts of the field and to cross each other in all directions. At first, they are tapered away at their ends and do not form continuous lines. After examining more than a hundred such specimens I have come to the conclusion their earliest appearance and their closest formation are undoubtedly round the blood plates. There they can be first clearly seen and very often the prickles of the changed blood-plates can be observed to be continuous with fibrin filaments. The early fibrin formation has no special reference to the leucocytes as the needles

1. Plate I

nuclei of large finely granular cells with single nuclei. These small lymphoid bodies were shown by means of acetic acid to be of different nature from the nuclei of the lymph corpuscles and they were besides smaller in size than the latter. A few ordinary blood plates were also seen. The lymph corpuscles varied in size from 5 - 11μ, most having a single nucleus almost filling the cell, while a few of the larger had a broad band of surrounding protoplasm of very finely granular character. Other glands showed the same characters but the small lymphoid bodies were fewer in number. When stained in dried films, these small bodies were seen of much paler colour than the nuclei of the lymph corpuscles. A very few typical multinucleated leucocytes were seen lying amongst the other corpuscles.

The chief change was the increase in the number of nucleated red corpuscles in the bone marrow and in the spleen. That in the latter situation they were being formed and not simply present in the blood of the organ, is, I think, proved by the fact that they were more numerous than in the blood, and their origin could be traced from colourless cells not present in the blood. The great number of blood plates in the blood of the

Exper. III Dog I wt. 6·2 kilog.

Date Feb	Red Corpuscles	Leucocytes	Blood-plates	Prep 1	Prep 2	Prep 3	% Nucleated Red Corpuscles
6	7820,000	14800	408000	1:494	1:18	1:2	—
7	7455000	19500	398000	1:382	1:18	1:2·1	—
	Bled 1 p.m. 150 c.c. of blood into shaum. Allowed to drink						
5 p.m. 11	5866000	26500	334000	1:221	1:17	1:4·9	—
8	5520000	22000	300000	1:251	1:18	1:4·3	—
9	5408000	26000		1:208	—	1:3	—
10	5672000	19000	350000	1:298	1:16	1:2·8	—
13	5870000	14000	396000	1:419	1:15	1:2·1	—
	Bled at 12-30 p.m. 140 c.c. blood. Not allowed to drink						
5 p.m. 11	5109000	25000	—	1:204		1:3·9	Present
14	4352000	17000	316000	1:256	1:13	1:3·1	"
15	4566000	15500	298000	1:295	1:15		"
16	4835000	9000	377200	1:537	1:12		"
	Bled Feb. 17. at 1·15 p.m. 140 cc. blood. Not allowed to drink						
4-30 p.m. 17	4,400,000	19,500	—	1:226	1:	1:4·4	"
18	3753000	13500	350,000	1:277	1:11	1:3·4	"
19	2884000	10500	400000	1:274	1:7·2	1:2·6	"
20	2929000	11500	484000	1:255	1:6·	1:2	"
21	3018000	10500	380000	1:287	1:7·9		"
22	3523000	13000		1:271			"
24	4280000	10000	400000	1:428	1:10·7	1:2·1	"

spleen pulp and of the small lymphoid bodies in the lymphatic glands is also noteworthy

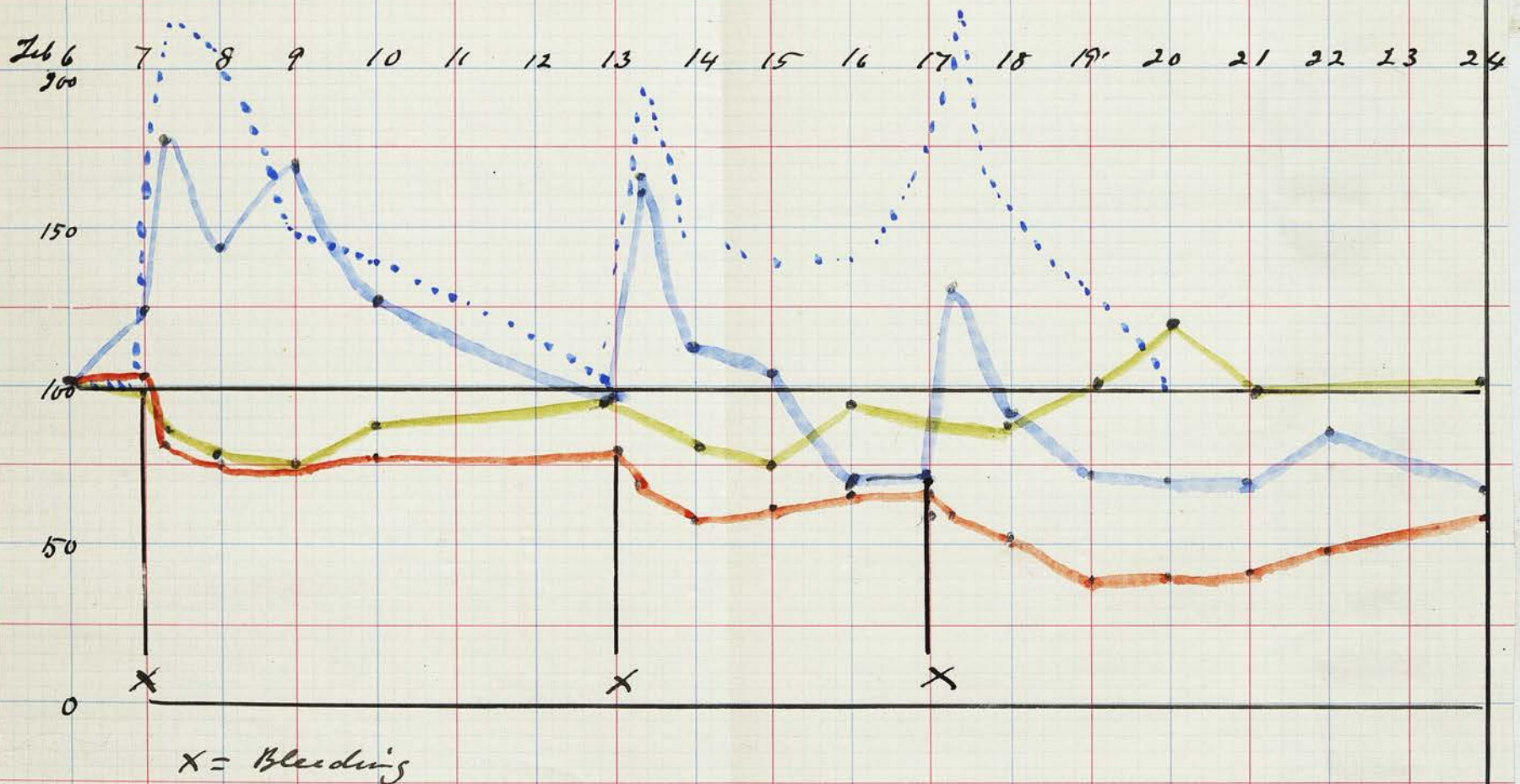
Experiment III Dog I

My object was to produce by means of three successive haemorrhages a degree of anaemia more pronounced than in the previous case. The animal was the same as that employed in experiment I, which showed such remarkable powers of blood restoration. Even however, with these three haemorrhages the corpuscles did not fall to a lower level than in the previous case, the lowest number reached (Feb. 19th) being 38% of the normal. As in the first experiment, the regeneration went on very rapidly and according it was in a very favourable condition for an examination of the blood-forming apparatus

The first bleeding was on Feb 7th at 1 p.m. and the blood examined at 5 p.m. shewed the following changes. The red corpuscles had already fallen considerably in number and the blood plates nearly proportionately with them. At the same time the leucocytes had increased markedly. The minimum was reached by the red corpuscles two days later and two days later still distinct signs of regeneration were noted By the same time the white corpuscles had re-

(Summed

Expt. ~~III~~ Dog I



X = Bleeding

their normal number. During all this period the blood plates never rose to their original number.
As regards the character of the blood when examined fresh and undiluted, there was nothing abnormal about the red corpuscles (on Feb 10th) beyond an increased softness and want of elasticity. Their size was practically unaltered. The leucocytes were more numerous in the field especially the large varieties. The blood plates did not seem more numerous nor did their size exceed the normal.
Stained specimens showed that on the same day after the haemorrhage along with the leucocytosis there was a great increase in the proportion of multinucleated forms. Many of the uninucleated were of large size and many showed indications of a change into the multinucleated variety. Some were of oval shape with the dark nuclear substance aggregated at the two poles as if they were in the process of division. V. Pl. 9 Fig 2 b & c
The increase in the proportion of multinucleated forms fell during the following days and late medicate forms became much less common.
On Feb 10th red corpuscles could be seen in the stained specimens which took the stain more deeply and tended to become folded and of irregular shape.
I could find no nucleated red corpuscles during

this period

The second bleeding was on Feb 13th 140 c.c. of blood being withdrawn which caused a fall in the number of red corpuscles per c.mm. of 22%. The minimum was reached on the day after the haemorrhage and two days later there was a decided increase A marked Leucocytosis occurred on the day of the the haemorrhage but had quite disappeared on the third day after. The number of blood plates again fell with that of the red corpuscles and rose gradually afterwards but did not exceed their original number.

On Feb 17th A few of the red corpuscles were about 6·5-7μ in diameter but there were none smaller. No poikilocytes were present.

On Feb 17th (immediately before the third haemorrhage) there was the same condition. Many of the red corpuscles were also softer than usual.

I also examined the blood carefully in Hayem's solution with the same result as regards the size and shape of the red corpuscles. The red corpuscles were relatively a little increased in number but were of usual size.

Stained specimens showed the same changes on the part of the leucocytes during the leucocytosis

as before

Nucleated red corpuscles were found for the first time on Feb 13th 1880. immediately after the second haemorrhage

The third bleeding was on Feb. 17th at 1·15 p.m. 140 c.c. of blood being removed.

About three hours afterwards the number of red corpuscles had fallen a little (the animal was not allowed to drink, whereas the leucocytes had more than doubled their number. The minimum number of red corpuscles was found on the second day after the bleeding and thereafter a rapid increase in the number of the red corpuscles took place, more than a million per c.m.m. being added in the course of four days. The leucocytes fell to their normal number two days after the haemorrhage. The blood plates steadily increased in number reaching a maximum on Feb 20th their number then exceeding considerably the original number

In the fresh blood examined on Feb 20th the red corpuscles shewed nothing abnormal beyond an increased softness and stickiness. They were remarkably regular both in size and in shape. The blood-plates were in great numbers and shewed the usual characters. I also examined the blood carefully in Hayem's fluid and found that none of the blood plates

exceeded 4 μ in diameter. They were all very faintly granular and without a trace of haemoglobin.

On Feb 23rd The blood was again examined fresh and in Hayem's fluid with a similar result

Specimens dried and stained shewed that the leucocytes underwent the same changes as before

Nucleated red corpuscles, which were seen first on Feb 13th., were present in all the films examined during this period, and also non-nucleated red corpuscles of the more darkly stained variety.

I have specimens of the blood during the height of the anaemia stained in methyl blue in which the red corpuscles are practically all of the same size and regular in shape. While most of these however are stained of a pale greenish colour some are stained of a greyish blue and more deeply Amongst them though in very small numbers are seen a few nucleated red corpuscles whose perinuclear portion is of the same colour and appearance as those latter That those more darkly stained are the younger corpuscles I think is beyond a doubt.

The blood plates when stained shewed their usual characters. They were scarcely so large as in September II v. Pl 30

The chief points of importance may be summed up thus As the anaemia was produced the average size of the

red corpuscles was unaltered and no irregular forms were seen. A similar statement is true with regard to all the time during which regeneration of the blood was going on. Corpuscles however appeared in the blood which stained more darkly, and a little later also nucleated red corpuscles were seen whose perinuclear portion resembled those non-nucleated corpuscles in character and in staining reactions.

A marked leucocytosis was seen after each haemorrhage, coming on very rapidly, and lasting a few days. During most of the time before the death of the animal, when the number of red corpuscles was rapidly increasing, there was no leucocytosis.

Only after the third haemorrhage did the blood-plates exceed their original number. Their average size was a little increased. There was however a marked difference between the size of the largest blood-plates and the smallest red corpuscles. The characters of the former were unaltered.

The dog was killed on Feb 26th

Unfortunately the animal suddenly died when being anaesthetised and I was unable to examine the contents of the splenic artery and

them during life as I intended

The following are the chief facts noted post-mortem

Red marrow - The cavities of all the long bones were filled with red marrow as in Experiment

The fresh marrow examined immediately after death without any reagent showed the usual colourless elements of the marrow with red corpuscles interspersed between them. A very large number indeed of the cells were nucleated as shown by the pale coloured spot towards their centre in which were seen fine granules, arranged so as to suggest the reticulated appearance when stained.

In the methyl-violet-salt solution, ordinary red corpuscles were seen but they showed rather more variation in size than in the blood of dog 6.5-9μ. Nucleated red corpuscles were present in very great numbers. They were indeed more numerous than in any specimen of marrow which I have ever examined. A large number could be seen in every field of the microscope. They showed considerable uniformity in appearance. Their size was 7-9μ and in most the diameter of the nucleus was 4 to 5μ. In some it had the characteristic reticulated appearance, in others it was smaller and more homogeneous. A considerable

number under in process of division. I saw one or two large oval nucleated red corpuscles with a somewhat small nucleus at each pole — an appearance which would rather suggest direct division. There were also nuclei of the same character without any protoplasm visible around. Also intermediate forms between the Colourless and the nucleated red corpuscles. There was no pigment cells in this case. Stained specimens same confirmatory appearances.

The spleen was a little large and certainly redder than normal. It contained a few nucleated red corpuscles in addition to the usual elements.

Stained films of blood from the splenic vein showed nucleated red corpuscles in one large number. Specimens stained in methyl blue, in fuchsin with methyl blue, and in Gentian violet showed that the non-nucleated corpuscles were of two varieties differing in the depth of staining, and that the peripheral portion of the nucleated red corpuscles was stained like the variety which took the stain more deeply. Blood plates were also seen which differed entirely in their appearance and staining from the nuclei of the red corpuscles. Evidently large numbers of nucleated red corpuscles

V. Pl 32 Fig 3

are not more numerous around them than
in any other part of the field, Undoubtedly
so far we can be sure in this manner the
formation of fibrin generally sets out from and
is densest around the heaps of altered plaques
Areas can however generally be found, free from plaques
in the centre of which fibrin formation is going
on whilst at the periphery no fibrin needles are
yet visible Whilst the fibrin formation has
been going on changes have been slowly pro-
ceeding in the heaps of plaques. They have be-
come more granular and amorphous looking,
the outlines of the composing elements having quite
disappeared, so that nothing but a granular mass
can be seen with small projections around their
edges which are continuous with fibrin needles.
If the process be watched longer small vesicles will
be seen to form as Bizzozero describes chiefly around
the edges of the granular masses but also on
their surface. These are like small mucin-blebs
Still later the granule-heaps disintegrate and
form débris. Sometimes the heaps decrease in size
somewhat, but this is much more probably due
to an actual contraction and condensation of
the heaps than to many suppose to a

passed into the splenic vein at the time of death.

Films of the splenic pulp stained nucleated red corpuscles in considerable number and in specimens double-stained with fuchsin and methyl blue when origin could be traced from columnar cells, as the haemoglobin-containing portion is stained of a deep ruby red while the protoplasm of the latter is a dull purple.

The iliac mesenteric lymphatic glands stained the usual elements with a few of the small lymphoid elements described before and a few blood plates. These latter smaller elements were not however so numerous as in the previous case. There were no nucleated red corpuscles to be seen.

The point of greatest interest and importance in this experiment is that, while on the one hand regeneration of the blood was progressing more rapidly than in any case which I have seen, the number, on the other hand the number of nucleated red corpuscles especially in the bone marrow was only few the largest I have ever met with. (The number of nucleated red corpuscles in the circulating blood does not appear to have any direct relation to the number in the bone marrow, as they were more numerous in

Experiment IV Dog III wt. 4.8 kilg.

March	Red Corpuscles	Leucocytes	Blood plates	Prop 1	Prop 2	Prop 3	Nucleated Red Corpuscles
12	5820000	19800	408000	1:294	1:14	1:2	—
13	5955000	23500	398000	1:253	1:15		—
	Bled 1.p.m. 130 c.c. blood with drawn						
14	3372000	28000	480000	1:120	1:7		—
15	3425000	26500	456000	1:129	1:7.5	1:4.6	—
17	3546000	21300	550000	1:166	1:6.4		—
	Bled 1.30 p.m. 115 c.c. blood with drawn						
18	2525000	33000	471000	1:76	1:5.3	1:5	Present
19	2555000	23000	450000	1:111	1:5.6	1:3.7	"
20	2676000	21000	487000	1:127	1:5.5		"
21	2681000	16000	400,000	1:168	1:6.7		"
22	3100000	20000	400000?	1:155	1:7.7		"
25	3362000	17000	328000	1:198	1:10.2		"
26	3379000	10000	304000	1:338	1:11.1		"

the blood in the previous experiment than in this)

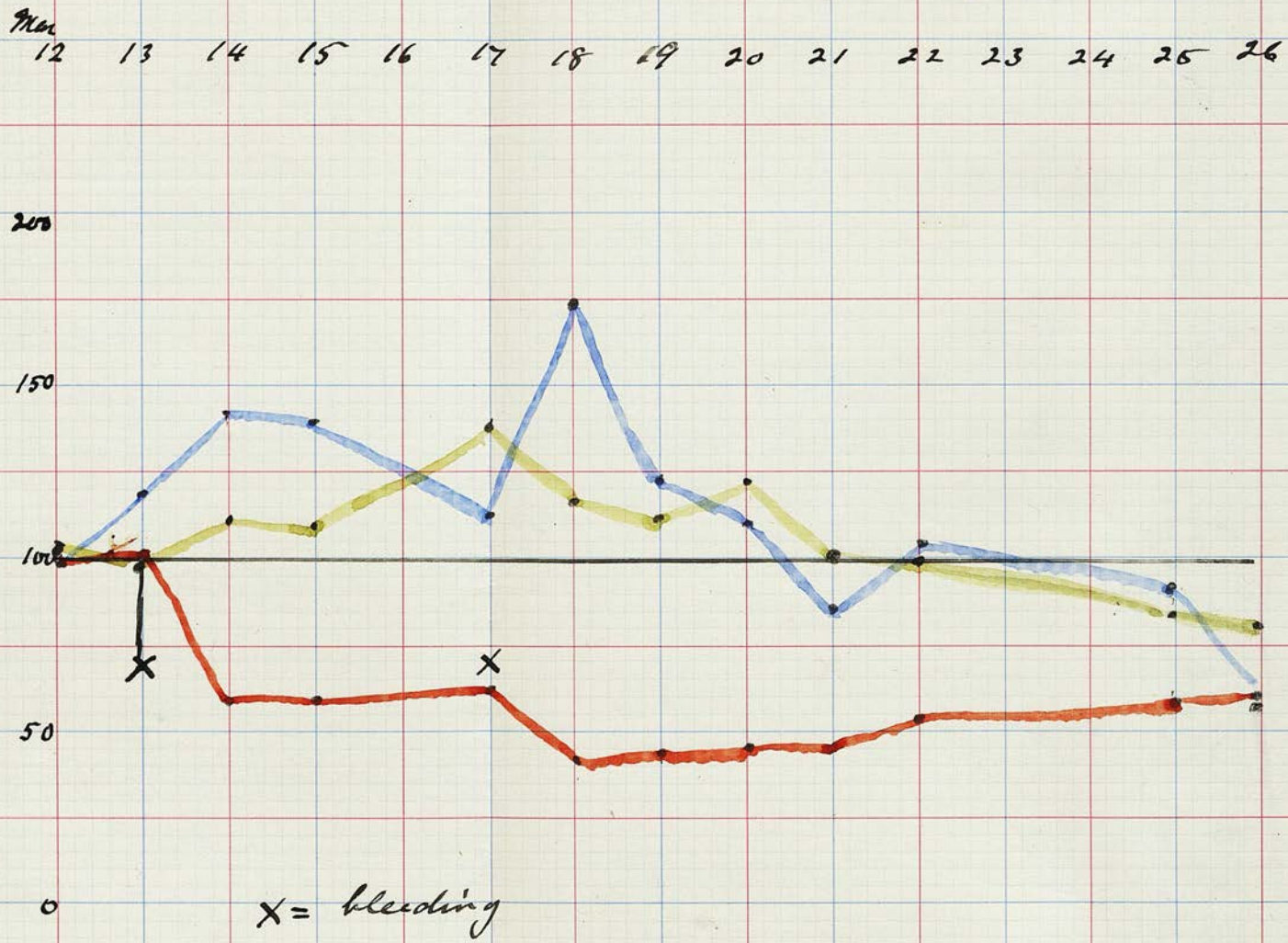
Experiment ~~XXX~~ IV.

The animal was a small fox terrier weighing 4·8 kilos and not more than eight months old. It was bled on two occasions.

On March 13th 130 c.c. of blood were taken from the right jugular vein. The minimum was reached on the day after by the red corpuscles, there being a loss of 44 p.c. of the original number per c.m.m. The leucocytes showed the usual increase with the maximum on the day after the haemorrhage. The blood plates in this case, differently from the previous ones, showed a considerable increase on the day after and on subsequent days.

The second bleeding was on March 17th which caused a fall of about 30 p.c. in the red corpuscles, the amount of blood withdrawn being about 110 c.c. The minimum was reached on the day after. About a week later there was scarcely a gain of a million per c.m.m. — showing that the regeneration was not going so rapid as in the previous experiment. The usual leucocytosis, most marked on the day after the bleeding, was seen

Dog III Exp. IV



subsequently diminishing. The number of the blood plates remained markedly increased for several days but gradually fell later, till before the death of the animal, it was much below the normal. On March 17th the red corpuscles were unaltered in character beyond showing often a somewhat softer consistence. On March 19th a considerable number were undersized — 6 μ in diameter and as few reached 8·5 μ. ~~On March 25th they were~~ A few were also irregular in shape. The same condition was seen on the following days.

The blood plates were of usual size on March 17th but larger forms — up to 4 μ in diameter were seen on March 19th, and on March 25th they were still larger. Accordingly on March 26th I examined the blood carefully in the fresh condition, in Hayem's fluid, in osmic acid solution and in the Sod. sulph. solution. In all these fluids the large blood plates were seen, nearly all of the largest size being of oval shape. In the osmic acid solution some were 6·5 μ long by 5 μ broad, being in fact as large as the smallest red corpuscles present. In all these solutions however they had all the usual characters of blood-plates and were sharply

v. Pl. 1 fig 4

depriced from the red corpuscles in all their respects.
In the fresh blood they changed their form as rapidly
as the smallest blood-plates.

Dried films showed that the alterations in the Leuco-
cytes were the same after the hemorrhages as in the
previous experiments. On March 15th two days
after the first bleeding the proportion of the two varieties
of leucocytes
was 1:4.6 2 c. the multinucleated corpuscles were much
increased in number. No nucleated red corpuscles were
seen. A few ordinary red corpuscles were more darkly
stained than the others. Blood plates of rather larger size
than usual but with the ordinary characters were seen.
On March 18th a few nucleated red corpuscles were seen
for the first time. Also many ordinary red corpuscles of dark
colour. Blood plates were beautifully seen up to 4·5 μ in
diameter. On all subsequent dates on which dried films
were made and examined viz March 19th 20th 21st 25th 26th
nucleated red corpuscles were formed in the blood. The
variations in the size of the corpuscles were also seen well.
On March 25th large blood plates were seen as described
above. Nearly all the larger forms were of oval shape
some measured 6 μ in their long axis and 5 μ in their
short. In their staining reactions they differed as much
from the red corpuscles

Summary The behaviour of the red corpuscles and Leuco-
cytes

was in close accord with what was observed in previous experiments, only the red corpuscles during the anaemia shewed much more variation in size and some irregularities in shape. Nucleated red corpuscles of typical appearance were seen immediately after the second haemorrhage and on the following days. The changes in the blood plates are more of interest. They increased in number after the first bleeding and remained increased till after the second haemorrhage when they diminished in number. Also after the second bleeding many much larger than usual were seen. There however shewed no alteration in character.

The rate of regeneration was not so rapid as in the previous experiment

The animal was killed under anaesthetics on March 27th. Before killing it I examined the blood circulating in the Spleen by making a small puncture in its capsule. The blood thus obtained contained a good number of nucleated red corpuscles, and the ordinary colourless corpuscles lymph elements &c. as in Experiment I. I saw also cells containing plague like bodies in their interior & Sub

I also made examinations of the Leucocytes and blood plates in the blood of the Splenic artery of the Splenic vein and of the Splenic substance obtained as above

I examined the blood first of the vein, then of the spleen substance and lastly of the artery. The results are

Blood of	Leucocytes	Blood-plates	Prop. of uninucleated to multi-nucleated leucocytes
Splenic artery	24,400	197,000	1 : 2·9
Spleen-substance	30,800	325,000	1 : 4·8
Splenic vein	25200	302000	1 : 1·1

This shews that in the passage of the blood through the spleen a considerable number of blood-plates is added to it, while the number of the leucocytes is very little increased. The numbers of the elements in the artery closely correspond to those of the blood taken from the ear, whilst those of the vein are the mean of two separate estimations which closely agreed with one another.

I also made films and examined the characters of the elements present. The blood of the artery shewed practically the same characters as that taken from the ear, the multinucleated leucocytes being much more numerous than the uninucleated. There were a few nucleated red corpuscles present.

In the blood from the splenic substance the large number of uninucleated corpuscles was the most striking feature. Some of these were of much larger size than those found in the ordinary capillary blood and sometimes two or three were lying close together. A considerable number of these no doubt were loosened from the spleen pulp

pulp and carried out in the blood. Nucleated red corpuscles were in considerable number, ordinary red corpuscles more darkly stained than the others in large numbers. There were many blood plates of large size.

In the splenic vein the proportion of mononucleated corpuscles was much higher than in the artery though the total number of white corpuscles was very little increased. Many uninucleated corpuscles were of very large size than usual and many in process of transformation into the multinucleated variety. Nucleated red corpuscles were scarcely more numerous than in the artery. Many darkly stained red corpuscles were seen.

In the splenic vein after death I found a few nucleated red corpuscles and many more colourless cells than during life. Also an interesting fact is that these colourless cells could be still further increased in number by pressing the organ and even spindle cells from the tissue of the spleen pulp could be made to appear.

The spleen weighed 19.5 grammes being .4 p.c. of the body weight. It was a little redder than usual but otherwise unaltered in appearance. In the vein pulp nucleated red corpuscles and colourless cells of similar structure were seen, in addition to the usual splenic corpuscles. Blood-plates were few in number showing that those

in

the blood from the splenic substance during life belonged rather to the blood in the spleen than to the pulp tissue.

Dried specimens of the spleen pulp showed nucleated red corpuscles in considerable numbers. They were best seen by methyl-blue-fuchsin staining, with which the haemoglobin-containing substance was stained a bright crimson. Their origin could be traced in the usual way from colourless cells. Their nuclei as well as those of their predecessors were deeply stained, the protoplasm of the latter being of a light purple tint, while the ordinary splenic corpuscles were stained diffusely and less deeply. The deeply stained variety of red corpuscle was also well seen.

The results of these observations with regard to the spleen may be summarised thus

(1) The number of leucocytes in the blood is increased very slightly in its passage through the spleen

(2) Their characters are however altered, multinucleated corpuscles being taken from the blood and uninucleated added to it as the former are not transformed directly into the latter and as the latter are constantly being formed in the splenic pulp, multinucleated leucocytes must apparently be broken down in the spleen

(3) Many colourless cells pass into the splenic vein

from the pulp at the death of the animal. This fact probably explains the statements made by some as to the blood of the vein being much richer in leucocytes than that of the artery

(4) Blood plates are added to the blood in its passage through the spleen. These are very abundant during life in the blood of the pulp and at the time of death nearly all pass into the vein

(5) The spleen after haemorrhage contains nucleated red corpuscles whose origin can be traced from colourless cells.

The bone-marrow showed analogous changes to those found in the previous experiment. The shafts of all the long bones contained red lymphoid marrow.

This was found to contain large numbers of nucleated red corpuscles of normal varieties. In most the protoplasm was well coloured with haemoglobin and the nucleus was generally about 4 μ in diameter. Some were in course of division. Their origin could be well traced from colourless cells, both in the methyl-violet-salt solution and in dried films. Many of these colourless predecessors seemed to be derived from larger cells of somewhat similar structure by a process of division. The nucleated red corpuscles were more numerous in the marrow from the shaft of the femur than from the vertebrae

transformation into fibrin

The early changes in the blood plates - the increased granularity and refractility, the assumption of the stellate form and the differentiation into two portions - take place with great rapidity, whilst the changes after the formation of fibrin has commenced are much more gradual. During the whole period no change can be seen in the leucocytes and certainly no signs of breaking down after the blood is shed can be seen

The assumption of the stellate form by the blood-plates has by some supposed to be due to part of their contents diffusing outwards - an explanation which is certainly plausible. The differentiation seems to be due to some change in their constitution on their death (?) by which the granules are allowed to come together by molecular attraction. Agent suggestion of the process is seen when water is applied (v. Sab) and in this latter case such an explanation would be applicable. Neither change can be considered to be a vital process as some (v.s. Hayem) suppose

These changes described distinguish the blood-plates at once from other small bodies such as small red corpuscles, fragments of red corpuscles or of leucocytes.

The number of nucleated red corpuscles found in the bone marrow in this experiment was considerably less than in the previous and this is probably to be associated with the fact the process of the regeneration of the blood was much less rapid.

Lymphatic Glands. In order to examine the elements properly first I excised one of the glands in the neck when the animal was still alive and immediately mixed a little of its juice with the methyl-violet-salt solution. I found the following elements. Small lymphoid bodies as described before were (v. Pl. 28) finely granular, they were perfectly circular, homogeneous or faintly granular and the largest were about 4 μ in diameter. The ordinary mononucleated lymph-corpuscles were mostly 6-7.5 μ in diameter and had the usual characters. Some, however, were considerably larger. There were also finely granular corpuscles containing a large single nucleus surrounded by a broad band of protoplasm. These were about 9-11 μ in diameter. In some of these, as well as in some finely granular corpuscles in which the nucleus was invisible, I found two or three plate like bodies lying very similar in appearance to the small lymphoid bodies lying free. (v. plate 28) On the addition of dilute acetic acid

where lymphoid bodies became more transparent thus differing markedly from the nuclei of the lymph corpuscles.

In dried films stained with Gentian violet, these small elements were well seen. They took the stain in varying degrees of depth but always much less deeply than the nuclei of the lymph corpuscles. Pl 28 Fig 1

In the thoracic duct immediately after death I found colourless corpuscles and red corpuscles as in Paper II. No blood plates were seen. In stained specimens, nearly all the colourless corpuscles were uninucleated, very few indeed being multinucleated. They were of various sizes but most were under 8 μ. I saw no nucleated red corpuscles.

In this as in the previous experiment the changes which could be brought into association with the regeneration of the blood were formed in the spleen and in bone marrow. In both of these, nucleated red corpuscles were more numerous than in health. Their origin could be traced from colourless cells and some were also seen in process of division.

No explanation of the large size of the blood plates could be found.

Exper V Rabbit 1

Date	Red Corpuscles	Leucocytes	Blood-platelets	Prep 1	Prep 2	Mic. Red Corps
Feb 30	5813,000	24500	588000	1: 242	1: 9.9	None
Mar 1	5863,000	21,000	610,000	1: 250	1: 9.6	—

Bled 12.30 p.m. 45 c.c. blood.

Immed after						
	5,338,000	21,000	~~688,000~~	1: 253	1:	—
" 2	3,364,000	23,500	682,000	1: 143.	1: 4.9	—
3	2,722,000	30,000	696,000	1: 90	1: 3.9	—
4	3080,000	25000	660,000	1: 120	1: 4.7	—
5	3645000	13000	530,000	1: 234	1: 5.7	—
7	2571000	11500	836,000	1: 324	1: 4.2	—
8	4,218,000	11,000	847,000	1: 383	1: 4.9	—
10	4445,000	10250	768000	1: 433	1: 5.8	—

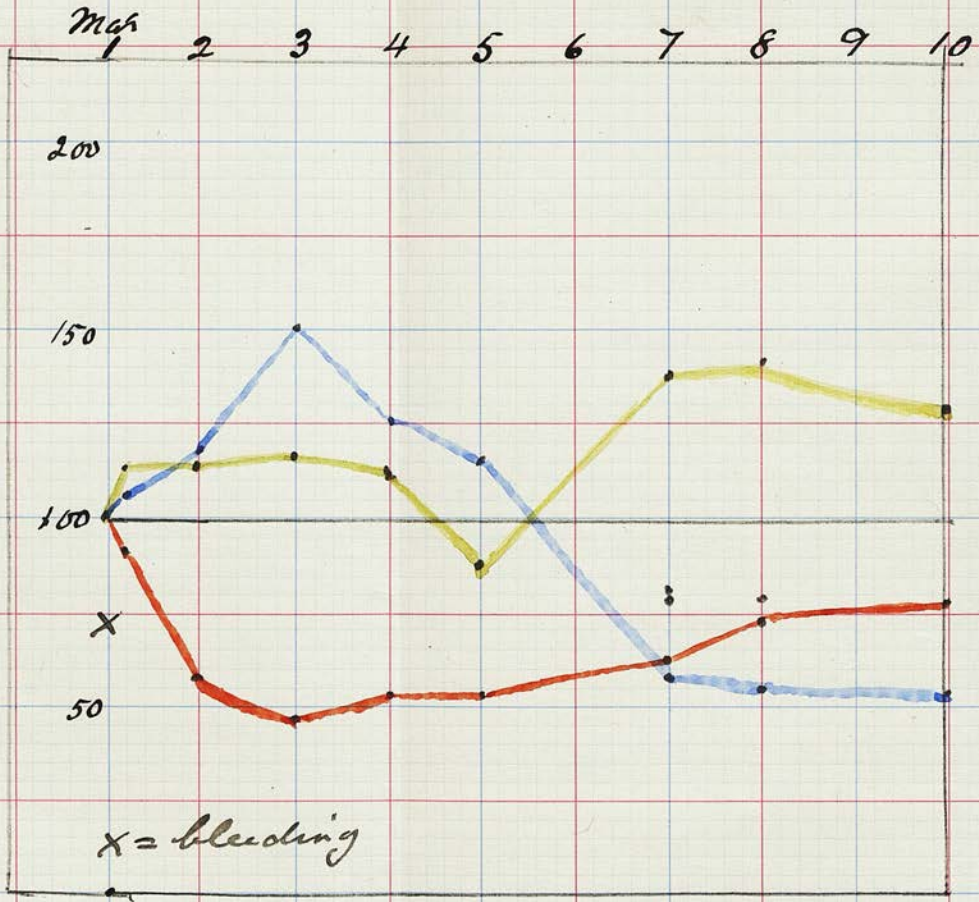
Experiment 5 Rabbit I

The animal was a healthy male rabbit weighing 2·2 kilos.

The blood examined before-hand ~~Stained~~ in its normal state shewed the red corpuscles to be of regular shape and practically all about 7·4 μ in diameter. The leucocytes were chiefly of two sizes as in the dog, the larger being 8–8·5 μ in diameter. Most of the larger size contained numerous coarse granules though not quite so coarse as those of the coarsely granular corpuscles of human blood. Stained films of the blood of the rabbit are much less satisfactory than of the dog, as both the red corpuscles and the blood-plates are of less firm consistence and stain with less definiteness. The leucocytes appear of two chief varieties viz the uninuclear and the multinuclear and in both of these are seen numerous granules which are stained of a deep purple colour with methyl blue. In the case of the multinuclear these granules are formed in the protoplasm chiefly at the surface and not, so far as I have been able to determine in the nuclei. V. Pl. 10

The animal was bled on March 18th at 12-30 p. m. 45 c.c. or 2 p. c. of the body weight being withdrawn from the jugular vein. Immediately after the

Exper V Rabbit I



bleeding the red corpuscles showed a fall of ~~about~~ half a million per c.m.m. The minimum was reached two days after the bleeding, when a much greater degree was shown than in preceding and corresponding bleedings in dogs. Afterwards repair set in and proceeded with great rapidity, about a million and a half corpuscles per c.m.m being added in the course of five days. The explanation of the marked fall in the number of the red corpuscles and of the rapid rise will be discussed later (v.p.) p.?

The leucocytes showed an increase after the bleeding, the maximum being on the second day after. The blood plates rose steadily in number and remained more numerous than normal (with the exception of one enumeration) till the animal was killed.

On March 3rd the fresh blood examined & undiluted showed already variations in the size of the corpuscles 4½ 6-8.5 μ. Most were undersized. They were also softer than usual. With the exception of the increased number of the leucocytes and the blood plates there was no other change. The latter were of usual appearance

On March 7th the blood was examined in Hayem's fluid. They ~~were~~ blood-plates were in enormous numbers but scarcely any reached, and none exceeded, 3 μ in diameter. The red corpuscles showed the same characters as (before

March 10th The red corpuscles varied considerably in Size 6-8.5μ. They shewed great softness and a tendency to early cremation. The blood plates as before were in very large numbers but were not of larger size than usual.

Dried films before the bleeding shewed the normal blood to have the following characters. The leucocytes were of the usual five classes uni- & multi-nucleated forms with intermediate forms in small numbers. A very large proportion of these contained coarse granules which stained a deep purple colour with methyl blue. These granules were found in both the uni- & the multinucleated leucocytes. A few of the red corpuscles shewed a slightly darker staining than the others.

On March 3rd. (after the bleeding) the leucocytes shewed the same changes though less marked than the days with the increase in the number of the leucocytes there was an increase in the proportion of multinucleated forms. Some of the multinucleated corpuscles had as many as seven nuclei. Intermediate forms were more abundant also. Red corpuscles of the more darkly stained variety were more numerous.

On March 4th Red corpuscles of the more darkly stained variety were present in enormous numbers. The leucocytes were as on March 3rd.

On March 10th Multinucleated leucocytes were much

fewer in number. So also the darkly stained red corpuscles though numerous were less so than before.

Throughout no nucleated red corpuscles were seen.

The blood-plates showed the same characters throughout on all occasions of examination.

The animal was killed on March 10th

Before its death I examined the blood in the splenic vein and in the splenic substance (by a prick in its capsule) and counted the leucocytes and the blood-plates. The numbers were

Blood from	Leucocytes	Blood-plates
Splenic vein	8300	756,000
Splenic substance	11500	804,000

The blood from the splenic substance swelling contained cells which had been washed out from the pulp, some of them cells were much larger than in the ordinary blood, reaching 16-18 μ in diameter.

Stained specimens showed that in the blood from the spleen substance most of the colourless cells were of the mononuclear variety; multinuclear leucocytes were few in number. But there were also seen those very large cells mentioned, which were darkly stained but showed map-like outlines on their surface inclosing more darkly stained areas. Cells of this nature belong to the spleen

and do not pass into the general circulation. I saw no nucleated red corpuscles and none of their colourless predecessors. Normoblasts & normal red corpuscles were in large numbers. The blood-plates were in large heaps of the usual character.

In the blood from the splenic vein, the mononuclear leucocytes either exceeded the multinuclear in number. Those large cells seen in the blood from the splenic substance were not present here. There were no nucleated red corpuscles.

I also examined the pulp of the spleen post-mortem and could find no nucleated red corpuscles.

Many of these facts with regard to the leucocytes and blood-plates are confirmatory of what was found in the case of the dog. Dogs III

Most interesting and important changes were found in the red marrow. (In the rabbit the red marrow is present relatively in large quantity, forming a pulpy tissue filling the cavities of all the long bones, from which it can be easily removed en masse).

When it was examined microscopically it showed as usual small lymphoid elements up to 4·5 μ in diameter homogeneous or finely granular. Marrow cells containing large numbers of coarse granules were also, as in the normal marrow present in large numbers. These were 8–14 μ in diameter. Striations marked off V. Pl. 26

in character from these, were large round cells containing one large spherical nucleus. Both nucleus and protoplasm had a fine ground-glass-like appearance. The largest of these cells were 12 μ in diameter. They varied in size down to 8 μ. Many of the smaller members of this class were distinctly tinted with haemoglobin. Many were also in process of division. I saw e.g. one of 8-form containing two distinct nuclei, the long axis being 18 μ and each nucleus 5·5 μ in diameter, with the protoplasm just tinted with haemoglobin. All stages of colouration with haemoglobin were seen — as a rule the smaller the cell proved the deeper the tint. Typical nucleated red corpuscles were present in large numbers. Most being 7·5 to 8·5 in diameter, the smallest nucleated red corpuscles was 6·5 μ in diameter with a relatively large nucleus. Many of these typical nucleated red corpuscles were seen in process of division, I saw one e.g. of 8 form measuring 8·6 μ in long axis with each nucleus 3 μ in diameter. In most of the nucleated red corpuscles the nucleus had the characteristic reticulated appearance though this was less marked than in those of the dog. I saw some with the nucleoles in the form of granules and in some did I see emigration of the nucleus. In some however the nucleus was of homogeneous appearance and less deeply stained. Many of the

(3) The blood-plates increased in number but were not altered in size or in character

ordinary red corpuscles were as small as 6 μ in diameter. The size of the non-nucleated red corpuscles was, on the whole, less than that of the nucleated and it was practically impossible to find nucleated red corpuscles corresponding to the smallest non-nucleated forms.

After the process of formation of the nucleated red corpuscles could be exceedingly satisfactorily traced and the process of division both in the nucleated red corpuscles and in their predecessors seemed much commoner than in the case of dogs. No segmenting cells were seen.

The lymphatic glands contained blastemal elements bearing granular corpuscles being very few in numbers (as contrasted with the condition in the bone marrow). There were no nucleated red corpuscles present.

Summary. In the blood the chief changes observed after the bleeding were the following.

(1) The red corpuscles showed greater variations in size (6-8·5 μ) and forms of less firm consistence and staining either more deeply appeared in large numbers. There were no nucleated red corpuscles seen.

(2) The leucocytes increased in number and the proportion of multinucleated forms became higher.

In the 'blood forming organs' the chief change was in the bone marrow. It there consisted in an increase in the number of the nucleated red corpuscles and

of

Eberth has shown that the change in form can be induced intravascularly by running a stream of cold salt solution over the mesentery of an animal He has also found that within the uninjured vessels of a piece of excised omentum the red corpuscles become crenated while the blood plates still possess their normal shape and also that in pressing with tolerably hard (cover) the red corpuscles break down in the circulating blood while the blood plates are unaffected.

The stickiness which the blood plates acquire in shed blood is a well marked feature and it is due in great degree their importance in pathological processes They can be seen to stick to the coverglass with great tenacity as is shown by the fact that when once adherent they cannot be dislodged by striking the cover-glass, nor even can they be washed away by a stream of water though they ultimately become broken up and dissolved by it. Within the vessels in normal conditions however they show no tendency to adhere to one another or to other objects. The red corpuscles have a great tendency to adhere to one another by their surfaces in a characteristic manner, while the blood-plates always attach themselves to one another by their edges or become united in an irregular manner. Neither is the crenation of the red corpuscles

Experiment VI Rabbit 2

Date	Red Corpuscles	Leucocytes	Blood-plates	Prep 1	Prep 2	Mic. Red Corps
Mar 12	6,501,000	10,000	563000	1:650	1:11	None
– 13	6091,000	9000	450000	1:676	1: 13	
– 14	6181000	9000	486000	1:686	1: 13	

Bled. 40 c. c. blood with churn

15	3,531,000	21,000	484,000	1:168	1: 7·3
17	3235000	15,000	620,000	1:214	1: 6·2
18	2,846,000	11,000	582,000	1:258	1: 4·9
20	3,490,000	8,000	740,000	1:436	1: 4·7
21	3455000	8,000	762,000	1:431	1: 4·6
22	3,701,000	8500	741000	1:435	1: 5·0
24	4,075,000	7,000	686,000	1:576	1: 5·9

their colourless predecessors with an increased frequency of both varieties of cells in process of division.

There was no evidence whatever of any formation of red corpuscles taking place either in the Spleen or in the lymphatic glands.

Note. The weight of the Spleen was 1·8 grammes, of the marrow scooped out from the cavity of the femur 1·8 gramme. i.e. the marrow pulp of the two femora exceeded the Spleen in weight.

Experiment VI Rabbit II

The animal was a healthy female rabbit weighing 1·85 kilog.

This Experiment was of practically the same nature as the previous. The operation of bleeding was performed once only viz on March 14th when 40 c.c. of blood (being 2·1 p.c. of the body weight) were taken from the left jugular vein.

Before the bleeding the blood shewed the normal characters as described in the previous experiment. The red corpuscles were regular in size and shape being nearly all 7·4 μ in diameter. In Stained specimens nearly all the red corpuscles had exactly the same appearance, but a few stained a little more deeply and of a more bluish tint than the others. Enumeration of the varieties of leucocytes was not satisfactory but the proportion of multinucleated forms

Exper. VI Rabbit II



forms was not high. A large number contained darkly stained granules as is usual.

After the bleeding the following changes were seen. The fall of the red corpuscles was very marked on the day after the bleeding but ~~and~~ the minimum was not reached till the ~~second~~ third day after, when the number of red corpuscles per cmm was ~~little more~~ less than half of the normal. Thereafter a steady rise in their number took place so that when the animal was killed - ten days after the haemorrhage - a great improvement was noticed. Here again a rapid leucocytosis was seen - most marked on the day after the haemorrhage, afterwards gradually diminishing. The blood-plates behaved much as in the previous case. A slight diminution in their number was noted on the day after the bleeding, but afterwards a considerable though somewhat irregular increase took place, which reached its maximum on the 7th day after - the same day, in fact as in the previous experiment. The difference in the time of appearance of the increase of the leucocytes and of the blood-plates is here well brought out in V. Chart

The blood was examined fresh at various stages. On March 18th the increase in the colourless elements of the blood was well seen. The red corpuscles were darker than

cised and retained their form less perfectly & good few were undersized

On March 21. Variations in the size of the red corpuscles were more common – many being 6–8·3 μ in diameter – a considerable number were oversized. There were also some slight irregularities in shape. The number of blood-plates was enormous, a considerable proportion of the field being occupied by masses composed of them. A similar condition was seen on March 24th

~~Stained films showed that after the haemorrhage~~

I examined the blood-plates carefully in Hayem's and the methyl-Sod. Sulph Solution and found very few having a diameter exceeding 2·5 μ while perhaps as regards size intervened between the largest and the smallest red corpuscles, whilst the characters of the two elements were widely different.

Stained films showed that on the day after the haemorrhage there was a great increase in the proportion of multinucleated corpuscles – this increase gradually diminishing on subsequent days as the leucocytosis disappeared. On March 17th there were seen a great number of red corpuscles which stained with methyl blue more darkly and of a bluish tint. These were evidently of less stable consistence than the ordinary red corpuscles as they were liable

Many of the largest corpuscles present traces of this nature

liable to become deformed in the process of drying.
These were seen in large numbers on subsequent
dates of examination till the animal was killed
No nucleated red corpuscles were seen. The blood-
plates showed the usual staining reactions and
appeared to fuse into irregular masses even more
readily than usual

The animal was killed under anæsthetics on May 24th.
Before its death I enumerated the leucocytes and
blood plates in the blood from the splenic substance
and in that from the splenic vein. The results are

Blood from	Leucocytes	Blood-plates
Splenic substance	11,500	744,000
" vein	6,500	736,000

This agrees closely with the results obtained in the
previous experiment though here the difference in
the number of the blood-plates is not so marked.
As before, the blood from the substance was found
(by means of stained films) to contain a larger
proportion of mononucleated corpuscles, some of which
were of much larger size, than the blood from the
vein. The proportion of mononucleated corpuscles
in the splenic vein however was higher than in the
ordinary capillary blood of the body.

I also excised a portion of the spleen containing

the blood which was circulating in it. I examined this blood, which was being pressed out of it, and then dissociated and examined a small portion of the Splenic substance. I found that in the blood (which could be readily pressed out) there were many coarsely granular leucocytes as well as more homogeneous corpuscles in which a single nucleus was visible in the Methyl-violet Salt-Solution. There were also many blood plates. In the Splenic tissue proper, however, I found exceedingly few coarsely granular corpuscles but nearly all the corpuscles being of the homogeneous uninucleated variety. The Blood plates were few in number. I therefore concluded that neither coarsely granular corpuscles nor blood-plates were proper elements of the Splenic tissue but that they belonged to the blood lying in the vessels and in the pulp. In no part of the Spleen which I made examinations in various ways did I find any nucleated red corpuscles.

In the red bone Marrow. I found the same striking changes as in the previous case. There was a great number of nucleated red corpuscles of normal appearance. There were also many colourless cells of homogeneous or gumed-glass appearance containing a single round nucleus surrounded by

a ~~[illegible]~~ kernel of protoplasm. The smallest of these cells showed a yellowish tint in the surrounding protoplasm, and all stages could be seen to the typical nucleated red corpuscle. A great number both of these colourless cells and of the nucleated red corpuscles were in process of division. Some of the cells just showing a trace of haemoglobin were 10 μ in diameter, whilst those nucleated red corpuscles with the perinuclear portion of a deep yellow colour were 8 μ or a little less. The process therefore seemed to be a rapid proliferation of colourless cells, by which many smaller cells of similar structure were formed; these smaller cells gradually acquiring haemoglobin and thus coming to form nucleated red corpuscles. The ordinary non-nucleated corpuscles = 6-8·6 μ in diameter, showed variations similar to, but more numerous than those in the blood. v. Pl 31 Fig 1

Coarsely granular cells were numerous as is usual. There were few blood-plates in the marrow of this duck.

In the dried specimens stained with methyl blue enormous numbers of the ordinary red corpuscles in the bone marrow stained darkly like the perinuclear portion of the nucleated red corpuscles v. plate 31 Fig 1

In the lymphatic glands no nucleated red corpuscles were formed. Nearly all the cells were

ordinary small lymphoid corpuscles. There were also many small lymphoid elements 2-3 μ in diam (Ehrlich) There were very few coarsely granular corpuscles

Summary The changes in the blood were practically the same as in the previous Case. The Spleen appeared to take no part in the formation of nucleated red corpuscles whilst in the bone marrow the process appeared very active. The non-nucleated corpuscles when first formed are probably those corpuscles described as being softer and staining more deeply with Methyl blue — this probably being due to their containing less formed material.

Note I may add that the Spleen weighed 1·5 grammes whilst the marrow pulp from the two femurs alone weighed 1·4 grammes

Summary of Experiments & General Conclusion

The changes caused by haemorrhage may be classified firstly as those more direct and partly physical due to the withdrawal of blood from the vessels and Secondly those associated with regeneration of the blood lost. We shall first consider the more immediate effects of the withdrawal of blood. In all the cases in which the blood was examined within a few hours after the bleeding a considerable fall in the number of corpuscles per. C. mm. had taken place, while in the case of Exper II. operated when the examination was made immediately afterwards no change was noticed. The fall in the number of corpuscles per c.m.m. can only take place by the dilution of the blood i.e. by an addition of fluid to the contents of the vessels and the anaemia will be greatest when when this dilution has reached its maximum. In the cases of the dogs in which there were eight ~~haemorrhages~~ bleedings, this point was reached in four cases on the day after the bleeding, in two cases two days after and in the remaining case three days after. It is interesting to note that in Exper I & Exper III (1) where the animal was allowed to drink copiously after the operation the anaemia supervened most rapidly. In the former case 2½ hrs after the bleeding half of the total fall

[1] Tyn Virchows Archiv. Bd 84. S 207

[2] Hmerfantt Virchows Archiv Bd 76. S. 310

[3] Jenaer Archiv f. Physiol. (Du Bois Reymond)
1878 S. 41

per c.m.m had taken place and in the latter nearly the whole, whilst in Exper III (2) 4½ hrs after the bleeding only about a half and in Exper III (3) three hours after only a third. In these two latter cases the animal was not allowed to drink. This is evidently to be explained by the addition of fluid to the blood which was provided for by the fluid taken into the stomach.

Lymph formed after bleeding dogs that the minimum number of corpuscles per c.m.m. was reached 2-6 days after and Hæmenfauth 1-9 days, but in these cases much larger quantities of blood were withdrawn generally 3·5 to 5 p.c. of the body weight. Hæmenfauth found that in some cases immediately after the bleeding no alteration in the number of corpuscles, in others a slight decrease while lymph generally formed a perceptible decrease (This decrease might be accounted for by the accident of the bleeding which took some time for its performance Howell showed by a number of experiments on dogs that so long as the blood pressure remained constant during the bleeding the haemoglobin did not diminish but when the bleeding exceeded a certain limit there was a rapid fall both in the blood pressure and in the haemoglobin i.e. the blood began to be dilated. In the case of three rabbits which I bled the number of corpuscles

strictly comparable with the change of form which the blood-plates undergo. The former is seen only when evaporation of the blood serum is allowed to take place or under certain other conditions and the projections are formed all over the surface of the corpuscles, while the latter is seen uniformly when the blood is shed and affects chiefly the outline of the blood-plates.

A little distilled water added to a preparation of blood made in the above manner causes marked changes. The red corpuscles are carried along in the currents which are formed and quickly become decolorised though their outlines can be seen for some time afterwards. The leucocytes swell up considerably the nucleus or nuclei become visible and the granules in the protoplasm exhibit Brownian movement. The blood-plates undergo interesting changes. If fibrin formation has become, both the heaps of blood-plates and the fibrin needles are more clearly visible, evidently due to the alteration of the refractive index of the medium. In some places nothing is seen beyond this increased definiteness. In others, and the same happens with isolated plagues, there is seen a transformation of the elements into clear vesicles which have a dot or point either in the centre or towards the periphery a small collection of granules. These vesicles may

Pl. I. Fig 6

fell to a level lower comparatively than in the case of the dogs and it was noteworthy that no considerable fall was found immediately after the bleeding. (Hunerfauth & Ellenndt also found in the case of rabbits a considerable fall immediately after bleeding them.) So again during the process of regeneration the number of the corpuscles in the rabbits increased sometimes with astonishing rapidity. (v
These phenomena may probably be explained by the fact that the vaso-motor system in the rabbit is much less stable than in the dog. Probably not only does fluid begin to enter the blood at once after the haemorrhage but it does so to an extent greater than is sufficient to restore the bulk of the blood to its normal, so that part of the anaemia is not a true anaemia but is due to increased bulk of circulating fluid. So also in the process of recovery in rabbits the rapid rise in the number of corpuscles per c.m.m. may be partly due to diminution in the amount of fluid contents in the vessels, associated with restored vaso-motor tone and not simply to the addition of corpuscles to the blood. It is certain that in the rabbit the number of corpuscles varies in a much more marked manner than in the dog or in man and some such explanation would be required.

Another very striking change associated with the

withdrawal of blood is the marked Leucocytosis which follows. This is always well marked, the number of Leucocytes being often more than doubled within a few hours after the bleeding. In four of the eight bleedings in dogs the maximum number of Leucocytes was found on the day of the bleeding — within a few hours after — and in the other four on the day after (In these last cases however the blood was not examined on the same day after the haemorrhage). The Leucocytosis lasts a few days and then disappears. On the other hand immediately after the bleeding there is no increase in the number of Leucocytes. I was glad to find that Lyon had obtained practically the same results. In all his cases, with one exception, a decrease in the number of white corpuscles was found immediately after the bleeding whilst a marked Leucocytosis followed later, generally within a few hours. Such a phenomenon is evidently to be associated with the withdrawal of blood and not with its restoration. What is the cause of this Leucocytosis? It has been ascribed by some to the well known properties of the Leucocytes so that they pass less easily out of the vessels than the red corpuscles. This explanation is rendered useless by the fact that immediately after the bleeding there is no increase as there would certainly (certainly

be if the Leucocytes were retained within the vessels. Others have ascribed it to a "sweeping out" of the lymphatic system consequent on the loss of fluid from the vessels. This theory is only plausible at first sight. It is quite inconceivable what forces could bring into play a more rapid flow of lymph through the lymphatics after haemorrhage. The fluid enters the vessels either directly from the tissues or from the alimentary canal. In the former case it is inconceivable that Leucocytes may pass back along with it. There is another explanation possible which so far as I know has been overlooked. After loss of blood the blood pressure is maintained by a contraction of the arterioles so as to accommodate the capacity of the vessels to the diminished contents. It is a well-known fact in most conditions the spleen contracts along with the arteries and accordingly in this case it will do so. The effect will be to cause a large number of Colourless Corpuscles to enter the circulation and as this passing out would be a gradual one it would take some time ere the Number per c.m.m. were actually increased. That Leucocytes can thus be squeezed out of the Spleen is proved by the fact that they are found in increased numbers in the Splenic vein after death and their number can be

still further increased by squeezing the Spleen.
The character of the Leucocytes added might throw some light on the Subject. Now, when the Leucocytosis is well established it is found that the increase is almost entirely on the part of the multinucleated corpuscles, that the number of large uninucleated forms is also increased as is also the number of intermediate forms, some of the latter evidently being in the process of division. Now so far as I know multinucleated corpuscles occur in large numbers only in the blood, in the lymphatics, & and Spleen pulp they are practically all uninucleated. Consequently the multinucleated leucocytes added to the blood after haemorrhage must be derived either from uninucleated corpuscles or from pre-existing forms by division. That the former method is the more probable is shewn by the large number of intermediate forms found in the blood after haemorrhage and I have observed that in the splenic vein many of the uninucleated corpuscles shew signs of transformation while exceedingly few in the thoracic duct do. Probably the uninucleated corpuscles from the Spleen develope much more rapidly into multinucleated corpuscles than those from lymphatic glands. I think it most probable for these

reasons that the leucocytes added to the blood after haemorrhage are derived chiefly from the spleen in the uninucleated condition and that they rapidly become multinucleated. In a few days when the number of leucocytes falls the proportion of multinucleated corpuscles also falls.

After the haemorrhage the blood plates shew a decrease for closely proportionate to that of the red corpuscles. (Hayem has found the same change)

We have now to consider what changes were found in the blood during the process of regeneration. During this period the blood-plates are sometimes increased in number sometimes not. In the Exper II there was a marked increase in their number from the time of the second bleeding, in Exper III a rise was noticed till after the third bleeding and in Exper IV a rise was noticed after the first bleeding. In the last case the increase lasted for some time, but the number of blood plates had fallen below normal when the number of the red corpuscles was still increasing. In the case of the rabbits the number of the blood plates was increased. In Exper I their number was distinctly diminished after the haemorrhage and remained so during the Restoration of the blood (which

took place with great rapidity. This shews that the increase in the number of the blood-plates during the regeneration of the blood is a common phenomenon yet it is not constantly present as Hayem believed.

~~No alteration in the leucocytes could be brought into relation with the process of regeneration~~. In Exps. II & IX the size of the blood-plates was much increased some in the latter case reaching 5μ in diameter. Their general properties however were not altered. In the other cases little or no alteration in their size was seen

No alteration in the leucocytes could be brought into relation with the process of regeneration.

As regards the red corpuscles, variations in their size were seen chiefly in Exper IX but even here the smallest were 6μ in diameter. Nothing was more clearly proved than, that the number of corpuscles might be rapidly increasing without the appearance of small forms in the blood. Young red corpuscles are therefore not necessarily of small size. Irregular forms were seen only in Exper IX in small number.

In the case of the rabbits considerable variations in size were seen especially in Exper VI where a considerable number were over size (a little over 8μ) as

well as undersigned.

An important fact is that in stained specimens a certain number of the red corpuscles were stained differently from the others. They took the stain either more deeply, and in methyl-blue preparations had a bluish grey tint, and in fuchsin preparations a deeper crimson than the ordinary corpuscles. They were also of softer consistence and were more liable to be folded or deformed in the film preparations. They were formed in all cases after haemorrhage. They were also formed in great numbers in the spleen & bone marrow of the anaemic animal. The point of interest connected with these corpuscles is the fact that in their mode of staining and in their general properties they resembled the perinuclear portion of the nucleated red corpuscles.

Nucleated red corpuscles appeared in all cases in the blood in dogs where marked anaemia was produced (less than 50% C. of the normal). They were never very numerous, perhaps not more than one or two to a hundred leucocytes. In appearance they resembled those of foetal life and those of the adult bone marrow. In this respect my results agree with those of Ponfick though he found them more numerous than I have done. I never saw any nucleated red corpuscles

in the blood of rabbits though ordinary red corpuscles occurred with altered appearance as above described Poikilocytes occurred in one case only Exper IV and that their number was very small.

With regard to the blood forming organs examined, undoubtedly the most important changes were found in the bone marrow. These consisted in a marked increase in the number of nucleated red corpuscles. Both in dogs and in rabbits there seemed to be derived from colourless cells possessing the same general structure. The origin of these colourless cells from larger cells by division of the latter could be specially well traced in the bone marrow of both rabbits. Also multiplication of the nucleated red corpuscles by division though found in all cases was more common in the case of the rabbits. It is specially to be noted that of all the cases, nucleated red corpuscles were by far most numerous in the bone marrow of Dog L (Exper III) and in the blood of the same animal destruction of the red corpuscles was most rapid. In dogs, the same process of formation, though to a less extent, goes on in the Spleen (which is in them a proportionately a much larger organ than in rabbits) but in rabbits I found no evidence that it had any haemopoietic function. Probably

these facts explain why nucleated red corpuscles so much more readily appear in the blood of the dog than of the rabbit. The number of nucleated red corpuscles which I saw with small granular nuclei was small- smaller than I we would expect on the theory that the nucleus gradually disappears by a process of atrophy. The rate of transformation however may be much more rapid than we imagine. I saw a large number of nucleated red corpuscles however in which the nucleus was somewhat homogeneous and ill defined, and stained less deeply than usual, having in fact the appearance as if it were being transformed in blood in the same way as the surrounding protoplasm. These results with regard to the bone marrow are in general accordance with those of others e.g. Neumann, Bizzozero, Litten & Orth, Seelmeyer, Tschirjew, Eckhard Feltmann, Gibson and others. The theory, therefore, that red corpuscles are invariably derived from nucleated red corpuscles is in harmony with biological principles and is amply supported by facts. The facts, that blood regeneration can take place without increase in the size and of number of the blood-plates, and that forms intermediate in quality are cannot be formed, are fatal to Hayem's theory, which in the presence of the other is quite untenable

I may repeat here the chief facts which I gathered from clinical observation which seemed to give support to Hayem's theory and consider also what theories regarding the origin of the blood-plates are probable.

I found e.g. that in cases of traumatic anaemia the number of the blood-plates was generally considerably increased and that their number decreased as the red corpuscles were restored. In such cases, however, the patients were not in a condition so nearly normal as can be obtained in experiments on animals. Besides, they were generally most numerous in cases of long-standing and often in association with pinkish cysts. In chlorosis too when blood-regeneration is going on their number is generally much increased but, on the other hand, the same condition is found in cases of chlorosis before treatment when the condition is rather becoming worse. A somewhat similar condition is found in the anaemia associated with cachexia where the anaemia is even progressive. Can these facts be explained on Hayem's theory? Hayem says that in such cases the blood-plates are produced in large numbers but fail to develope into the higher forms i.e. the red corpuscles, and the the explanation is plausible enough if the theory were correct. In pernicious anaemia too especially espec(ially

approach the size of a red blood corpuscle, though v. Pl. 1 fig 6
generally they are smaller, and shew a considerable
resemblance to the decolorized red corpuscles lying
around. Their origin however can always be clearly
traced. These clear vesicles can only be seen with
a careful examination and in a good light. The
addition of a little methyl-violet shews them
much more distinctly. It tints especially the granular
part and makes the outline much more clearly
visible. When this change has taken place a group
of blood-plates often presents the appearance of a
grape-like cluster of clear vesicles with a number
of small granular patches interspersed, doubtless
one for each vesicle. Sometimes a vesicle may
be seen to lose its granular part and then it
presents simply a regular circular outline with
no visible structure. This process can be seen in
blood which has been freshly drawn and treated
with water as well as after fibrin formation has tak-
ken. The change is probably due to an aggregation
at one part of the the granular material which occurs
in the blood-plate, the protoplasm in which it lies
becoming swollen up by endosmosis and rendered
more fluid at the same time. A similar change takes
place on the application of dilute acetic acid v. Pl. 2

if the case be a severe one the number of the blood-plates is often exceedingly small, and in the cases which are of a somewhat indefinite nature their number is a little though not markedly reduced. If pernicious pernicious anaemia, as seems almost proved, consists in a destruction of red corpuscles why do we not find as is common in traumatic anaemia, compensatory changes which would be indicated by an increased number of blood plates? Such a condition is only explicable on the supposition that at that stage of the disease the production of blood-plates was exhausted. In Malarial anaemia also the number of blood-plates is small, and here, at least in the persistent chronic form, the condition must be an impairment of the haemopoietic function. If we study the progress of certain cases very striking facts are found. I have seen cases of chlorosis, malarial anaemia and pernicious anaemia in which the number of the blood-plates increased more proportionately when that of the red corpuscles during the process of improvement and in one case of pernicious anaemia in which the red corpuscles had nearly returned to normal the blood-plates were in great numbers. I have also recorded a case of leucocythaemia in which towards its close the anaemia very rapidly

advanced, whilst the blood-plates almost disappeared from the blood. Still all these facts simply indicate concomitant changes in the two elements, explainable on a fuller knowledge of their physiology and not a transformation of the one into the other. Hayem in his work Du Sang figures the blood from an anaemic dog, and shews many small red corpuscles as small as the blood-plates but they are associated with poikilocytes, and this condition, so far as my experiment and clinical observations shew, is only present after along with some degree of Cachexia, generally after numerous haemorrhages. I do not therefore deny that, in some cases of anaemia due to haemorrhage, red corpuscles may be formed of as small diameter as the largest blood-plates but I strongly insist that rapid regeneration of the blood may be going on, and this is the common condition in a normal animal, without the presence of small red corpuscles and in the blood and without the fact, and sometimes also the number, (as Expt I) of the blood-plates being increased.

I have not seen the slightest evidence in favour of Afanassiews theory that the blood plate develops into a nucleated red corpuscle. This is virtually to make the blood-plates identical with the

small colourless cells in the bone-marrow which I have described (as have many others) as developing into nucleated red corpuscles. Now in Hayem's sodium-salt solution which he employed in his experiments the large blood-plates & these cells have a certain resemblance but the staining reactions and the general characters of the two bodies when examined in other ways are entirely different. &c. &c. So also the nucleus of the red corpuscle is widely different from the blood-plate, and if the former is set free from the nucleated red corpuscle in the process of development into the non-nucleated state it can only become a blood plate by undergoing important alterations, of which there is no evidence.*

A similar objection to that last stated may be brought against the theory that blood plates are the discharged nuclei of multinucleated leucocytes. Certain facts would make such an origin probable. The two elements show many concomitant variations in disease. In acute suppuration, in some other acute cases with leucocytosis, in some cases of lymphadenoma, and in a case of leucocythaemia I found the leucocytes and blood plates increased together; whilst in pernicious and in malarial anaemia there was a decrease (in

in the number of both. On the other hand in another case of lencocythæmia the blood plates were at first of normal number and afterwards much decreased, though the lencocytes increased in number. Now the two cases of lencocythæmia differed from one another in this, that in the former case there were many multinucleated lencocytes in the latter very few. So also in Exps. II, IV I found that the number of multinucleated lencocytes diminished in the blood during its passage through the Spleen whilst the number of blood-plates was increased. All these facts would make it probable that the blood-plates were the freed nuclei. In chronic however there is often a marked more or less scarcity of blood-plates with a scarcity of lencocytes especially of the multinucleated variety. But a careful study shews that the blood-plates differ in their structure, in staining reactions, and in their behaviours to acetic acid, markedly from the nuclei of lencocytes, so that I find it impossible to believe that the blood plates can have such an origin, unless the nuclei when set free undergo important changes of a degenerative (?) nature.

Regarding the theory most recently suggested by Hayem, that the blood-plates are derived from

ordinary non-nucleated red corpuscles, I can only say that I have never seen any appearance which would suggest the occurrence of such an occurrence or in any way resembling that figured by him in his work

There are therefore objections to the origin of the blood-plates from any of the elements formed in the blood, yet my observations shew that they belong properly to the blood and not to the blood forming organs, for if the latter be examined microscopically immediately after death when most of the circulating blood has passed out, very few blood-plates are seen. If, therefore, they are really derived from these organs they must change their character as they pass into the blood. Now in all the blood-forming organs, though in greatest numbers in the lymphatic glands, I have found small lymphoid bodies - not nuclei - of nearly the same size as the blood plates and these are probably of the same morphological nature as the blood plates. These are possibly derived from large cells, which Hayem describes as occurring in the lymphatic glands, but which are also found in the spleen and in the bone marrow. These cells can sometimes be seen to contain 1-3 such bodies of similar appearance. These small lymphoid bodies however do not change their form as the

v. page 273

v. p.

blood-plates do and they differ from them in certain other respects. Moreover I have found neither blood-plates nor these bodies in the thoracic duct and Hayem & Bizzozero also describe the lymph as being free from blood-plates. However from the Spleen and from the bone marrow these bodies might pass into the blood and become blood plates

I have simply stated the facts in favour of various possible theories but am not prepared to say which is the correct one as objections can be brought against all. The difficulty of the matter is shewn by the fact, that, though the blood-plates have been the subject of much research during the last few years, scarcely any statement as to their origin has been advanced with any degree of assurance. The possibility that they may be some albuminoid by a process of precipitation must not be overlooked, as they are certainly not nucleated unless in a much altered condition and neither have they the properties of the surrounding protoplasm or of the leucocytes.

I may briefly summarise my conclusions as to the development of the ordinary red corpuscles. In foetal and adult life I alike, I believe that their only mode of origin is from nucleated red corpuscles. In foetal life these nucleated red corpuscles

are abundant in the blood, and at a later period when they are scanty in the blood they are still numerous in the liver. About the time of birth, the place of transformation of nucleated into non-nucleated corpuscles is transferred to the bone marrow, where nucleated red corpuscles are formed, and the process of transformation goes on, during the whole of adult life. The nucleus of the nucleated red corpuscle is lost either by a process of atrophy or by its being simply pushed out with haemoglobin like by the surrounding protoplasm and thus losing its distinctive characters. The corpuscle which has just lost its nucleus still contains some living (?) protoplasm which gradually disappears during its subsequent existence. The result is that the young corpuscle stains more deeply than the older corpuscles and is besides of less firm consistence (A somewhat similar change ~~is seen~~ of staining reaction is seen in the development of squamous epithelium, the cells on the surface containing more formed material than the cells below) The nucleated red corpuscles are greatly increased in number often haemorrhage and some may pass into the circulating blood. This latter event happens sometimes in the human

(* This is in accordance with Lövitt remarks)

and with comparative frequency in the dog. I have never seen it in the case of the rabbit. Also often hemorrhage in a healthy animal many non-nucleated red corpuscles with altered staining reaction are formed in the blood. These corpuscles resemble in their staining the much of perinuclear portion of the nucleated red corpuscles and always make their appearance before the latter. Small red corpuscles, on the other hand, rather indicate a deterioration of the haemopoietic function than the presence of young forms in the blood. In the dog, the spleen has a similar function, though in minor degree, to the bone marrow. I have seen no signs of such a function being possessed by the human spleen or by the spleen of the rabbit.

The nucleated red corpuscles are denuclefom colourless cells of somewhat similar structure and both forms multiply by division. These colourless cells (furbelosen Vorstufen of Hirst) are of different structure from any of the forms of leucocytes of the blood and I have never seen any of them present in the general circulation. The leucocytes are, I believe quite a separate direct class of elements, and have no genetic relationship

Neumann. Virchows Archiv. April 1890

So far as I know, to the red corpuscles. This point is still the subject of discussion and Remann in his last paper rather inclines to believe that the small mininucleated corpuscles can pass out of the vessels and develope into red corpuscles. From my own observations however I am inclined to believe that the all the small mininucleated corpuscles increase in size and develope into the multinucleated variety, whereas the colorless predecessors of the nucleated red corpuscles are separate elements and are formed only in the place of development of the latter.

It is my pleasing duty in conclusion to record my gratitude to those who have aided me in my work. My best thanks are due to Prof. Greenfield who originally suggested the subject to me and has superintended most of my work, and also to Prof. Rutherford for kind assistance and advice chiefly in connection with the experimental part.

I have also to thank the Physicians & Surgeons of the Royal Infirmary, by whose kindness I have been allowed to examine cases in their wards.

Plate I Blood-plates in various conditions

Fig. 1 From fresh human blood

" 2 From human blood a few minutes after withdrawal from the body.

3 From human blood in osmic acid solution (1%)

4 From blood of anaemic dog in OS. AC. Sol

5 From human blood in Sod. Sulph. Sol. tinted with methyl violet

6 After application of water, then tinted with methyl violet

r = red corpuscle

l = Leucocyte

Plate 2. Blood-plates from blood of dog after treatment with acetic acid. Note the contrast in characters to the nuclei of the Leucocyte (l)

Their outline is rather too strongly marked

Note All these plates are drawn with magnifying power Seibert's numeral 7a. Zeiss Oc 4 unless otherwise stated

The swelling takes place as before but the contour of the vesicle is more easily seen than when treated with water. I think that in the employment of both reagents, the vesicle formed is larger and the granular mass smaller and more distinct if the reagent is added after some time than immediately on the blood being shed. The use of these reagents brings out a striking difference between the blood-plates and the nuclei of the leucocytes.

Alcohol beyond increasing the granularity ~~of the~~ causes little change in the blood-plates. It clings the fibrin needles very beautifully into view. A strong solution of carbonate of soda causes crenation of the red corpuscles and increased granularity of the blood plates whilst weak alkaline solutions cause their complete disappearance.

The changes described on the shedding of the blood can be retarded or prevented by the use of certain artificial media. These media, to speak shortly, are those which delay or prevent coagulation of the blood. Numerous certain combinations have been formed especially suitable, of which one of the most useful and best known is that of Hayem, as follows.

Aq. destill	parts 200
Sod Chlor	1
Sod Sulph.	5
Mag chlorid	5

Pl. I

Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 1



Plate
Fig 3. Silk fibres held for a few seconds in the blood flowing from the artery of a dog.
Note the large masses of adherent blood plates

p = blood-plates

r = red corpuscles

Plate 4.

Cell corpuscular elements from normal human blood.

Prepared by the method of sliding over a chilled cell. Fuchsin (alcoholic Sol)

l. = Leucocytes

p = blood-plates

r = red corpuscles

Pl 3



Pl. 4

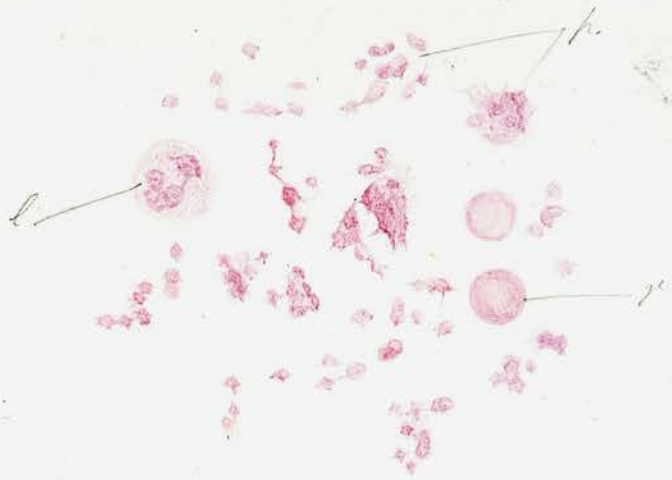


Plate 5. Corpuscular elements of blood, from a case of traumatic anaemia. Case I

Gen. mod. (watery sol.)

l = leucocyte

r = red corpuscle

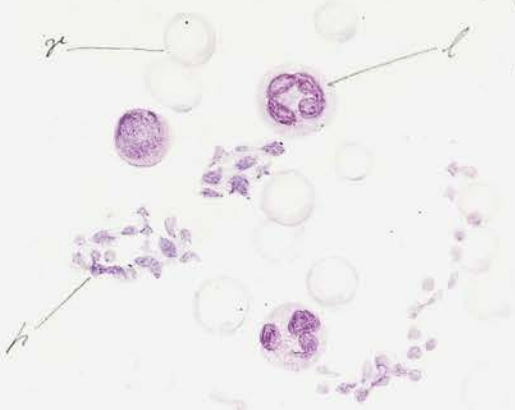
p = blood-plate

Plate VI do.

Methyl-blue-fuchsin.

Note that the blood-plates are stained differently from the nuclei of leucocytes

Pl. 5



Pl. 6

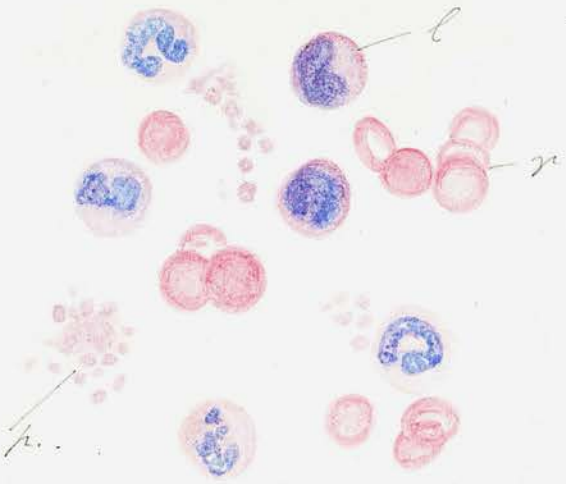


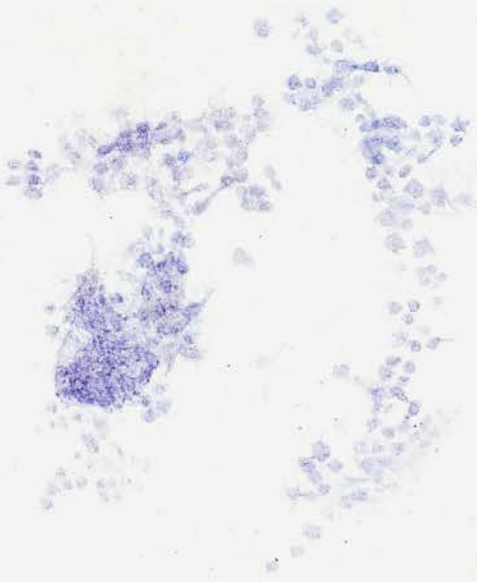
Plate 7. Blood plates from a case of severe Secondary anaemia Case 35.

Fixed in Hayem's Sol. Stained with Gent. viol. Note. All these blood plates were in one field of the Microscope.

Plate 8. Group of blood-plates from blood of anaemic dog. Exper II.

Gent. viol (watery Sol.)

Pl. 7



Pl. 8

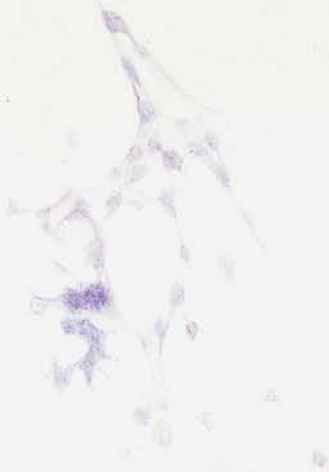


Plate 9.

Fig. 1 Leucocyte from normal blood.
Methyl-blue.

Fig 2. (a) Leucocyte dividing from blood of rabbit

(b) (c) Leucocytes from blood of dog, probably in process of division.

(d) Curious appearance of a leucocyte from blood of dog.

(e) Leucocyte dividing - division almost completed - from blood of dog.
In Osmic acid

Plate 10. Leucocytes from blood of rabbit containing coarse granules

Fig 1

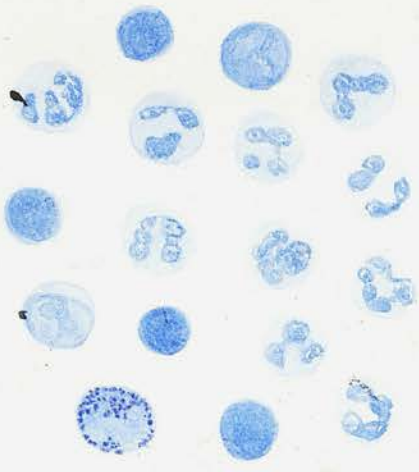
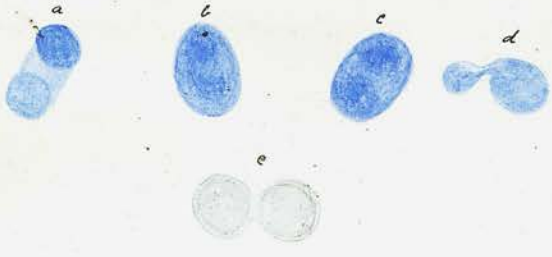


Fig 2



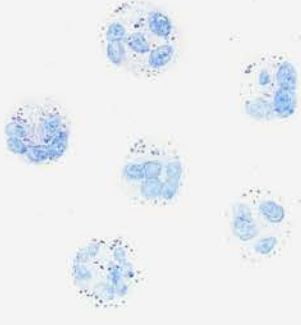


Fig. Plate 11. Shews development of multi-nucleated Leucocytes from mininucleated corpuscles, by a process of differentiation.
Methyl-blue-fuchsin

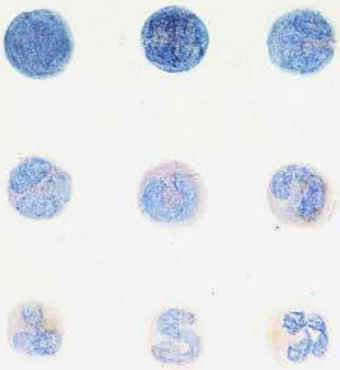
Plate 12. Leucocytes from a case of suppuration, shewing increase in the proportion of multinucleated forms.
(Somewhat more highly magnified than in the other plates)

It is useful both as a reagent in which to examine the blood in a fluid state and also as a fixing agent for dried specimens (& such) Hayem recommends that it should be mixed in the proportion of two parts to a hundred parts of the fluid to one of blood. The elements are well preserved though not perfectly so. Lakes has shewn that the central depression of the red corpuscles becomes deepened and their rim broadened.

Osmic acid in 1 p.c. Solution is said to fix all the elements of the blood in their normal condition. I think this statement to be doubtful at least with regard to the red corpuscles which tend to become folded in it whilst their diameter is certainly a little diminished. Hayem thinks that in this Solution the blood plates become somewhat shrunken (retracted) while in the osmic acid Solution they tend to be swollen (globules) and to lose the excavity which he alleges they possess. In the latter Solution they are exceedingly well preserved and measurements may be taken. They appear as small discs or lens-shaped bodies, composed of a finely granular material but otherwise shewing no structure. My belief is that some especially the larger, are really of oval (and not round) shape. In fact

v. Pl. 1. fig 3

Pl. 11



Pl. 12

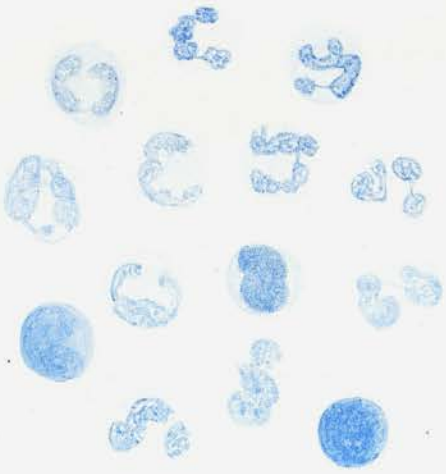


Plate 13. Leucocytes from a case of Pernicious Anaemia - Increase in the proportion of small uninucleated forms with diminution in the number of leucocytes per c.m.m. Note that the multinucleated are well marked off from the uninucleated and have as a rule many nuclei. Methyl-blue.

(Number of leucocytes = 1500 per c.m.m.)

Case 13

Plate 14. Leucocytes from a case of Leucocythemia.

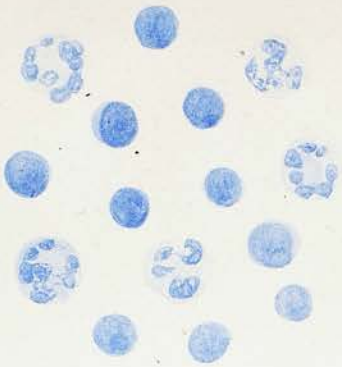
Case 51

Observe the great variety of forms and the presence of very large uninucleated corpuscles ('mnsters) -

(Number of leucocytes = 152,000 per c.m.m.)

Methyl-blue

Pl. 13



Pl. 14

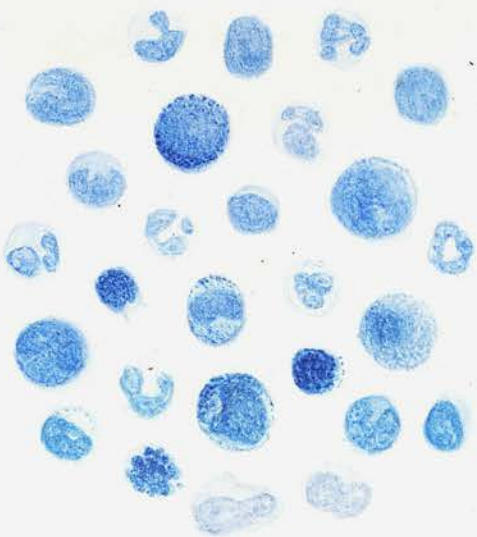


Plate 15

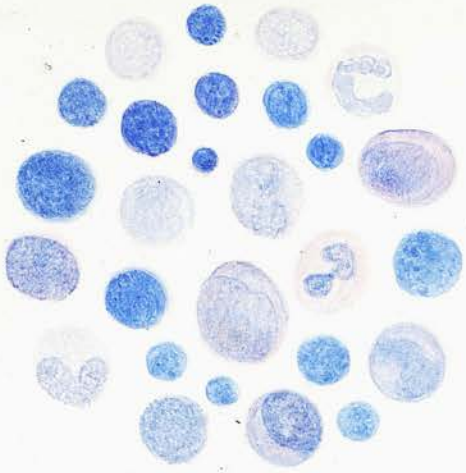
Leucocytes from a case of Leucocythaemia
Case 52.

Methyl-blue-fuchsin

Note the great paucity of multinucleated corpuscles (contrast with Pl 12) and the great number of mononucleated corpuscles of very different sizes. Number = 379,000 per c. m. m.

Plate 16. Low power view of a field in a dry-preparation. Same case.
The red corpuscles are bright red
" leucocytes " blue
Methyl-blue-fuchsin

Pl. 15



Pl. 16



Plate 17. Poikilocytes from a case of pernicious anaemia Case 14.

Fuchsin (alcoh. sol).

All the corpuscles were in one field of the microscope.

Plate 18 Poikilocytes from a case of traumatic anaemia Case 4

Selected from fresh blood.

(Rather less highly magnified than in other plates)

Pl. 17



Pl 18



Plate 19. Nucleated red corpuscles from human blood

Fig 1 Traumatic anaemia. Case 1

Meth. blue

r^1 = ordinary red corpuscle

r^2 " " darkly stained

Fig 2 do do

Fuchsin-Meth. blue

l = Leucocyte

Fig 3 From a case of pernicious anaemia. Case 13

Meth. blue

'Poikiloblasts' & 'Megaloblasts'

Plate 20. From a Case of pernicious anaemia Case 14

Nucleated red corpuscles of various forms

Some containing dark granules.

Meth-blue.

Pl. 19

Fig 1

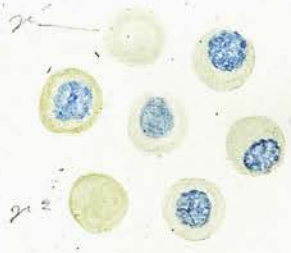


Fig 2

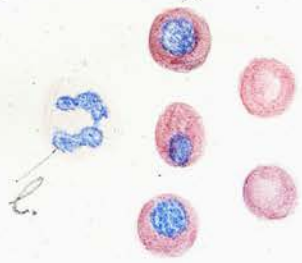
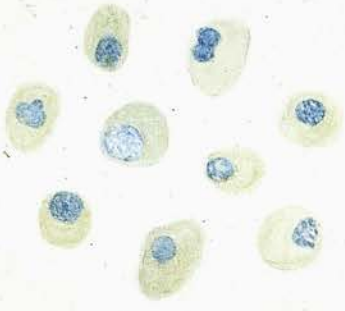


Fig 3



Pl. 20

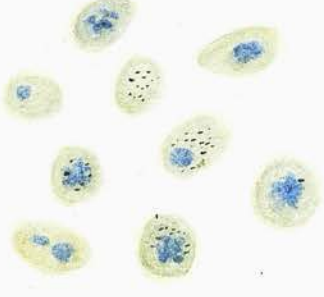


Plate 21 Type of blood in Secondary anaemia

r = red corpuscle

p = blood-plates

l = leucocyte

Plate 22 " " " Pernicious anaemia

n.r. = nucleated red corpuscle

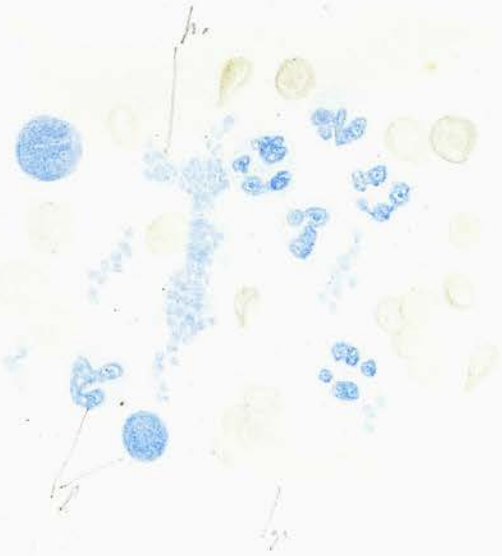
in the blood of a dog which I examined in which the blood plates were of exceptionally large size all the largest forms appeared to be of oval shape. Hewitt however says that they are all round. In this solution I have never seen any trace of a concavity on their surface which some of- ten they possess In a solution of sulphate of soda on the other hand a concavity can be sometimes seen with tolerable distinctness though I have never been able to see a double concavity on profile view. As however they have a more changed form in the latter solution and as most of those who have seen them in their unchanged condition within the vessels deny the existence of concavity we may conclude that when seen it is artificial and is probably only on one side, being caused in fact by a slight bending of the body. Neither in preserving fluids nor in fresh blood have I seen any trace of coloration in the blood-plates - though in osmic acid after a time both blood-plates and leucocytes alike acquire a somewhat brownish tint.

Vol. 1. fig 4

Vol 2 fig 5

The fluid employed by Bizzozero is a Methyl-violet - Solution composed of a 6 p. c.

Pl. 21



Pl. 22.

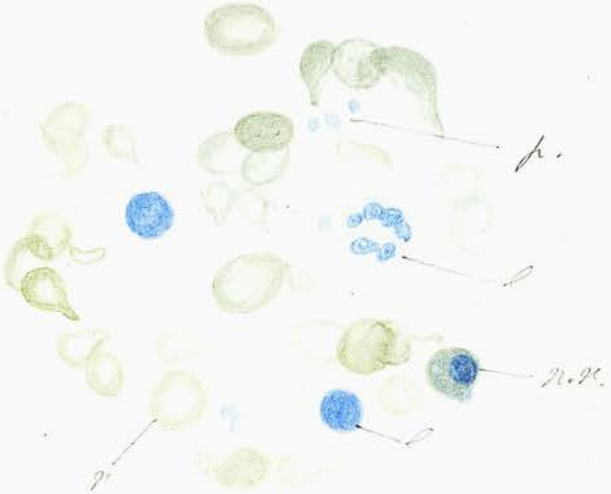


Plate 23. Cell-elements from the blood of a foetal liver (human 4½ months)
Methyl-violet-salt-solution

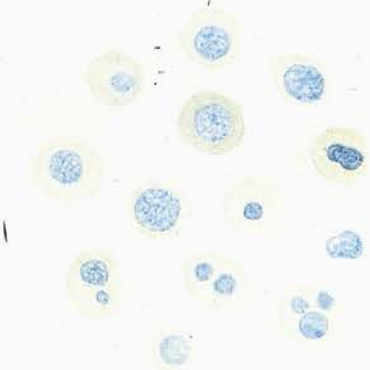
Plate 24 Fig 1. Nucleated red corpuscles from the blood of a foetal liver (same case)
Methyl-blue

Fig 2. " from the blood of heart. (same case)
Fuchsin-methyl-blue

Pl. 23

Pl. 24

Figs. 1



Figs 2

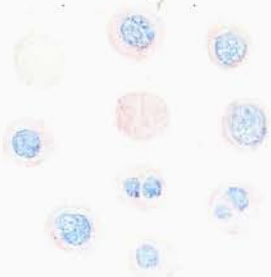


Plate 25 Elements from bone marrow of a dog.

Methyl-violet-Salt Solution

Fig 1 Small lymphoid bodies

Fig 2 Colourless predecessors of nucleated red corpuscles

Fig 3 Ordinary marrow cells

Fig 4 Nucleated red Corpuscles

Fig 5 Pigment-holding cells

Plate 26 Elements from red marrow of an anaemic rabbit dog. In same Solution. Ziegler V

Fig 1. = as 4 Figs 1 & 2 Pl. 25

Fig 2 Nucleated red corpuscles, many in course of division

Fig 3. Large finely granular marrow cells

Fig 4 Coarsely granular "

Pl. 25

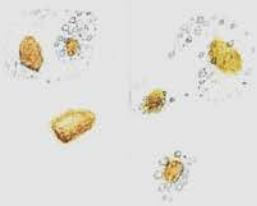
Fig 1

Fig 2

Fig 3

Fig 4

Fig 5



Pl. 26

Fig. 1

Fig. 2

Fig 3

Fig 4

Plate 27. Cell elements from the blood of spleen of anaemic dog. Eyepiece III Methyl blue

r = red corpuscle

n r = nucleated "

c.c. = colourless predecessor of previous

u.l. = uninucleated leucocyte

m.l. = multinucleated "

p = blood plates

x = (?) large "

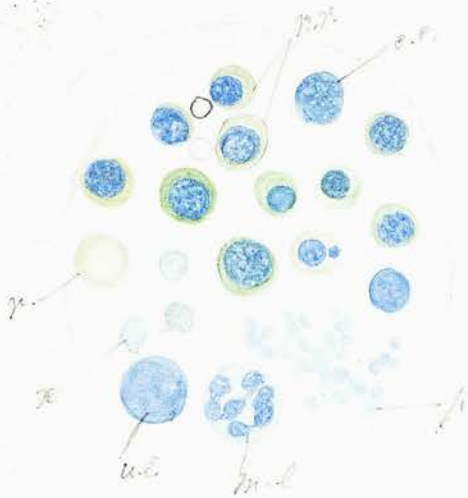
Plate 28. Fig 1 Elements from lymphatic gland, showing various forms of lymph corpuscles and small lymphoid bodies (l. bo). Dry III Eyepiece IV

Gent. viol (watery solution

Fig 2. lb = small lymphoid bodies from lymphatic gland

d = large colourless cells containing somewhat similar bodies.

Pl. 27



Pl. 28

Fig. 1

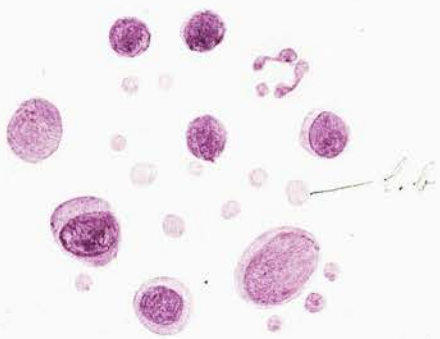


Fig. 2



Plate 29. Blood from anaemic dog. Zeiss II

Gent.-viol. (watery sol.)

Note the large size of the blood plates

p = blood plates

r = red corpuscles

l = leucocytes

Plate 30 Blood from anaemic dog. Zeiss III

Fixed in Hayem's fluid. Gent viol.

Only red corpuscles and blood-plates

are seen

Pl 29



Pl. 30

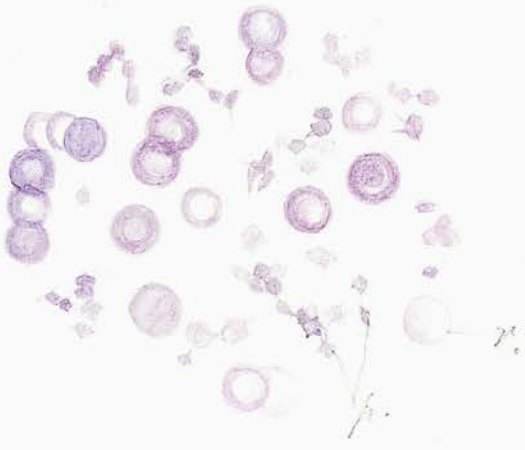


Plate 31. Fig 1. Nucleated red corpuscles in process of division from the red marrow of an anaemic rabbit. Exper VI. Methyl-blue (dry prep)
r = ordinary red corpuscles

Fig 2 Development of nucleated red corpuscles from colourless cells with reticulated nucleus, from the spleen of an anaemic dog. Exper II. Methyl. blue
c.c. = Colourless cells
n.r. = Nucleated red corpuscles
r = ordinary "

Fig 3 Nucleated red corpuscles from Exper III spleen of an anaemic dog. Division just completed
Methyl blue fuchsin.

Plate 32 Nucleated and ordinary red corpuscles from dogs, the two nucleated corpuscles shewing two shades of staining, the one resembling the perinuclear portion of the nucleated corpuscles.
Fig 1 From spleen of dog II Exper III
Methyl-blue
" 2 From bone marrow of dog II Exper II
Methyl-blue-fuchsin.
" 3 From splenic vein (post mortem) of
dog I Exper III
Gentian violet (watery solution)
Some blood plates at lower part

Solution of Sodium chloride to which methyl-violet has been added in the proportion of 1:5000 or Gentian violet in the proportion of 1:3000.
Afanassiew has invented a fluid which — a peptone-salt-solution — which he considers superior to any of the others. He takes advantage of the fact discovered by Pfeiffer that the injection of peptone into the blood of an animal prevents change in the blood plates when the blood is shed, the fact that such a procedure prevents coagulation having been previously discovered by Schmidt-Mulheim. Afanassiew's solution is composed of a .6 p.c. solution of Sod. chlorid. to which .6 p.c. dry peptone has been added. This is coloured with methyl-violet (about 1:10000) and a little carbolic acid or corrosive sublimate is added to prevent decomposition. I have used this fluid, both in qualitative and quantitative examinations, and have found it very suitable. In most of my enumerations however I have employed the ordinary haemocytometer fluid (Sol. to Sol. Sod. Sulph S.G. 1022 with Chloral added 1:1000) tinted with methyl violet. In this fluid the blood plates retain their

Pl 31

Fig. 1

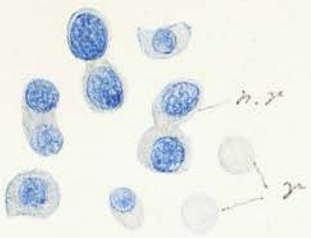




Fig 2

Fig 3



Pl. 32

Fig. 1



Fig 2

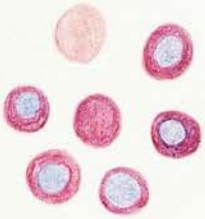


Fig 3

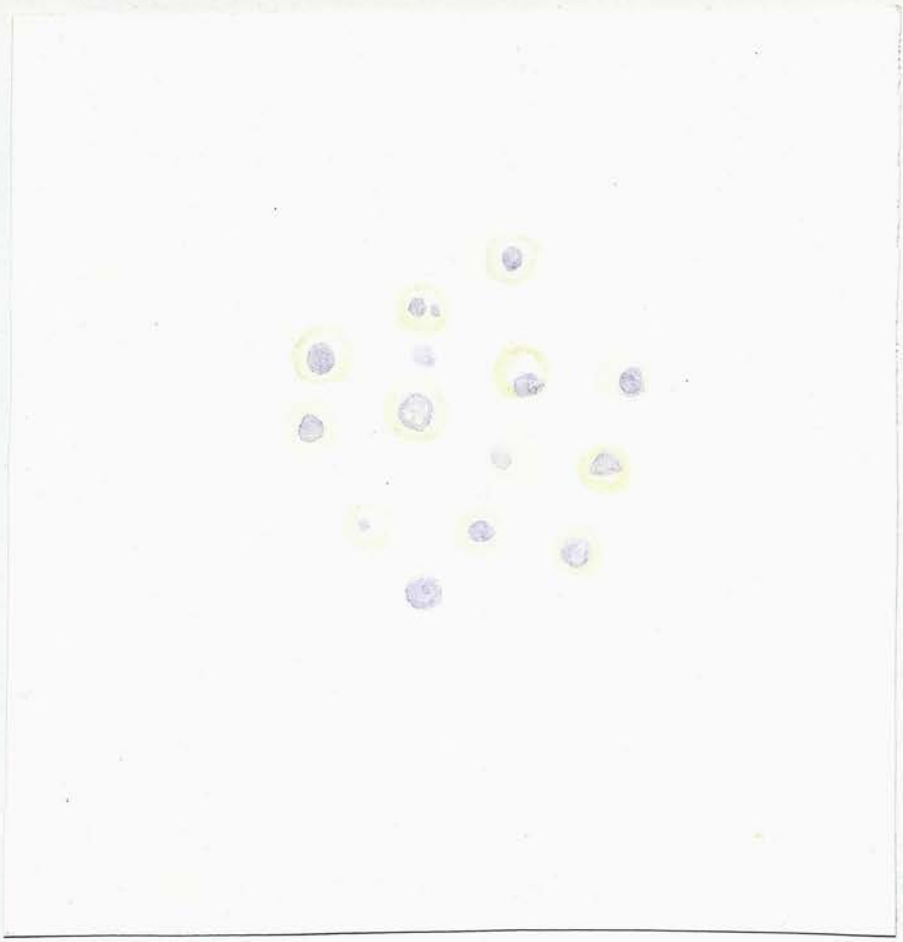


Plate 33. Nucleated red corpuscles of typical appearance from the bone marrow in a case of traumatic anaemia

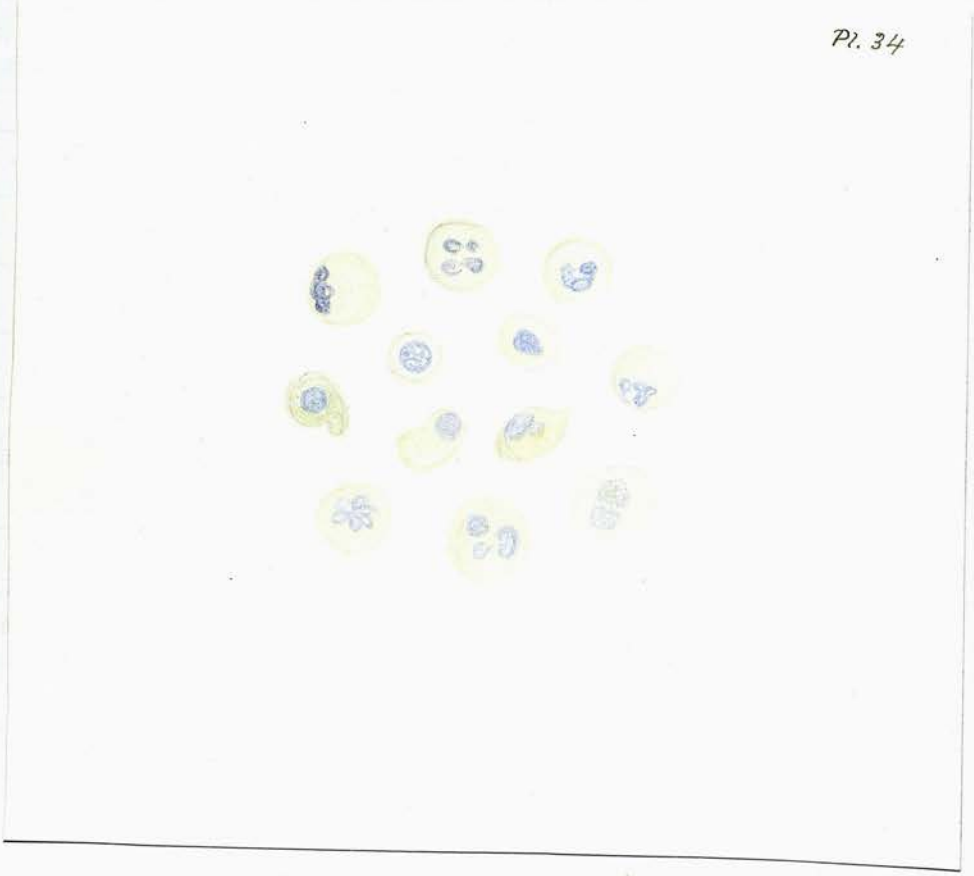
Methyl violet-Salt-Solution

Pl. 34. Large nucleated red corpuscles from the bone marrow in a case of Leucocythaemia. Note the peculiar arrangement of the nuclei.

Methyl-violet-Salt Solution.



Pl. 34



Pl. 35. Nucleated Cell-elements from the bone marrow in a case of pernicious anaemia.

Methyl-blue (dry prep.)

n.r. = nucleated red corpuscle

r = ordinary

c.c. = small Columbus cell.

Pl 36. Pigment- and Corpuscle-holding cells from the bone marrow in a case of cirrhosis of liver with jaundice.

Note that in some of the cells the outlines of the contained corpuscles can be seen, whilst in others only pigment

Pl. 35



Pl. 36



Pl 37. Schematic view of Staining Reactions of corpuscular elements of the blood

1 Ordinary red Corpuscles

2 Nucleated "

3 Blood-plates

4 Uninucleated-Leucocytes

5 Multinucleated "

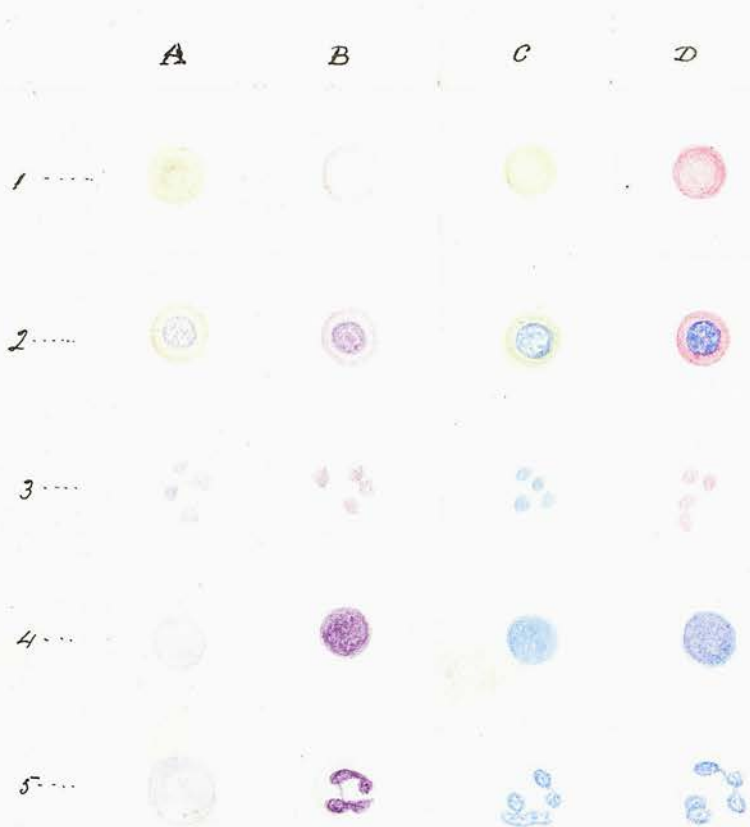
A Sod. Sulph. Sol. tinted with methyl violet

B gent. viol. (watery Sol)

C Methyl-blue (alcoholic Sol.)

D Methyl-blue-fuchsin (alcoholic sols)

} dry preps.


A
B
C
D
1
2
3
4
5

form little altered for several hours. In it they appear as little discs round or somewhat oval sometimes finely and uniformly granular, sometimes shewing central differentiation, and of a pale bluish tint. Generally their outline shews some irregularity or angularity and occasionally their surface presents an evident concavity.

None of these fluids should be kept for a long time and they ought to be repeatedly filtered. Especially the last mentioned.

The form of the blood plates can also be preserved and their characters studied by means of dried films of blood prepared in the manner described (p. 8 sup.); the films can thereafter be stained and their staining reactions compared with those of the other elements of the blood. The following are some of the chief forms in which the blood-plates are thus met with:- If the blood have been mixed with osmic acid before being dried the blood plates are seen as normal discs uniformly darkly stained and presenting no trace of differentiation or of any structure whatever. This we may consider to be the

blood plate in its truest and least altered condition. In specimens simply dried before being stained the blood-plates present various appearances according to the rapidity of the process of drying. Some appear round as in the previous case, some stellate and are uniformly stained throughout, whilst others of either of these shapes present towards the centre a portion rather more darkly stained though not sharply defined. Some of those which have a rounded outline shew slight spin(ous) processes and are evidently in a stage intermediate to the round and stellate varieties. Groups of blood-plates are also seen. Sometimes they are joined by their processes, sometimes they have become fused and form the well known Kölliker kanfen in which however the individual elements can be better seen than in the unstained condition. v. Pl. plates

Their staining reactions form a subject of great importance, regarding which there has been much variety of statement. Ranvier for example says that they are unaffected by staining reagents and therefore approach the protoplasm of the leucocytes in their chemical

Characters

Ranvier considers that they stain like fibrin, while Hayem finds only a trifling difference between their staining and that of the red corpuscles, - not sufficient to establish any chemical difference between them. According to Afanassiew, they stain in the same way as the nuclei of the white corpuscles, whilst Eberth and Schimmelbusch note some minor differences between them. My own results are that the conditions of staining affect materially the results and thus the above difference of statement can be explained to some extent

As compared first of all with the red corpuscles they stain in the same way if the blood be first received into osmic acid, dried, and stained with a diffuse stain such as gentian violet. On the other hand in the diluted sulphate of soda solution, or in Afanassiews solution, the blood-plates are stained a pale greyish blue while the red corpuscles are completely unstained. In dried specimens of stained with alcoholic solution of methyl-blue the blood plates are of a bright-blue colour and distinctly seen, the red corpuscles show a pale transparent green colour and are ill defined

v. Plates.

If stained with alcoholic solution of gentian violet they are of a bright crimson colour like the nuclei of the leucocytes and stained throughout, the red corpuscles are stained a duller and more purplish tint and often only on the surface. Many other examples of difference might be cited but these are quite sufficient to shew that the blood-plates and the red corpuscles are widely different from each other in their staining reactions and I have never seen any gradations between the two. Some of these differences are manifestly due to the presence of haemoglobin in the red corpuscles and to its absence in the blood plates but others indicate widely different structure and composition.

In no case in dried specimens do they stain exactly like the protoplasm of the leucocytes. They stain readily with the aniline dyes, whereas the protoplasm of the leucocytes remains unstained or is very feebly tinted. The nearest approach of the two to one another is seen in staining dried specimens with a weak watery solution of gentian violet. Even here however though a few of the blood-plates are like the protoplasm of the

Leucocytes in colour yet most are more darkly stained and if we consider their thickness we see that they really stain much more deeply than the protoplasm. For this reason, if for no others they could not be held to be detached pieces of protoplasm, pseudopodia of the white corpuscles &c.

Their staining reactions resemble most closely those of the nuclei of the leucocytes but are not identical with them. In staining with alcoholic solution of methyl blue or gentian violet the blood-plates and the nuclei have the same colour. Single blood-plates are less deeply coloured but this is due to their thickness for if we compare a heap of blood plates with the nuclei we find that they closely correspond. The same statement is true of anilin stains generally. The chief differences between the staining reactions of these elements are the following In double staining with fuchsin and Methyl blue v. Pl. 6 if a part of the film be selected where the nuclei are quite blue it will be found that the blood-plates are of a distinctly crimson colour and in double staining with gentian violet and methyl blue the where the nuclei are blue

blue the blood-plates are violet. Again if dried films be stained with weak watery solution of Gentian violet the nuclei hence a violet tint which the blood plates have not. If the substance of the blood plates have differentiated into two parts the more granular stains more deeply and I will show below that in the nuclei of the leucocytes there are two substances one of which stains deeply the other not. These two substances, however, in the case of the nuclei can by double staining be stained of different colours whereas the blood-plate shows always only one colour. I conclude therefore the blood plates are composed of a substance closely allied to nuclear substance ~~in its chemical nature~~ so far as its staining reactions show yet they are not identical with those of the nuclei of leucocytes. The behaviour of the two structures to acetic acid is moreover entirely different.

The size of the blood-plates varies proportionately more than that of the other elements of the blood. In the human subject in normal conditions it may be said to be from a little over $\frac{1}{4}\mu$ to about 3μ (1·8 - 3·6 Hlava & Schmidt, 1·5 - 3·5 Osler) Most are about 2μ in diameter. In cases of anaemia

blood-plates of larger size are often seen. In the blood of an anaemic dog I have seen them reaching 5 μ in diameter. With regard to the blood plates in other animals there appears to be a pretty general consensus of opinion that their size varies proportionately with that of the red corpuscles. Also in animals with nucleated red corpuscles the blood plates are said to be nucleated also (See the papers of Hayem Bizzozero & Hunter & Schimmelbusch)

The number of the blood-plates in health may be said to be 200,000 - 250,000 per c.m.m. though considerable margins on either side of these figures must be allowed. Hayem places the average number at 235,000, Hayem 255,000, Fusari 200,000 and Osler gives the number as 200,000 300,000. I have accordingly avoided speaking of their number as increased or decreased in pathological conditions unless the alteration is well marked.

In anemia ~~the~~ also their number varies considerably. I have seen one dog where blood plates remained steadily about at 150,000, while in another of the same breed the average number per c.mm. was about 400,000.

'v. op. cit Sub-'

Relation of the Blood-plates to Coagulation & Thrombosis

As the relation of certain of the corpuscular elements of the blood to the process of coagulation must be viewed from so many different aspects I shall not attempt a full discussion of the question but shall state briefly the ~~chief~~ main facts for and against the two chief theories.

Many facts have been adduced in favour of the theory that the blood-plates are the elements causally related to the process. Such a theory on the other hand has been objected to chiefly by those who fail to recognise the blood plates as real corpuscular elements and consider them destruction products, but also by some who look upon them as separate elements (eg. Hlava & Schmidt Sz.) Hlava eg. grants that they take part in the process of coagulation, but affirms that they are derived from leucocytes on the shedding of the blood, and Gibson also considers their being the cause as matter of certainty but yet does not admit their existence as separate elements. Those authors are accordingly in favour of the theory if the separate existence of the blood-plates is proved. With regard to the actual facts in favour of this theory the

following is a short summary of the most important

The blood plates are stated by many to be the only elements which alter their morphological characters before and during the process of coagulation. This is in direct antagonism to the theory of Schmidt and his followers according to which a very large number of leucocytes break down on the shedding of the blood. (Hayl 95. divides the leucocytes into α and β leucocytes according as they do or do not break down during coagulation) The various facts on which this theory is based I need not repeat here. This breaking down of leucocytes has been seen by no one, so far as I know, on observation of the freshly drawn blood though it can be examined within a few seconds from the time of its shedding and its occurence has been denied by most modern observers e.g. Hayem, Halla, Laker Osler Hunter & many others. Moreover when the blood is received into various preserving fluids the leucocytes are always in the same proportion whether the blood is mixed instantly with the preserving fluid or after some time. So also Biggens and others who have washed the corpuscular elements into vascularly while the

flow

't Huyen - De Sang p. 287

was retarded or arrested have found the Leucocytes to be apparently in much the same proportion as in the blood when shed. Hayem describes an interesting experiment bearing on this question. He excised a large vein and full of blood and kept it suspended vertically in a cold chamber (ice) for eighteen hours. By a puncture in the wall at the proper level he withdrew some of the plasmatic layer which is a clear fluid containing a very few Leucocytes. At the ordinary temperature an hour afterwards this fluid showed a well marked fibrin formation. The Leucocytes did not break down and become fewer in number during the process. He concludes from this that the formation of fibrin is not dependent upon a breaking down of the Leucocytes. That the blood-plates on the other hand do change their form is a fact admitted by all. Also the fibrin filaments are observed around the heaps of changed blood plates and in many cases the projections of the latter can be seen to be continuous with the fibrin filaments. In pathological conditions (eg. Severe Secondary anaemia) where the blood-plates are very numerous the fibrin network is very dense whereas where in conditions where they are few in number the

[1] Haycraft. Brit. Med. Journ. 1888 II

[2] Ruggeri v. Gal op. cit.

fibrin filaments are also very scanty. This rule however is not without exceptions, as I have seen (eg. in cases of pneumonia) the fibrin formation take place very rapidly and be very abundant while the blood plates were few in number.

Also, and this is a very important fact the means methods which prevent or delay coagulation also prevent or delay changes in the blood-plates Such are the addition of neutral Salt Solutions to the blood, exposure to cold, the addition of peptone to the blood and lastly the method of receiving the blood into oil (Hayecraft). Briggen whipped freshly shed blood with fine threads and found that these first became covered with blood-plates and thereafter fibrin was deposited upon them Also by running a mixture of blood and normal Salt Solution over fine threads he found & saw under the microscope, the blood plates deposited first on the threads and thereafter fibrin filaments. By defibrinating pieces of vessel and examining the contained blood at intervals. he found, that so long as the blood remained fluid the blood-plates retained their normal form. He further made experiments by means of peptone the fluid capable of coagulating on the addition of fibrin ferment

To various samples of this fluid he added, Saliva pieces of lymphatic glands, Spleen &c and threads covered with blood plates by whipping the blood. He found that coagulation commenced soonest and was most marked in the fluid to which the threads had been added. Saliva which contains no blood-plates however induced coagulation but Buzzozero considered that it might hold in solution the fibrin ferment derived from them. He does not deny the possibility that other elements may have the power of inducing coagulation but considers for the above reasons that the chief rôle is played by the blood-plates. Hayem also admits that leucocytes may furnish some material instrumental in bringing about coagulation, as in normal lymph which is spontaneously coagulable outside of the body, no blood-plates are present. In the blood however the blood plates are the chief agents. Eberth and Schimmelbusch on the other hand bring as facts against this theory that in the heart of a dog or cat 12 hrs after death little pieces of coagula are found with no alteration in the blood-plates and that in the blood of the portal veins no coagulation

' v. op. cit. Sub

may take place and yet the blood plates may shew alterations in form. They consider coagulation to be a process of crystallisation with which the blood plates have nothing to do. Even those facts which they mention, however, I think might be explained under Bizzozero's theory

On the whole therefore I think there is a preponderance of evidence that the blood-plates are the chief agents in the coagulation of the blood. It is however a subject on which fresh research is necessary especially on the part of those who have an intimate knowledge of the characters of the blood-plates.

A short account may be given of the part which they play in thrombosis, about which there is much greater unanimity amongst those who are competent to judge. Hayem, Bizzozero and more recently Eberth and Schimmelbusch have arrived at the same conclusion viz that the blood plates within the vessels be brought into contact with a foreign body or the vessel wall in an abnormal condition they undergo the same changes as they do outside of the vessels, keeping themselves up by their adhesive properties and passing together into a granular mass. If a thread be held for a

short time in the blood flowing from an artery
and be washed immediately afterwards in
Hayem's fluid, it will be found on microscopic
examination to be covered with blood-plates
more or less changed in form. I have tried
this for various lengths of time and have been
much struck with the myriads of these bodies which
gather in the course of a few seconds (v. plate 3)
Nothing can be more convincing of the
possibility of their being important factors in
pathological processes. So also I have inserted
a silk thread through the wall of a vein bringing
it out again at some distance (as Bizzozero did)
and have found that after a few minutes its
surface is covered with a smooth layer which on
microscopic examination is found to consist almost
exclusively of blood plates. On the surface separate
elements are well seen but deeper they are fused
together and their individuality is lost. Eberth and
Schimmelbusch have studied experimentally the
production of thrombi in the small and large
vessels both in cold-blooded animals and
in mammals. The thrombi were produced by
injuring the vessel-walls in various ways. In the
case of the small vessels the formation was watched

v. pl 3

under the microscope by the process described ~~p~~ below page 9, in the case of the large vessels, the portion of vessel injured along with the contained blood was hardened and sections were cut. In all cases they arrived at practically the same result viz that the condition necessary to the formation of thrombi was that blood plates be brought into contact with abnormal vessel walls. ~~In normal~~ In normal conditions, except at the branching of vessels where there are eddies, the blood plates circulate in the axial stream and do not touch the vessel walls, but if through any cause the stream be slowed sufficiently the blood-plates appear at the periphery. For example, if the vessel wall be painted with caustic, so that the internal coat is cauterised yet remains in position no thrombus is formed, but if it becomes detached and projects into the stream a thrombus is formed around it. So also if the internal coat be ruptured by a ligature which is afterwards removed so that the stream is restored through the vessel a thrombus ~~arises~~ forms round the edges of the ruptured coat which constitute the lumen of the vessel. In such thrombi the presence of leucocytes red corpuscles or fibrin threads is accidental.

Eberth and Schimmelbusch, and also Osler, have obtained confirmatory results by the post mortem examination of thrombi from various situations. These results are all in accordance with the known properties of the blood-plates and their number is certainly sufficient for the ~~process~~ purpose. It still remains to determine the exact limits of the part played by the blood-plates in the formation of the various varieties of thrombi. I have seen e.g. a thrombus from the right side of the heart ~~of~~ which the leucocytes certainly appeared to constitute a very large portion.

In the same way it has been experimentally proved that the blood plates have for similar reasons a most important function in the closure of wounds in vessels and in the stoppage of hæmorrhage.

Chapter III The Blood-plates - (1) Historical

Long before the nature of the blood-plates as separate elements of the blood was recognised, the granule-masses derived from them had been described by various observers and even their variations in disease noted. In giving an account of the literature of the subject we find that the earliest notices are chiefly of the blood plates in their altered condition and we have to determine whether the structures described are really the elements in question. As Osler remarks, the time since their first notice may be divided conveniently into three periods, the first extending up to the time of publication of Hayems papers, when the first full histological description of them as separate elements was given, the second like the appearance of Bizzozero's paper in Virchow's Archiv 1882, & the third since the latter date. During the second period they have attracted little attention, while during the last period they have been the subject of many papers and have provoked much discussion. Opposition to Bizzozero's views has come partly from

members of the Dorpat School who have endeavoured to identify them as destruction products and partly from Lovit who denies their existence in the blood in its normal state and attributes their appearance to the effects of abnormal conditions. The discussion extends close up to the present time as Lovits last paper appeared in Virchows Archiv for Bd. 117 1889

It will be seen that there are three subjects to which the blood plates have been brought into close relation by various investigators viz the development of red corpuscles, the formation of thrombi, and the coagulation of the blood. Perhaps so far back as forty years ago, they were observed, and affirmed to be the progenitors of the red corpuscles, whilst the close relation which they had to the fibrin threads in coagulating blood was also early recognised. These subjects, together with the question as to their pre-existence in the circulating blood, afford the points on which most discussion has taken place

Perhaps the earliest notice which can be connected with this subject is contained

Donné De l'origine des globules du sang.
Comptes rendus 1842 p 366

Zimmermann Zur Blutkörperchenfrage
Virchows Archiv. Bd 18. p. 21

in a paper by Donné on the origin of the globules du Sang, in which he describes an element present in the blood besides the well known red & white corpuscles. These globulins du chyle as he calls them are little grains about 3 μ in diameter and are like the globules found in the chyle. They are poured into the blood constantly with the chyle unite in threes and fours and become enclosed by an albuminous layer to form the white corpuscles, which in their turn become red corpuscles. It is quite impossible to say whether these bodies described by Donné are really fat-globules or blood-plates. The latter supposition would imply great inaccuracy of observation, but equally so does that which supposes the white corpuscles to be fat globules (nuclei) surrounded by an albuminous layer. The resemblance of the blood plates to nuclei has already been referred to.

About 1846 Zimmermann described a corpuscle which he called Elementar körperchen or Elementarbläschen. He wrote in Reich Magaz. f. d. gesam. Heilkunde which

Virchows Cellular Pathology p. 224

I have not been able to see. In 1860 however owing to a criticism by Virchow his views were repeated in Virchows Archiv. In that paper he refers to somewhat similar structures having been mentioned by Gerlach Arnold & Andral More special reference is made to Fr. Simon who had described small vesicular bodies formed in the blood after it had been allowed to flow into a solution of ferrocyanide of potassium and had assumed that the fibrin-material of the blood had been precipitated in that form. Zimmermann repeated the experiment & confirmed the observation. He however came to the conclusion that they are not composed of fibrin, though they are enclosed in the fibrin threads on coagulation of the blood and there often shoot out in a radiating manner from them. Unless therefore he says there be two kinds of coagulable protein bodies in the blood one of which coagulates in the form of molecules when the blood is shed even in the presence of neutral salt solutions and another later in the form of threads we must assume that these bodies are present in the circulating blood of

blood. They are round oval or rod-shaped and vary greatly in size, the smallest being 2-3 μ in diameter and quite colorless the others larger and feebly tinted with yellow. The latter are further developments of the former and ultimately become red corpuscles. Their various reactions correspond with those of the blood-plates. He believes that they are formed in the lymphatic system and enter the blood by the thoracic duct. He also proves that they are not composed of fat and finds that they are small vesicles filled with contents. He found them of different sizes in various animals and even in those with nucleated red corpuscles they were present and all stages could be found to the adult red corpuscles. His two chief tenets are that they are present in normal blood, not being formed by precipitation and that they are the forerunners of the red corpuscles. That the colorless forms of the Elementar-Körnchen are really blood-plates I think cannot be doubted even Dr. Schultze who discredits entirely Zimmermanns theory as to the method of blood formation admits that the small colorless forms are most probably identical with his Körnchenhaufen (6 and). He considers

[1] Beale Trans. of Phil Soc. in Quart Journ of Med Sc.
1864

[2] Kölliker Gewebelehre p. 220

that the larger forms, described as coloured yellow are derived from the red corpuscles by the method employed. It will be seen how closely in accordance with Zimmermanns theory is that of Hayem and to Schultze's criticism how similar are those passed on Hayem's hæmatoblasts.

Of very doubtful nature are the elementary granules described by Beale. There are according to him in the blood a great number of small spherical masses of germinal matter of the same appearance and refractive index as the white corpuscles. Some of these under certain conditions grow into white corpuscles whilst others become red corpuscles. The masses figured by him in his papers, however, appear much smaller in proportion to the red corpuscles than are the blood plates, and have scarcely their appearance.

Besides the coloured elements of the blood, there are, according to Kölliker, colourless elements in the blood of two kinds viz. 'Elementarkörnchen fettiger Natur und wirkliche Zellen'. The former are like those formed in the chyle and are composed of globules of fat with an albuminous envelope. As Schultze says these cannot be the same as the 'Körnchen' described by him

Schultze Archiv f. Mikrosk Anat. Bd. I. p 36. 1865

though it is difficult to understand how Kölliker so often met with them. Much more closely resembling the blood-plates are the free nuclei which he describes as occurring in the lymph 2·2 to 4·5 μ in diameter.

Under the name of Kernchen bildungen Schultze describes elements present in the blood in addition to the red and the white corpuscles, and these are without doubt the same structures as the blood plates. He finds in normal blood numerous heaps of colourless elements which vary greatly in size according to the number of elements composing the heap. These elements (Körperchen) measure 1–2 μ in diameter as a rule and there may be twenty or thirty in a heap. His account of their shape, size, general appearance & behaviour to reagents agrees very fully with the facts known of the Blutplättchen. He also notes that they can be recognised in films of blood which have been dried. They have no power of amoeboid movement when examined on the warm stage. In describing the change in form which they undergo he observes that on the commencement of coagulation numerous fibrin filaments run

through the heaps and in many cases present the appearance of originating from them As regards the nature of the elements he considers that they are composed of an albuminoid substance and that they are rather to be looked upon as destruction products (Detritus bildungen) than as separately existing elements. Yet he advances this view with caution, as is shewn by his hesitation to apply a suggestive name, and his preference for an indifferent one (as he himself says) viz. 'Kornchenbildungen'. This refers with regard to Zimmermanns corpuscles have already been given. He recommends his Kornchenbildungen as good material for study and mentions that it may be more than fortuitous, that he found them specially numerous in the blood of an anaemic woman. Concerning their staining reactions, their preservation in fixing fluids and their presence in circulating blood he had no knowledge. Had this been the case we may fairly assume that his view that they are detritus-products, advanced so cautiously would have been quite changed.

With regard to the nature of the bodies described

[1] Bulletin Central f. d. med Wis. 1868 p. 345

[2] Ruis Zur pathologischen Anatomie des Blutes
Archiv f. Anat. u. Phys. 1872. p. 237

by Bettelheim it is impossible to give an opinion. 'Stäbchen ~~artige~~ formige Körperchen' of the size of half a red corpuscle or less were found by him in the spaces between the rouleaux of red corpuscles both in health and in disease. These bodies had a to and fro movement. The blood plates certainly have such a form when seen edgewise but on the other hand they do not exhibit any such movement.

In 1872 Reis wrote a paper containing a description of certain bodies formed by him in the blood, and important statements with regard to their variations in disease. He considered them to be derived from the breaking down of white corpuscles in certain conditions and called them therefore Zerfallskörperchen. He looked upon them as practically abnormal elements. In harmony with this view he found them numerous in circumstances where owing to the state of the blood the vitality of the white corpuscles was affected. There are two principal groups of such conditions. First, in acute diseases they are present only in small numbers during the beginning and height of the fever, but become very numerous towards the end.

Their increase, therefore, is not due to the high temperature but to the degree of malnutrition produced. (A similar fact has been observed by later observers but a different explanation has been given) Second they are increased in wasting diseases. Such as phthisis Carcinoma &c They are also increased in leucocythaemia and in anaemia due to loss of blood. He says that all transition forms can be traced between the perfect white corpuscles and those small bodies He also describes their behaviour to reagents, and ingeniously explains their differentiation into a large clear and a small granular part on the application of acetic acid, by saying that the greater part is related to the stroma of the white corpuscle a small part to the nuclei. It is however difficult to understand how a small part of nuclear substance should be found in each Kölliker in such a regular manner He considers from their comparative scarceness in normal conditions that they have nothing to do with coagulation but he mentions that in pathological conditions they may come to form emboli in the small vessels. From the description given by him and from the drawings

[1] Virchow Centralbl. f. med. Wiss. 1872 S. 115

[2] Hedzinsky " " 1873 S. 147

[3] Zeph Tschinsky " " 1874 p. 657

which are appended to his paper it is apparent that his Zerfallskörperchen are really blood-plates in their altered condition.

About the same time are a number of short notices on the same subject. Gastropexe mentions somewhat similar bodies as being characteristic of the blood of syphilitic patients and [2] Hayem describes small round cells (Sang kleine runde Zellchen") which are found in the spaces between the red corpuscles These the latter says were never absent in the specimens of blood which he examined. He also notes that on the formation of fibrin they apply themselves like knots to the fibrin threads at the points where these cross one another. He considers them to be derivatives of the white corpuscles. It is probable, especially from the relation to the fibrin threads which he notes, that the structures here described are really blood plates. [3] Laptschinsky also in a paper on the alterations of the blood in disease makes reference to the Körner haufen. He says that they are specially numerous in cases of high temperature either during the fever or after defervescence. They are present

'65 Gr. Proc. of Roy. Soc. 1874

Osler & Schaefer. Centralb. f. med Wiss. 1873, 1874

according to him in small numbers in cachectic conditions where there is no fever. He describes a case of tubercular meningitis in which the detritus masses in the blood were in some places so great that not a single white corpuscle was to be seen. It is doubtful from this whether he was not really describing under the name of Körnchenhaufen detritus masses of leucocytes as he himself believed. This statement with regard to cachectic conditions is quite exceptional.

It was in the condition of granule masses that these bodies were known to most observers of the above mentioned observers and their corpuscular nature was almost entirely overlooked. In 1874 Osler wrote a fine description of these masses and the changes which they undergo in certain conditions. He recognised that these masses were composed of elements of a corpuscular nature whose diameter varied from an eighth to a half of that of a red corpuscle. He perceived that it was quite impossible for such masses to pass through the capillaries and an investigation formed that it was only when the blood was shed that they occurred in the aggregate form. Within the vessels of the subcutaneous tissue of the new-born rat he observed the same

Ramuz Traité technique d'histologie p. 213

structures but quite separate and having no tendency to adhere to one another. To him belongs the credit of first observing them within the vessels in their unchanged form and of clearly recognising that their change of properties and of appearance was caused by the shedding of the blood. He further described changes which the masses underwent after being kept at a temperature of 37°C in salt solution ($\frac{3}{4}$–$\frac{1}{2}$ %) He found that they broke up into variously shaped filaments which with swellings on them which detached themselves and moved away from the heap so that the surrounding fluid became swarming with them. He considered them to be living organisms. In his later papers he makes no mention of this phenomenon.

"Ranvier describes, in his work on histology, 'granulations libres' of two kinds as occurring in the blood besides the ordinary corpuscles. The first are spherical like little drops of fat while the second are angular and present an appearance like the débris of the white corpuscles from which however they differ in their physico-chemical reactions. The chief point of interest in his description is that when fibrin formation

'Hagem Archiv. de physiol. 1876. 1879

takes place he finds that the fibrin filaments always set out from these angular bodies and never have any special relation to the red or white corpuscles. He finds their staining reactions the same as those of fibrin and explains their appearing rather more deeply stained by their greater thickness. He considers them to be preformed pieces of fibrin which act as centres of coagulation. As they are seen as soon as the blood is shed, he inclines to believe that they exist in the circulating blood.

It is to Hayem that we are indebted for a complete description of these elements and for placing them on a firm histological basis as normal elements of the blood. His papers were communicated towards the end of 1877 and published in the following year. The two facts which he especially strove to prove that these elements are the progenitors of the red corpuscles and that they are the means by which coagulation is brought to pass. He calls them therefore haematoblasts, and describes them as round biconcave discs tinged with haemoglobin, their characters being most marked in those of largest size. The colour is most marked when they are examined quite fresh and is soon dissolved out by the

surrounding plasma. Most preserving fluids act markedly in the direction of removing the haemoglobin. The size which he gives is on the whole greater than that given by most observers, being in fresh blood prevented from coagulating by a low temperature 1·5–4·5 μ. In dried specimens, which change to any he considers, a more accurate means of measurement he finds some haematoblasts with a diameter as great as 5·75 μ while he gives the average diameter as 3 μ. They are non-nucleated and probably consist of a stroma with contents. The change of shape is associated with a retraction of the stroma and an exudation of a thick substance which connects various haematoblasts together. The biconcavity is rather exaggerated he thinks in his preserving fluid while in osmic acid it is lessened many of the haematoblasts becoming swollen and vesicular. (Osmic acid however is generally recognised to be the best preserving agent which we have for such purposes) The haematoblasts many of which are large biconcave and containing haemoglobin become red corpuscles by a change in their physical properties, becoming more stable and acquiring the characters of the adult red

corpuscles

In certain forms of anaemia therefore, he observes, there are present small red corpuscles in the blood of a diameter smaller than that of the largest haematoblasts and as both of these bodies are often increased together he considers that in such conditions there is quite sufficient, if not an actually increased production of the progenitors of the red corpuscles but there is a failure of complete development into the higher forms. Such is Hayems theory, which I have given at some length as its importance is considerable. It may be noted however that the chief points in support of his theory viz. the large size of the haematoblasts, their biconcavity and their colour are exactly those on which most observers differ from him. In support of the second of his propositions viz. that they are the agents concerned in the formation of fibrin he brings forth weighty evidence. They are the only elements of the blood which undergo any change during the process, the fibrin filaments shoot out from them, where their change is retarded fibrin formation is incomplete and where it is prevented no fibrin filaments are seen at all. He also examined the blood of various animals and never found

[2] Huyens Archiv. de phys. norm. et path. 1883 p 363

[3] Jendrassik Berlin klin Woch. 1879 S. 653

[3] Reis " " 1878 p. 696

these bodies absent. He also says that they vary in size with the red corpuscles and where those are oval (as in the Camel) the haemotoblasts have that form also. Later Hayem by prolonged staining with haematoxylin thought that he revealed the presence of a small central body in the haemotoblasts which stained darkly and which he supposed to be a nucleus. Ehrlich and Ogmanowicz agree in believing this to be merely a small collection of the granular matter which takes the stain more readily than the rest of their substance.

In 1879 Leube[2] reported a case of essential anaemia with a great increase of the Hämoglobingehalt in the blood. From the history of the case, however, and from the description of the condition of the blood I agree with Reis in believing that this was not a true case of pernicious anaemia but of anaemia secondary to disease of the stomach. Between the theories of Hayem and of Reis as to the nature of these bodies he does not definitely decide.[3] Reis about the same time repeats and amplifies his former views. He considers that along with the altered condition of the blood there must also be an increase in the number of the white corpuscles in order to produce these ele-

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[1] Ehrlich Berlin klin. Woch. 1880 p. 405
" 1881 p. 43

[2] Neumann Zeitschrift f. klin Med. Bd iii S 411

[3] Pouchet Journ of Mic Sc. 1880 p. 221 ff.

in great numbers and he enumerates a number of diseases in favour of his views the most[illegible] pernicious anaemia and Chlorosis together as diseases in which there is a decrease both of the Hæmoglobin körperchen and of the white corpuscles. Ehrlich on the other hand considers these bodies to be derivatives of the red corpuscles In certain conditions he finds that the red corpuscles undergo a certain degeneration by which their staining reactions are altered. They thereafter break down and form the products described by Ries.

[2] Neumann criticises the theory of Hayem and considers that the intermediate forms which he observed between the haemoblasts and the red corpuscles are really artificial products formed from the red corpuscles by the method employed - the same objection in fact as he brings against Zimmermann's method and theory

[3] Pouchet writing shortly after Hayem supports his theory to a great extent. He finds the bodies which Hayem describes and calls them globulets He agrees [illegible] with him that they are the progenitors of the red corpuscles, enlarging in size by a growth comparable to that of a group of bodies similarly

'Norris The Physiology & Pathology of the Blood p. 71

formed by the union of albuminins and crystalline compounds" described by M Harting and others. He believes that they are not cells or their derivatives but are formed in the plasma of the blood in a manner analogous to the fibrin formation of fibrin. Poncket is one of the few writers who supports Hayems theory of direct transformation of haematoblasts into red corpuscles though he differs from him in his views on the origin of the former.

With regard to the invisible corpuscle of Norris and his theory as to blood formation, I shall only mention the parts which have a direct relation to the present subject. According to Norris blood regeneration takes place by the gradual assumption of haemoglobin by colourless discs which circulate in the blood with the other elements but which are invisible owing to their complete similarity in colour and refrangibility to the blood serum. They are poured into the blood from the lymphatic system being produced from cells in the glands. These cells (primary lymph cells) are mononucleated with a thin rim of surrounding protoplasm and are transformed by a process of enucleation so that there is a setting free of the nucleus which becomes a colourless disc

Bizzozero. Ueber einen neuen Formbestandtheil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung. Bd XC 1882. p. 261

How it is from the breaking down of the discs of the 'lower series' (i.e. those which are beginning to acquire haemoglobin that Norris considers Hayem's haematoblasts are formed. These discs, he says, disintegrate on the shedding of the blood and appear in the form of rosettes or granules. From these latter he describes like Hayem the shooting out of the fibrin threads. He confirms Hayems description of the changes which take place in the haematoblasts but considers that they are not the progenitors of the red corpuscles, but are the destruction-products of the elements which are. I have seen no evidence in support of this view of the origin of the haematoblasts.

In 1882 Bizzozero published his well known paper on a new element of the blood to which he gave the name of blood-plate. A discussion followed between Bizzozero and Norris as to the priority of discovery of the third corpuscular element. There is no doubt that Norris published the description of his element first but ~~there is no doubt~~ it is no less certain that it is quite a different structure from that of Bizzozero, with regard to the element which he describes. Bizzozero does not hold that no description had been given of it previously - on the contrary he fully acknowledges the work of

previous observers, but merely lays claim to the credit of first observing and describing it in the circulating blood. For this purpose he examined microscopically the mesentery of a chloralized guinea-pig the mesentery being spread on a cork frame and kept moist by a stream of '6% Sodium-Chloride Solution. Selecting a part where the individual capillaries could be well seen and where the stream in them was slow, he found circulating along with the red and white corpuscles another element viz colourless flat discs or less frequently lens-shaped bodies, of about a third to one half the diameter of a red corpuscle. They mingled freely with the other elements, and seemed to have no preference for the axial or the peripheral parts of the stream. In such conditions they were of definite and smooth outline and regular in shape. In this experiment there is only one objection viz that the abnormal conditions - exposure to cold and mechanical influence - in which the vessel walls were placed might induce destructive changes in some of the other elements, particularly in the leucocytes, otherwise he demonstrated conclusively that those bodies whose peculiar

shape and appearance had been so often noted in shed blood, existed in the circulating blood with a perfectly regular and definite form. In various animals whose blood he examined, he found that their size varied with that of the red corpuscles but he considers the evidence altogether insufficient to establish any genetic relationship between the two elements. In opposition to Hayem he holds that they are quite flat and never concave, also that they are quite colourless - the only circumstance in which they appear coloured being when tinted by the haemoglobin dissolved out of the red corpuscles. Highly interesting are his experiments with relation to the formation of thrombi; using a chloralised rabbit in the manner mentioned above he made pressure with a needle point on the wall of a small artery so as to make a projection of the vessel wall into the blood stream. He observed that the Blutplättchen collected over the spot and adhered to one another so as to form a small swelling which might come to block the vessel at that point or be carried away by the stream and form a Blutplättchen Embolus. A thrombus thus formed was found to contain

Zuckerkandl Sitzungsb. der k. Akad. d. Wiss. zu Wien
" Bd. 93. III Abtheil. S. 21
" Centralblatt der Medicin S. 694 1889

a few leucocytes but was chiefly composed of blood-plates. Again he put a thread through the jugular vein of a and found that if it were allowed to remain in the blood stream for fifteen minutes or so it was covered with blood-plates, but if it remained in the vessel for about an hour the outer part of the concretion on it was composed of a layer of blood plates but in the centre they had become fused and changed into a granular mass. He therefore concluded that the chief role in the formation of thrombi was played by the Blutplättchen the participation by the leucocytes being only secondary and that the peculiar granular appearance of thrombi was due to the changes in the former elements when massed together. Reference to his theories of coagulation have been made above.

Zahn also believes that the blood-plates are distinct preformed elements of the blood. He calls them Blutscheibchen and describes them as mostly elliptical discs with a concave surface. In the latter point he agrees with Hayem but he differs from him in holding that they are devoid of haemoglobin, any yellow tinge which they may have being due to reflected light as the tint never deepens when they are heaped together in numbers. Their typical

Uebersicht der neuesten Arbeiten über die Blutgerinnung. (Runschenbach Hayl v Biggers)
Fortschritte der Medicin 1883

structure and distinct chemical relations he thinks speak against their being intermediate forms or destruction products. He also denies the instantaneous breaking down of the white corpuscles on the shedding of the blood and he thinks that the appearance of this being the case is due to the heaps of changed blood-plates.

Nicolai Heyl & Rauschenbach have written in opposition to Bizzozero's views especially in so far as the relation of the Blutplättchen to coagulation is concerned. A comparison of the work of these three writers by Weigert will be found in the Fortschritte der Medicin for 1883. The former of the two authors mentioned fails to find Bizzozero's elements in their typical form but has been familiar with the Körnchen for a long time and finds them especially numerous after the application of the methyl-violet-Salt-Solution which Bizzozero employed. Both believe that they are destruction products but admit that they may be formed intravascularly. Weigert himself believes that the evidence is in favour of the chief part in the coagulation of the blood being played by leucocytes. He thinks that the presence of the blood plates in the circulating blood as Bizzozero

Hewyt Inaug. Dis. Inpat 1883 (Ihmsen's reprint)

[2] Jahrb Sitzungsberichte &c cit supp.

experiments is due to the injury to which the vessels were exposed, causing the leucocytes to break down.

Another theory of the origin of the blood plates is given by Slowtz. Semmer made some experiments which are described in Pflügers Archiv 1875, in which he examined the plasma of horse's blood prevented from coagulating by means of cold and found therein red granule balls as the Körnerkugeln somewhat larger than leucocytes. These he looked upon as transition forms between red and white corpuscles. He believed that they existed in normal blood but disappeared in some way in the formation of fibrin. After some hours in the cold plasma they broke down into colorless granules. From the breaking down of these Körnerkugeln in shed blood Slowtz believed that the blood-plates arose and Feitag held a similar theory. Lukes Semmer failed to find these granule-balls in the normal ~~granules~~ blood of other animals and considers that those in the plasma of the blood of the horse are simply large coarsely granular leucocytes. The fact that the granules composing them differ markedly in many respects from the blood plates (Norris Stiffness that these granule-balls are formed from the breaking down of those

[1] Klaus Archiv f. exper Path 1883

[2] Fortschritte d. Medicin 1883 p. 341

[3] Zahn Virchows Archiv. Bd. 62. 1875

[3] Hallo Fortschritte d. Med. 1884 p. 202, 479

minisible discs which have acquired a little colour.

The following is a short Summary of the views of Hlava on the relation of the blood-plates to Coagulation.

The Blut plättchen, he finds must in circulating blood but are not so numerous as Bizzozero has described them to be and their form is not so constant. They are probably the nuclei of multinucleated leucocytes which have become free. The Körnchen kugeln seen in shed blood are for the most part formed from the leucocytes but also to a less extent from the blood-plates. The more numerous these Körnchenkugeln are in dried preparations the fewer are the leucocytes the multinucleated variety breaking down first. From the granule-masses thus formed fibrin formation takes place by a process of coagulation necrosis. The stages in the process he pursue are heaping up of leucocytes breaking down of their protoplasm ~~and the form~~ disappearance of their nuclei and the formation of the granular mass which becomes fibrin. With regard to the formation of white thrombi he agrees with Hahn that the leucocytes by heaping themselves up and fusing together are the chief agents in the process.

Hulla on the other hand holds that coagulation

Jaworowsky. Zur Frage d. nach dem dritten Formbestandtheil des Blutes beim Menschen und einigen Thieren. 1883 Russisch.

Ref Jahresber. f. Anat u. Phys. 1883

Ajmassian Ueber den dritten Formbestandtheil des Blutes.

Deutsches Archiv f. klin Med. 1884 Bd 35 p. 217

always starts from the blood-plates. They have a close relation to the white corpuscles and are probably derived from them though not after the blood is shed as there is no breaking down of the white corpuscles to found on examination. He found that they underwent certain alterations in disease to which reference will be made below

Tarchanoff has repeated Bizzozero's experiments on various animals and has found the blood plates in the circulating blood as the latter describes. He finds that fibrin formation always commences around them and considers them to be the chief factors in coagulation. They are not formed from the destruction of white corpuscles but he does not know their origin or fate.

Afanassiew published in 1884 a very interesting paper on the blood plates. Perceiving on the one hand the insufficiency of the evidence for Hayem's theory and on the other the important part which the nucleated red corpuscles of the red bone marrow played in blood formation he sought an explanation which would include an important part played by the blood-plates as well. From observations in disease as well as from direct experiment he found that in traumatic anaemia the

number of the blood plates and of the microcytes greatly increased The anaemia was produced both by the direct withdrawal of blood from dogs and by injection of pyrogallic acid — in all cases with a like result. In the blood in such conditions, he found also, that the blood-plates were much larger than normal so that a series could be found whose highest members had a diameter approaching that of a red corpuscle In the bone marrow (excised from a dog in such a condition) he found the same large plates but also further stages in which they had a clear narrow band surrounding their dull somewhat granular substance. In others this band could be seen to be coloured and thus the transformation of the blood-plates into nucleated red corpuscles could be traced. He found that a blood-plate and the nucleus of a red corpuscle when stained had exactly the same appearance The nucleated red corpuscle by losing its nucleus becomes an ordinary red corpuscle whilst the nucleus is again a blood-plate. When the process is rapid the corpuscle produced is smaller than usual and thus microcytes occur. A criticism of Hayem's theory will be given below.

Hewit- Beiträge zur Lehre von der Blutgerinnung
Sitzungsber. d. Kais. Akad. d. Wiss. zu Wien Bd XC.
III Abth. 3.80
" Fortschritte der Medicin 1885

Schmidt criticises the statements made regarding the blood plates from a peculiar standpoint. He holds that they do not exist preformed in normal blood but appear during the process of dying of the blood especially of the leucocytes, though their origin from the latter is not their only origin. They exist first as globulin discs which may be secreted by the leucocytes. They are dissolved in the blood at the temperature of the body, and only become visible after it has cooled somewhat and they have passed into the granular condition. After this they are no longer soluble on being heated to 40°C. The first requisite for their appearance is the presence of leucocytes in abnormal conditions & for this reason they have been seen circulating in the blood, the conditions when the observations were made always being abnormal. The bases made thus theory on observations made on peptonised blood i.e. the blood of an animal into which a certain amount of peptone has been injected. He made observations also on the blood of frogs with somewhat similar results only in them the discs are more slowly formed & more slowly become granular. He also claims to have pro

Mitchell Gibson. Journ of Anat. & Phys. 1886 p. 100

2 Osler. Cartwright Lectures. On certain Problems in the Physiology of the Blood Corpuscles
The Medical News 1886

duced similar bodies artificially by precipitating paraglobulin from a solution containing uric acid by means of sulphate of magnesium. He saw these bodies on microscopic examination of the precipitate. Lakes however who repeated the experiments found that such bodies only resembled blood-plates in appearance, being found to be of quite a different nature when tested by reagents. Sheppard Gibson (1885) recognises the existence of the bodies described by Bizzozero. These (the colourless microcytes) he found immediately after the shedding of the blood and believed that to them the coagulation of the blood was due. He considered them to be chiefly the nuclei of red corpuscles but thought they might also be the nuclei of leucocytes. He distinguishes them from the coloured microcytes which are fragments broken from crenated blood corpuscles.

[2] Cohen gives an interesting account of the third corpuscular element of the blood in the Cartwright lectures for 1886 in which the chief points of the subject are reviewed. He gives the results of his own observations of white thrombi formed on atheromatous ulcers, on the valves of the heart in aneurisms and in veins. These he found in

all

Theodor Schimmelbusch Die Thrombose
Stuttgart 1887

cases to be composed of blood-plates which had become fused and had in the deeper parts undergone a granular degeneration and occasionally liquefaction but in the superficial parts still allowed the outlines of the individual elements to be recognised. These facts are confirmatory of what Eberth & Schimmelbusch have experimentally proved with regard to the formation of white thrombi. They took the utmost precautions to obviate the objections made to Bizzozero's observations. The animal employed was immersed in a warm bath of ~~heat~~ normal salt solution kept at the temperature of the body and its body was prevented from polluting the fluid in any way by being wrapt in an india rubber sheet. The bottom of the bath was a thick glass plate which served at the same time as a microscope table; an immersion lens was used and the microscope tube brought into the bath. An incision was made in the middle line of the abdomen, a knuckle of bowel was squeezed gently into the surrounding fluid and its mesentery was carefully spread over a cork frame in the bottom of the bath so that it could be examined microscopically. In such circumstances they saw the blood plates circulating with the other elements

[1] Fuchs Virchows Archiv Bd 116 S. 28

In such favorable circumstances the observed the formation of thrombi in small vessels. In normal conditions the blood plates circulated with the blood-corpuscles in the centre of the vessel, being separated from the wall by a layer of plasma, but when the stream was slowed from any cause they fell out and appeared in the layer next the vessel wall. This fact together with the peculiar changes which they undergo, explains the chief facts with regard to thrombus formation. If an obstruction was offered to the blood stream it caused them to come in contact with it, and acting as a foreign body, caused the blood plates to change their character and to adhere to one another. Thrombi are thus formed by the accumulation and fusing together of blood-plates.

In spite of these experiments which ~~certainly~~ seem conclusive as regards the corpuscular nature of the blood plates, the most recent discussion has been concerning their actual existence in normal circulating blood. The question would appear to be settled by the observations of Zahn on the wing of the bat. In a thin portion of the membrane of the wing he was able to study the contents of the capillaries without the use of a cover glass or any medium. The observation was

Soweit Virchows Archiv Bd. 117. S 545

aided greatly by the rhythmical contractions which take place in the veins and which cause in some capillaries the flow of blood almost to come to a standstill. In such capillaries he was able clearly to see the blood-plates (Blutschaibchen) circulating with the other elements and presenting the usual appearances. Sometimes they occurred in small groups but this was due to their sticking between the red corpuscles which had temporarily come to a standstill, but they had no tendency to adhere to ~~them~~ one another or to other objects. The mesenteric vessels caught sometimes on the projection between the origin of two capillaries and bent over; when liberated they recovered their shape entirely like the red corpuscles. In some favourable cases he believes that he has seen a small depression in the centre of the blood plates when within the vessels. Since recently Stricker of Vienna claims to have discovered a method of examining the blood which proves their absence in its unchanged condition. The method is to receive a small drop of blood in oil of nearly the same specific gravity as the blood kept at a constant temperature of 37-38°C. If in these circumstances the blood is kept free from mechanical insult, he says that the blood plates

are absent, whereas if the temperature be lowered or if the drop of blood reaches the slide on which the oil rests they immediately appear. He also claims to have by this method watched the destruction of white corpuscles and the appearance of blood plates in their stead. He accordingly holds to his former position that they are either derived from the destruction of leucocytes or formed as a precipitate from the serum. So far as I know no criticism of these experiments has as yet appeared. I have not been able to test them as accurately but the objection at once presents itself that as Hurt could only examine a very thin layer of plasma round the edge of the drop, it would be a great assumption to conclude that they were absent simply because they could not be seen in that layer. Moreover as he speaks of small leucocytes 2-2·5μ in diameter as being present it is highly probable that these are really blood plates.

I think therefore that the observations of Eberth & Schimmelbusch on the one hand and those of Laker on the other hand furnish conclusive evidence in favour of their existence as normal corpuscular elements in the circulating blood.

Chapter IX The Leucocytes

There has been till within recent years a tendency to regard the white corpuscles of the blood as cells of the simplest structure and of somewhat ill-defined character, and to class them together as one class, for though some differences amongst them were known, these were generally considered to be of little importance. Yet in reality they present a most interesting and characteristic structure while their life history involves some of the most important facts in animal physiology and pathology and can only be worked out by means of a careful histological study by suitable methods. They may be studied in the fresh undiluted blood, by means of dried films, and in various preserving fluids. As the last method supplies but little information we shall confine our attention to the two former.

It has long been known that in the fresh blood are a few white corpuscles with coarse highly refractile granules whilst most of them possess fine granules or are almost homogeneous. In the latter large class at least two divisions can be made out with considerable distinctness. The one includes all the smallest leucocytes. They measure 6 to 7·5 μ in diameter, most being a little over 7 μ, and are nearly homogeneous presenting only a few

granules of extreme fineness. Sometimes a large nucleus is visible filling almost completely the corpuscle sometimes it is not. It can however always be revealed by the addition of weak acetic acid, which brings into view a narrow rim of homogeneous protoplasm surrounding it, which is swollen up by the action of the reagent. These corpuscles have a refractive index very near that of the blood serum. Consequently they may often escape observation on a hasty observation. The second division includes corpuscles of a larger size. These are mostly 8 or 10 μ in diameter are finely granular and in the fresh condition no nucleus is visible. They are much more rarely seen than the former as their refractive index differs more from that of the surrounding serum. Some are perfectly spherical, others show some irregularity of outline, the most common being a tailing out and shaving off at one side. This is probably due to amoeboid movement which has been arrested, the preserving fluids the corpuscles are always spherical. Acetic acid brings the nuclear substance into view as a highly refractile mass sometimes forming one large nucleus almost completely filling the cell sometimes broken up into several nuclei of smaller size and variously arranged.

The large finely granular

[1] Schultze Archiv f. Mik Anat. 1865 S 1

[2] Hayem Du Sang 1889 p. 103

The exact structure of the nuclei however cannot be studied by this method.

This division agrees closely with that of other authors. Schultze divides the finely granular corpuscles into three classes. The first includes the smallest corpuscles which are all mononucleated and possess no amoeboid movement. The second includes corpuscles which are about the size of a red corpuscle, are mononucleated with a little protoplasm surrounding the nucleus and on the warm stage exhibit changes of form but have no creeping movement. The large typically amoeboid corpuscles - 9-12μ in diameter with many nuclei constitute the third class. Hayem divides the leucocytes into three classes, the coarsely granular the finely granular, and the hyaline, the last class including the smallest corpuscles. I believe that Hayem is correct in fixing the diameter of the largest leucocytes at 10μ. It is quite exceptional to meet with forms of larger size, though the apparent diameter is increased by pressing them flat or by using artificial media which cause them to swell up. The coarsely granular corpuscles are generally 8-10μ in diameter. The granules generally cover only a part of the cell and sometimes the portion left uncovered has an appearance of

Seeling

Pinchot Quart Journ. of Mic Sc. 1880

the presence of nuclei. According to Schultze these corpuscles have protoplasm of firmer consistence than the finely granular corpuscles and the processes which they protrude on a warm stage have a more definite outline

In dried films stained with methyl blue corresponding varieties are seen. The smallest corpuscles are uniformly darkly stained and show no structure, being apparently composed simply of nuclear substance. Larger corpuscles are seen which have exactly the same characters, though in a few a trace of surrounding protoplasm can be seen ~~Most of the corpuscles however~~ There may be designated the smaller & larger uninucleated corpuscles respectively Most of the corpuscles however are clearly composed of protoplasm which stains very faintly in which the darkly stained nuclear substance lies variously arranged. The commonest ~~form~~ is that possessing several round or oval nuclei connected by bands or threads of nuclear substance, the arrangement exhibiting the greatest variety of fantastic forms Pouchet has described the typical leucocyte as possessing four nuclei but there is really no definite number and they are practically never found lying quite separate from one another

v. Pl. 9

Jastrowsky Virchows Archiv Bd 96 S 60

The size of the nuclei varies but they are generally 2-3 μ in diameter. Each nucleus has a definite structure which is seen best in thin parts of the film where the leucocytes are pressed out flat. It is composed of two substances one of which takes the stain deeply (chromatin substance) the other faintly. The former is generally arranged in fine granules some of which mark the outline of the nucleus, whilst others either form a network within or aggregated into a clump in the centre, evidently constituting a nucleolus. This deeply stained or chromatin substance would therefore appear sometimes to form a framework (Kernst) in the interstices of which the other substance lies, sometimes a sort of capsule filled with the other substance (stained) in the centre of which a portion of the chromatin substance is gathered together. Lavdowsky considers that the former condition is formed when the leucocyte is in process of division, the latter when it is at rest. In double staining with fuchsin and methyl-blue the general substance is stained a pale crimson colour while the chromatin substance is dark blue. The nuclei are connected by threads in various ways by threads of darkly stained nuclear substance and sometimes by paler bands.

Many, if not all, of the multinucleated forms are derived from the larger uninucleated forms as Ehrlich & Lewit hold, and the following would seem to be the method of transformation. One often 2. Pl. 11
sees a uninucleated corpuscle with an indentation of the nucleus at one point revealing thus a quantity of protoplasm which however is very darkly stained - much more so than that of the fully developed multinucleated corpuscle. This would appear to be the first stage. A further stage is seen in which protoplasm is visible all round the nuclear mass which has become a little smaller in size and shows signs of lobulation. In the further process the nuclear substance becomes more broken up, sometimes forming a horse shoe shaped structure sometimes more in the form of a ring, possessing thickenings at parts. More thicker parts gradually separate from one another, at first all being joined by threads, later some being quite free. By this stage the nuclei have their typical structure. During the process the staining of the surrounding protoplasm becomes lighter and lighter till in the fully developed corpuscle it is scarcely coloured at all by methyl-blue. The process is best studied in specimens double-

(stained

stained with fuchsin and methyl-blue in which the two substances, which stain differently, appear v. Plate 11
in the unnucleated corpuscle to be uniformly mixed and the process of development seems to be really one of differentiation whereby the chromatin substance gradually separates itself from the other till, in the final stage, it leaves it almost quite free. The process will be at once understood from plate 11 Forms intermediate between the unnucleated and the multinucleated corpuscles are often the largest corpuscles present, and their number varies in different conditions. They are well seen in the blood of the dog. v. Pl. 9 fig 1
The coarsely granular corpuscles when stained with methyl blue appear, in human blood, generally as bodies stained diffusely of a rather pale blue colour whose surface is covered to a greater or less extent by dark purple granules. Distinct nuclei are generally invisible. In the blood of the rabbit however corpuscles ~~are seen in great numbers~~ with similar granules in their protoplasm are found in great numbers and of all varieties corresponding to the various forms of ordinary finely granular corpuscles. v. Pl 10

It is difficult to determine whether or no the Leucocytes multiply by division in the blood and

of so to what extent. There is no evidence that a multinucleated Leucocyte breaks up into several small corpuscles, each nucleus taking with it a quantity of protoplasm as Norris believed. There are no corpuscles in the blood which could have such an origin. The smallest uninucleated corpuscles have no surrounding protoplasm round the nucleus the nucleus has quite a different structure, and their size is too great to admit of such an origin. Sometimes the nuclei of a multinucleated Leucocyte are arranged in two groups, each containing two or three at nuclei; and it is quite possible that such a corpuscle is in process of division into two. It is amongst the corpuscles which I have described as intermediate forms that division is probably most common. Such corpuscles are often of oval shape and the imperfectly differentiated nuclear substance is arranged in two masses one at each end. These corpuscles are also larger than the most of fully developed multinucleated forms and this fact would make the division more probable. Quite recently in the blood of an anaemic dog received into osmic acid I actually saw a corpuscle in which the process of division was almost completed, each half containing a large nucleus

v. Pl. 9 fig 2

v. Pl. 9 fig 3

Ymit Über die Bildung rother u. weiss. Blut-
Körperchen 3. 13

9 Arnold Virchows Archiv. Bd. 47. S. 107

almost completely filling it. The two halves were connected by a narrow band of protoplasm. I accordingly believe that actual division takes place in the blood, chiefly on the part of the uninucleated corpuscles and forms but little removal from them, but that the process is not a common one.

The fantastic arrangement of the nuclei of the multinucleated corpuscles has been variously interpreted. Foulis considers that the many morphous arrangement of the nuclear substance is really an evidence of degeneration, which terminates with the dissolution of the corpuscle though he has not seen this latter event. This view seems to me not to be in harmony with the facts. The development of the uninucleated corpuscle into the multinucleated is not by a process of fragmentation of the single nucleus, as Foulis describes, but by one of differentiation and elaboration to a ~~higher structure~~ more complicated structure. Such a process cannot be considered to be degenerative in nature.

Quite another view is held by Arnold concerning the appearances in the multinucleated corpuscles. He considers them to be stages in a process of nuclear division which he calls indirect fragmentation and which he distinguishes from indirect

segmentation. He has studied the process chiefly in the giant cells of the bone marrow and distinguishes four stages. First there is a stage in which the chromatic substance becomes more abundant, the chromatic threads more numerous. Second, an in-pushing of the nuclear wall between the chromatic threads takes place ~~Third, there is~~ a Hence very complicated figures often arise. Third, a drawing together of the chromatic substance at different points is seen. Hence arise deeply stained structures joined by more lightly stained threads. Fourth there is a cutting off of the protoplasm either within the cell or at the edge. It is a multipartite nuclear division by indirect fragmentation. Most of the corpuscles in the blood would be in the third stage. I have been unable to follow all the details of this description in the case of the leucocytes and as already said evidence that each nucleus gives rise to a separate corpuscle is entirely wanting. Arnold says that the leucocytes multiply also by direct division.

There can be no doubt that most of the leucocytes enter the blood in the uninucleated form from the lymphatic glands spleen and probably

also from the bone marrow. In the lymphatic glands of the dog examined immediately after death many of the corpuscles are of the small uninucleated variety, - a few are larger (8-10 μ), and are also uninucleated, whilst here and there a typical multinucleated leucocyte can be seen. These uninucleated corpuscles pass away by the efferent lymphatics. In the thoracic duct of a dog examined a few minutes after the death of the animal one finds practically all the colourless corpuscles present to be of the uninucleated variety, and of various sizes (7-10 μ), those of the smallest size being the most numerous. Few even of the largest size shew any signs of change into the multinucleated variety and multinucleated corpuscles are very rare. I have examined the contents of the thoracic duct on several occasions with a practically similar result. Again, the white cells from a scraping of the spleen after death are practically all uninucleated, but if it be cut during life, and the blood flowing from it be examined many multinucleated corpuscles are found with many uninucleated ones. This shews that these multinucleated corpuscles belong to the blood circulating

in the Spleen and pass out with it at the time of death. Examination of the contents of the Splenic artery and vein during life (v. sub) shewed that though the number of Leucocytes was only slightly increased in passing through the Spleen, the number of mononucleated forms was much increased while the multinucleated forms are actually much diminished in number. Are these multinucleated corpuscles which disappear from the blood transformed or broken down in the Spleen? Dr. Whiting has shewn me some specimens ~~of the~~ from Spleens of various animals, in which one could see in the centre of some of the Malpighian bodies numerous multinucleated corpuscles, round these a small zone of very small mononucleated corpuscles and outside numerous mononucleated corpuscles of larger size, the inference being that the multinucleated corpuscles in passing from the centre to the periphery ~~break down~~ break up into small mononucleated corpuscles. Yet I think that most of the multinucleated corpuscles which disappear from the blood in its passage through the Spleen are destroyed. Otherwise the number of mononucleated corpuscles passing out by the vein

'Smit Sitzungsber. d. kais. Akad. zu Wien
Bd 96 III Abth. S. 129

would be very much greater than it is. The presence of a great quantity of detritus material in the splenic pulp seems also to be in favour of a destruction actually taking place. Foulis quotes a similar statement with regard to the bone marrow. He found in the vein carrying the blood from the bone marrow a much larger proportion of unnucleated corpuscles than in the arterial blood. He found uni & multinucleated corpuscles in nearly equal numbers - a condition very similar to what I found in the case of the spleen. Uni-nucleated leucocytes according to Foulis are more numerous in the veins and in the right side of the heart than in the arterial system. All these facts go to shew that the transformation of the unnucleated corpuscles is a rapid one.

Hayem has shewn that the multinucleated corpuscles have the most active amoeboid movements. They probably pass out of the vessels into the tissues ~~and~~ in large numbers and if so most probably disintegrate there. To determine what variety of corpuscles is present in the afferent lymphatics I dissected out one of the large lymphatics running along the femoral

vessels of a rabbit. I cut it across and by squeezing the limb of the animal caused a considerable amount of lymph to flow out. This was caught on a coverglass held beneath the end of the vessel spread out into films and thereafter stained. In this lymph I found only uninucleated corpuscles with practically no protoplasm around the nucleus. In the efferent lymphatic of a dog examined below the knee, I found only one or two multinucleated corpuscles, all the others being uninucleated. In the case of the rabbit some of the lymph might have passed through gland structure but this could not have been so in the case of the dog. I therefore accordingly we may conclude that practically all the corpuscles in the efferent lymphatics are uninucleated.

I conclude therefore that uninucleated corpuscles are being continually added to the blood from the lymphatic glands spleen and bone marrow. In the blood some may multiply by division but most are transformed into multinucleated corpuscles. The process of transformation must be a rapid one seeing how few intermediate forms there are and the life of a leucocyte

Zunit. Op. cit.

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1 Ehrlich Zeitschrift f. klin. Med. Bd 1 1879 S. 553

Archiv f. Anat u. Phys 1879. S. 166, 157

(Physiolog. Abtheil.)

in probably in the blood is probably a short one
Multinucleated leucocytes must be destroyed in
large numbers somewhere I have already
given the reasons for believing that many are
destroyed in the spleen others probably disintegrate
in the tissues.

Jurist finds the corpuscles of lymphatic glands
to be of two varieties which he calls leucoblasts
and erythroblasts. The two varieties are also
formed in the thoracic duct but the former
in much larger numbers. Both varieties are
uninucleated but they differ in this that the
nucleus of the erythroblasts shows a reticulated
structure and they multiply by indirect divi-
sion, whilst the nucleus of the leucoblasts
contains a few granules and multiplication
takes place by direct division. The erythro-
blasts become transformed in the spleen bone
marrow and probably also in the blood into
nucleated red corpuscles. The leucoblasts are
enter the blood and develope into the multi-
nucleated leucocytes. Reference to this will
have to be made again.

Reference must also be made to Ehrlichs
researches on the relations of various corpuscles

to aniline pigments. He found that in some cells there were granules which had a peculiar affinity for acid stains e.g. Eosin indulin auranti &c. He called them therefore eosinophile granules and divided them into α and β granulations according as they had the greater attraction for eosin or indulin. Further he found that the α granules could be changed into β granules by heating which probably acted by causing them to lose water These eosinophile cells are found in small numbers in normal conditions but increase greatly in certain diseases, notably in leucocythaemia. They are present in great numbers in the blood of the rabbit. Most of the ordinary leucocytes of the blood contain granules which are stained by neutral dyes e.g. methyl blue and acid fuchsin combined He also describes cells derived from connective tissue corpuscles whose granules are stained by basic dyes e.g. dahlia, and calls them therefore basophile corpuscles. There have been unable to apply Ehrlichs methods in pathological conditions but they would probably furnish important results.

With regard to the number of the leucocytes

in normal human blood there can be no doubt that the original proportion to the red corpuscles given by Welcker and Moleschott - viz 1:335 and 1:357 respectively are much too high Hayem, Thoma & Halla place the normal proportion at from 1:1000 to 1:500. Afanassiew considers 1:600 the average normal proportion and Halla considers that if the proportion exceeds 1:400 the ~~non~~ condition is abnormal My results would lead me to say that the number rarely exceeds 10,000 per c.mm. i.e. a proportion of 1:500 without some pathological condition being present. The minimum in the other hand rarely falls below 6000 per c.mm.

The proportion of unnucleated to multinucleated corpuscles I found to be 1:2 in human blood, and in the blood of the dog practically the same. In the blood of the rabbit many of the corpuscles are so thickly beset with coarse granules which obscure their structure and make it often impossible to say definitely, except with regard to corpuscles in the thinner parts of the film, whether they are multinucleated or not.

Chapter V — The Red Corpuscles.

It is simply my intention to mention here the chief alterations which the red corpuscles undergo in disease and to explain certain terms applied to the altered forms. Regarding their normal characters I have nothing new to add.

The red corpuscles may become altered in size, form or physical and chemical characters. Abnormal elements allied to them may also appear in the blood. Alterations in size are very common in disease and to the corpuscles of abnormal size different names have been given.

The term megalocyte (globule géant of Hayem. Renamed blutkorperchen of Gram) is commonly applied to corpuscles over 8 μ in diameter and of a shape nearly normal. Their diameter may reach 14 μ or even higher. Their concavity is generally less marked than normal and their thickness is not increased in proportion to their size. About the term 'microcyte' there has been much confusion and it would be much better to discontinue its use altogether. It seems to have been applied to any small body in the blood resembling to any extent a red corpuscle in character or in shape. Of such bodies I can distinguish the following varieties at least.

Gram Fortschritte der Medicin 1884

Ueber die Grösse der rothen Blutkörperchen

2 Eichhorst Centralbl. f. med Wiss. 1876

(1) Small red corpuscles, often with convex surfaces sometimes quite spherical – (Hayem, Bluthkörperchen of Gram, Herlules Rains of Hayem) According to these two authors, whilst in the vessels they have concave surfaces and only when the blood is shed do they change their form. Similar forms are often assumed by the ordinary red corpuscles in urine

(2) Eichhorst corpuscles – These are small deeply stained bodies of spherical shape. Their diameter is about 3 μ. They were formed by Eichhorst in pernicious anaemia and were supposed by him to be characteristic of that disease. Hunter found them in most of his cases whilst others (e.g. Lepemann) have found small bodies but not deeply stained. They are certainly not always present. They indicate destructive rather than regenerative changes in the corpuscles

(3) Small rounded bodies often with a sort of stalk attached. These are buds which have become detached from poikilocytes. Their origin can often be traced.

(4) Irregular deeply stained bodies – fragments of broken down red corpuscles. They are formed in poisoning with toluylendiamin, pyrogallic acid &c. Similar bodies are sometimes found in typhoid fever

' Ayn assein (op cit)

genus (Afenassieff)

(5) Artificial products - presence of crenated corpuscles &c - Coloured microcytes of Gibson

(6) Colourless microcytes - Blutplättchen

Both the megalocytes and microcytes of class (1) are present as normal elements in the blood of the embryo

The term poikilocyte was first applied by Quincke to corpuscles of irregular shape. They were supposed at first to be characteristic of pernicious anaemia but are found in many other conditions (v Sub) They are often of the most bizarre forms. Their formation is said to be due to alterations in the physical characters of the serum but this is quite an insufficient explanation of the extraordinary forms which are met with. Hayem thinks that they are due to a too long survival of the characters of the haematoblasts, so that the substance of the corpuscles yields to external impressions, and that this is often associated with a want of sufficient haemoglobin. I have however never seen any which did not possess the ductility and elasticity of normal corpuscles. Afanassieff considers them real living elements (wirkliche lebendige Elemente) associated with

[1] Ehrlich Berlin. Klin. Woch 1880 S. 405

1881 S. 43

[2] Hayem Archiv de Phys. 1883

[3] Hunter The Pathology of Pernicious Anaemia

The Lancet 1888 ii

blood regeneration as contrasted with destruction products.

The presence of nucleated red corpuscles in abnormal conditions is a subject about which there has been as much difference of opinion. Ehrlich & Lazarus has found them in all varieties of anaemia and divides them into normoblasts and megaloblasts according to their size, the former being characteristic of secondary (simple) anaemia the latter of primary (pernicious) anaemia. A much rarer form he finds, is the irregular nucleated red corpuscle - the poikeloblast. Hayem on the other hand during an eight years experience in examination of blood only found them in seven cases three of which were cases of leucocythaemia. Osler in his tables gives cases of leucocythaemia, typhoid fever erysipelas scarlatina &c in which they were present. According to Hunter Quincke never found them in cases of pernicious anaemia whilst Eichorst found them in most but not all of his cases. They can be recognised by any of the above mentioned violet-tinted fluids, or in films dried and stained. I may say that my results closely agree with those of Ehrlich with regard to their characters and presence.

'Huyghens Comptes rendus 1880 p. 225

Other abnormalities may simply be mentioned. Corpuscles Cells containing red corpuscles have been seen in leucocythaemia (Osler). Hayem describes leucocytes coloured with haemoglobin and also irregularly spherical cells but with a central nucleus and an external part coloured with haemoglobin like a nucleated red corpuscle. He also mentions alterations in the adhesiveness and viscosity of the red corpuscles seen in "phlegmasiæ". Ehrlich describes a degeneration in the red corpuscles by which the affected parts stain a crimson colour in acetic eosin-haematoxylin, the normal corpuscles staining a bright orange colour. Also sometimes in staining with saturated watery solution of methyl blue a well marked protoplasmic network deeply stained is seen within the corpuscles but he scarcely looks upon this as a degeneration.

The well known alterations of the red corpuscles in malaria and the organisms which affect them are outside of the present subject.

Chap. VI The Red Bone-Marrow.

Whilst the origin and morphological nature of the red corpuscles of the mammalian adult have been subjects of almost endless discussion, there is considerable agreement regarding their development during early foetal life. It is a firmly established fact that at the earliest stage of foetal existence, all the red corpuscles of mammals are nucleated and of the same type as those of the lower vertebrates. These primitive nucleated red corpuscles of the embryo are derived according to Kölliker from colourless cells with granular contents and they very identical with the original formative cells ('Bildungszellen'). The protoplasm of these cells becomes homogeneous and acquires haemoglobin whilst the nucleus remains as the nucleus of the red corpuscle. They multiply by division and after a time all the trace of their development from colourless cells disappears. Soon however non-nucleated red corpuscles appear in the blood, and they gradually increase in number at the expense of the nucleated red corpuscles till at birth all the red corpuscles are said to be of the non-nucleated variety. The pig according to Neumann forms an exception to this rule, as nucleated red

'Köllikers Gewebelehre – S 637

2 Neumann Neue Beiträge zur Kenntniss der Blutbildung Archiv. der Heilkunde. 1874 S. 457

corpuscles can be formed in its blood for some time after birth. I have found nucleated red blood corpuscles though in very small numbers in the blood taken from the heart of rabbit killed at birth, whilst in the blood of the liver they were fairly numerous. Whilst however both varieties of red corpuscles are formed in the circulating blood, stay of a human foetus at the fourth month, in certain organs nucleated red corpuscles are formed in much larger proportions than in the blood. Kölliker attached special importance to the liver as a blood forming organ during foetal life and his view has been amply confirmed. He describes the formation of nucleated red corpuscles from colourless cells in a manner similar to their origin from the original granular cells. These colourless cells - the earliest lencocytes - he thinks are probably formed chiefly in the Spleen and carried to the liver where they undergo the transformation. An account of considerable length is given by [2]Neumann who found that an active formation of blood vessels and blood corpuscles together goes on within the liver during foetal life. The formed nucleated red corpuscles in great numbers in the blood of the liver during foetal life. That these were not simply carried

Zur Kenntniss der Bildung rother und weisser Blutkörperchen. 1883

aus d. Sitzber. der K. Akad. der Wissensch. zu Wien III Abth. 1883 Bd. 88

carried there from the spleen was formed by the fact that he found them to be much more numerous there in the spleen, whilst in the portal as well as in the hepatic veins they were relatively few in number. Further, he describes large cells lining the hepatic capillaries in which an endogenous formation of red corpuscles was going on. The exact process is not given, but he found in many such cells, nucleated red corpuscles lying similar to those within the capillaries - in some cases several such corpuscles in one cell. He also ~~found~~ describes small unnucleated colourless corpuscles but does not find a direct formation of these into coloured corpuscles. Hirt is unable to confirm Neumann's views with regard to the endogenous development of nucleated red corpuscles but still maintains the importance of the liver as a seat of formation both of coloured and colourless corpuscles during foetal life. The former he finds are derived from colourless cells (Farbloser Zellen) containing a single nucleus similar to that of the coloured corpuscles, by the surrounding protoplasm acquiring haemoglobin. The ~~to~~ nucleated red corpuscles as well as their colourless predecessors multiply by the process of indirect segmentation

and he distinguishes the latter from colourless blood corpuscles which multiply only by direct division. He found abundant division figures in the liver both of mammals and of lower vertebrates.

In the blood and organs of a human foetus about the fourth month I found conditions analogous to what Zürit describes. In the blood of the liver examined in methyl-violet-salt solution I found nucleated red corpuscles of various sizes & appearances in large numbers, the proportion of the nucleated to the non-nucleated corpuscles being about 1:8. Nucleated and non-nucleated corpuscles alike varied much in size viz 5-12μ in diameter. The nucleated corpuscles were similar in structure to those found in the adult bone marrow v. Gehuchten. The relative size of the nucleus varied much, in some being less than half of the cell in diameter, in others being surrounded by a very thin layer of homogeneous yellow protoplasm. By far the greater number possessed a single nucleus but I saw also forms with two or sometimes even three nuclei generally of different sizes. In most the nucleus had a very well defined margin and presented the coarsely or reticulated appearance so characteristic of the

2. Pl. 23

2. Pl. 24

nucleated red corpuscle, but in some it was of a homogeneous appearance with indefinite outline, and sometimes so faint as to be almost invisible. These latter cells were generally very deeply coloured with hæmoglobin and were probably in process of transformation into the non-nucleated variety. I found also colourless cells with a single nucleus of similar structure to that of the typical nucleated red corpuscle, and homogeneous surrounding protoplasm, and I believed that I could see all stages between the two, the homogeneous protoplasm of the colourless cells gradually acquiring a yellow tint which gradually deepened. Various degrees of coloration were found both in the large and in the small nucleated red corpuscles, the depth of colour having no relation to the size of the cell – a point to which further reference will be made. A few both of the colourless and coloured corpuscles were seen in the process of division. In films dried and stained of the blood from various organs, I found confirmatory appearances as regards the character of the nuclei of the red corpuscles as well as regards their multiplication by division. Similar nucleated red corpuscles were found in considerable numbers in the blood taken from the heart – but they were

v. pl. 24

Pfeiffer v. Ivan Ehlen die Isolirung der rothen Blutkörperchen &c.
Virchow's Archiv. Bd. 95 S. 1

very much fewer than in the liver. In the spleen and bone marrow nucleated red corpuscles were more numerous than in the blood from the heart but much less so than in the liver, and here also I saw transitional forms between colourless and coloured corpuscles. I was much struck with the relative paucity of colourless cells in the spleen as compared with that of the adult. The liver was a large organ filling a great proportion of the abdomen, contained much blood and was very rich in free cellular elements, whilst the spleen was a relatively small and unimportant looking organ Bizzozero and Torre found as the result of an extensive series of observations that in all vertebrates development of nucleated red corpuscles takes place through indirect division of pre-existing forms but that special organs are present in all in which these forms are more abundant In the adult stage such organs are the bone marrow in mammals reptiles birds and tailless amphibians, the spleen in tailless amphibians and in fishes, and in fishes also the lymphoid patches in the kidneys. In the embryonic condition division-forms are present in the blood — a condition which persists in the adult condition of the lower vertebrates

[1] Max Schmidt. Sulla origine dei globuli rossi del sangue
Respr. Virchow & Hirsch's Jahresber. 1879

[2] Bizzozero Ueber die Bildung der rothen Blutkörperchen
Virchows Archiv. Bd 95 S. 23

Foa & Salvioli[1] found in the mammalian embryo multiplication of nucleated red corpuscles by division* was most common in different parts of the body at different stages viz at the earliest period in the blood later in the liver, later still in the Spleen and shortly before birth in the bone marrow. Bizzozero[2] found an almost similar condition in the embryo of the frog and of the hen, with the exception that in the case of the latter the liver appeared to take little part in the process. Bizzozero however does not exclude the possibility that nucleated red corpuscles may be developed in other ways viz from colourless cells (NB. The term 'colourless cell' in this connection is not to be taken as identical with 'colourless corpuscle of the blood or 'leucocyte' in fact as I shall afterwards shew it is quite a different structure) but may therefore conclude, that in the mammalia during embryonic life non-nucleated red corpuscles are derived from nucleated ones by the loss of their nucleus, the latter corpuscles gradually diminishing in number till they finally disappear from the blood at or about the time of birth, that the process of blood formation is more active in certain organs especially in the liver, and that the nucleated red corpuscles multiply by division and are most probably

Neumann Centralb. f. d. med. Wissensch. 1868.

" Ueber die Bedeutung des Knochenmarkes für die Blutbildung. Archiv der Heilkunde 1869 Bd X S. 68

Litzmann Centralb. f. d. med Wiss. 1868

also formed from colourless cells of nearly the same structure. The manner in which the nucleated red corpuscle loses its nucleus will be considered later. Kölliker writing in 1867 said that after a careful search he had entirely failed to find nucleated red corpuscles in the mammalian adult. About a year later, Neumann announced that he had discovered nucleated red corpuscles in the bone marrow of the adult and shortly afterwards published an important paper on the structure and functions of the bone marrow. Neumann's discovery was confirmed a few months afterwards by an independent observation of Bizzozero's and since that time the red bone marrow has been the subject of numerous researches. These researches have had relation both to its normal histological characters, to its changes in disease and to changes produced by direct experiment, and these three points must be kept in view in considering its importance as a blood-forming organ. The result has been to throw a considerable amount of light on the development of the red corpuscles.

It will be convenient first of all to give a short description of the cellular elements found in the normal red bone marrow. I have studied

1 Ok cit

2 Reichms Archiv. Bd. 52 S. 156

its structure in the human subject, in dogs cats, and rabbits, but for convenience will take as the basis of the description the red marrow of the dog, which is practically similar to that of the human subject By the earlier observers eg. Neumann & Bizzozero the cellular cells of smaller size were all classed together 'lymph corpuscles of various sizes yet several varieties may be separated The cell elements however present such a variety of characters that I will not attempt to describe every form which may be seen but only those which after studying many specimens I consider to represent types. The a little of the red marrow scraped from the bone may be examined fresh, without any medium but it is more convenient to use some preserving fluid eg. Bizzozero's Methyl-violet 5-1 % Solution. 2. Pl. 25

1 The cellular elements of smallest size are lymphoid elements of 2 - 3·5 μ in diameter with a smooth and definite outline. They are generally quite homogeneous but they may contain a few fine granules generally at the edge, and stain a very faint violet colour in the Methyl-violet Solution. The addition of acetic acid strips makes them more transparent

2 Bodies composed almost entirely of a single nucleus there being no protoplasm visible around it in the

Obraszow Zur Morphologie der Blutbildung
Virchows Archiv Bd 84 S. 358

fixed condition. There are generally about 4-6 μ in diameter have a well marked border and the interior of the nucleus has a reticulated appearance evidently formed by an arrangement of fine granules These cells are the 'proteleucocytes' of Obrastzow. v. Pl. 25

3. Cells containing a nucleus similar to that described but showing besides a band of surrounding protoplasm of homogeneous appearance. These are generally 7-9 μ in diameter and the relative size of the nucleus varies, sometimes being surrounded by a very fine layer of protoplasm sometimes by a broad band. The size of the nucleus is generally 4-5½ in diameter. These cells are generally present in large numbers, and it is they which by many are considered to be the predecessors of the nucleated red corpuscles. They are often seen in the process of division. In the methyl-violet solution the nucleus is of a violet colour while the surrounding protoplasm is almost colourless.

One often sees a few cells of somewhat similar structure to these but of larger size and with granular protoplasm. (Such cells are very common in the bone marrow of the rabbit) v. Pl 26

4 'Ordinary marrow cells' generally of somewhat larger size than the previous viz 9-13 μ as a rule. They contain one two or three nuclei of a round

oval or kidney-shaped form (bläschenförmige Kerne) and both the nuclei and surrounding protoplasm contain fine granules. One often sees a few coarser granules in the nuclei and the line between nucleus and protoplasm is marked by numerous closely arranged granules. In the methyl violet solution both nuclei and protoplasm alike stain of a pale violet-blue. This class differs from the previous both as regards the structure of the nuclei and the character of the protoplasm. I have never seen the 8-form, indicating division, in cells of this class and a similar statement has been made by others. v. Pl. 25

Cells ~~exactly~~ very similar to those described but containing coarse granules, like the coarse granules of corpuscles of the blood, are seen in varying numbers in the red marrow of the dog. They are exceedingly common in the red marrow of the rabbit v. Pl. 26

5 & 6 Myeloplaques and giant cells (Riesenzellen) These two ~~cells~~ terms have been used in the same sense but as Boggero points out there are well marked differences between them. The former are only formed in contact with the bone are of irregular shape and contain many nuclei. They are exceedingly rare in the marrow squeezed out of a piece of bone. The latter are formed throughout the bone marrow, are

[1] Arnold. Virchows Archiv. Bd 97. S. 107

[2] Ibid. " " Bd 106 S. 354.

[3] Obraztzow op. cit

of round or oval form and contain one large nucleus which may show a stage of division. It is in these cells that Arnold has described the process of indirect fragmentation of the nucleus, and his views have been confirmed by Watney.[2] The two varieties are distinguished also by Obraszow[3] who distinguishes Myeloplaxen with granular protoplasm in which many nuclei are shewn and those with less granular protoplasm containing one large nucleus which is often seen in a process of budding

7 Cells containing pigment are said to be very rare in health, much more numerous in certain diseases In the dog I have found them in considerable number even in the healthy condition. The pigment is of brown or yellowish colour sometimes in fragments sometimes in a diffuse form throughout the cell. The cells containing are generally of somewhat indefinite character and often contain coarse granules besides. One often sees besides irregular heaps of coarse granules with similar pigment lying amongst them

8 Cells containing red corpuscles are also described by and are many supposed to represent a method of corpuscle formation. I have never seen such in the healthy condition, and in disease I have always been able to trace them as a stage towards the

Zeitschrift f. Virchows Archiv. Bd 105 S. 136

cells containing pigment. Gulmuyden on the other hand describes and figures such cells containing as many as twenty to thirty typical ordinary red corpuscles.

9, 10 Coming now to the red corpuscles we find that they are of two varieties viz the nucleated and the non-nucleated. Ordinary bi-concave non-nucleated corpuscles are present in considerable numbers. They present the usual characters but generally show more variation in size than do those in the blood. By far the greatest interest however attaches to the nucleated red corpuscles. In the marrow of the dog they are easily found. They are spherical bodies varying somewhat in size but generally about the same size as, or a little larger than, the ordinary red corpuscles. There is generally one round nucleus, often lying somewhat eccentrically. In the fresh marrow, examined without any medium, the nucleus comes into view as a colourless spot, containing fine granules arranged in a characteristic manner, but its characters are much better seen in the methyl-violet-solution. In this solution the nucleus is stained a pale violet colour. It has a well marked border and in its interior contains exceedingly fine granules so as to give it a reticulated

appearance. Its relative size varies somewhat but its diameter is generally about half of that of the corpuscle. Some corpuscles have a nucleus relatively much larger, and in such corpuscles the reticulated appearance of the nucleus is very distinct, while the narrow band of surrounding protoplasm is less deeply coloured than in the others. Other corpuscles show a nucleus of homogeneous appearance, and these are generally deeply coloured with haemoglobin. The nucleus of the red corpuscle is in its typical form similar in appearance to that of the colourless marrow-cells with homogeneous protoplasm described under above. The nucleated red corpuscles exhibit the same character of flexibility as the ordinary non nucleated corpuscles and their perinuclear portion behaves in the same manner to reagents. Corpuscles also are seen in which the surrounding protoplasm is just tinted with haemoglobin and all degrees of coloration are seen.

We shall now consider the relations of the various elements to one another, and more especially the origin and fate of the nucleated red corpuscles. We find that the distinction drawn between the colourless cells with homogeneous protoplasm and single nucleus of reticulated appearance and the ordinary marrow

[1] Smit Op. cit.

[2] Obrastzow "

[3] Archiv der Heilkunde 1869. S. 68

[4] Zuckert-Gerlach Journ. f. Anat. u. Phys. Jahrg XX

[5] Rindfleisch Archiv f. mikrosk. Anat. Bd XVII S. 1.

cells with granular protoplasm is of great importance and well recognised by most writers. The former are of most interest in relation to the nucleated red corpuscles - they are the farblosen Vorstufen of Litten, the blasse Markzellen of Obrastzow as contrasted with the gewöhnliche Markzellen. There are two chief theories concerning the development of the nucleated red corpuscles. The one is that they simply reproduce themselves by division, the other that they are derived from the colourless cells by the gradual acquisition of haemoglobin by the latter. Neumann[3] in his first paper on the bone marrow (1869) put forward the latter view, and it has been supported by a great number of observers eg. Bizzozero in his earlier papers, Obrastzow Litten, Gulmuyelen Osler and Tschait Gibson.[4] All these find intermediate forms between these colourless cells and nucleated red corpuscles. Rindfleisch[5] considers such a theory unnecessary as division-forms are found amongst the nucleated red corpuscles and Bizzozero more recently speaks in a similar way but without bringing any facts against it. I think that the existence of intermediate forms is beyond doubt as they can be seen in every preparation. The only possibility

Christen Op. cit.

of error is that the so-called intermediate forms are produced by the decoloration of the nucleated red corpuscles. Yet, I think, that this possibility is excluded by the fact, that they can be seen, however fresh the preparation is, and no process of decoloration can be seen on watching a red corpuscle for a long time. I have frequently seen lying together, a nucleus without visible protoplasm around it, a nucleus surrounded by colourless protoplasm, and one surrounded by yellow tinted protoplasm, the nucleus having the same structure and appearance in all. I have also seen the same appearance in the embryonic state which also Kölliker and Zoviit describe. It must also be borne in mind that it is the process by which nucleated red corpuscles are originally formed in early foetal life. 66

Obraszow who has made a most minute study of the elements of the bone marrow considers that the nucleus without visible protoplasm, which he calls a protolencocyte gradually acquires protoplasm around it, at first colourless but later containing haemoglobin. He however holds that during life both in the pale marrow cell and in the nucleated red corpuscle, the nucleus

(Substance

! Op. cit.

and the protoplasm are drawn uniformly mixed throughout the cell and that the separation take place only on the death of the cell, on account of a tendency of the nucleus to post-mortem concentration. His view is based on the fact, that in the perfectly fresh condition the nucleus is not visible, and that the bound of surrounding protoplasm, after it has become visible, can be seen to broaden. The latter fact can be explained by imbibition of the surrounding fluid, whilst the former probably only indicates a change in the relative refractive indices of nucleus and protoplasm. That an increased differentiation of nucleus from surrounding protoplasm takes place on the death of the cell I think highly probable; but in thin fixed specimens, made with great rapidity and stained, the nucleus can always be seen with distinctness. The same facts apply to the nucleated corpuscles of the lower vertebrates. 'Foa & Salvioli' also believe that the nucleated red corpuscle is derived from a colourless cell but trace the formation of the latter from a giant cell. From the large nucleus of the giant cell a bud forms which makes its way to the surface of the cell and becomes separated, covered with a thin layer of protoplasm

de Paris
Bouchet Soc. Medic. 1879 No 14.

Malassez Archiv. de Physiol. 1882 p. 2

This daughter cell, as it is called, then acquires haemoglobin and becomes a nucleated red corpuscle. I have not been able to confirm this theory. Pouchet maintains that the nucleated red corpuscle is derived from the smallest leucocyte of the blood (leucocyte hyalin) by a process which he describes as a haemoglobin degeneration a process, by which the periphery of the cell is impregnated with haemoglobin, which increases in amount at the expense of the nuclear substance till the latter finally disappears. The term which he uses is, I think, a most unfortunate one and I have seen nothing to indicate that any of the leucocytes of the blood have such a fate. Malassez on the other hand traces the formation of the nucleated red corpuscles from ordinary marrow cells. He studied the cell elements of the marrow by means of dried films, fixed with osmic acid and stained with eosin-haematoxylin, and found that in the largest marrow cells the nuclear substance (Stance) was in a diffuse form, that in the smaller, it became more defined, and that in those smaller still it assumed a reticulated form, which gradually passed into the nuclear form of the typical nucleated red corpuscle. Such evidence

' Op. cit.

I failed to find in the human bone marrow and in that of the dog, but in the marrow of the Rabbit as I will describe below appearances are found which lend support to such a theory; as one can see marrow cells much larger than ordinary nucleated red corpuscles very faintly tinged with haemoglobin. Yet I believe that the smaller cells in which the haemoglobin is in greater quantity are not formed by a process of concentration, but are the product of an actual division of the cells, the acquisition of haemoglobin progressing at the same time. While, however it is practically certain that nucleated red corpuscles are derived from colourless cells it is equally certain that they multiply by division. I have seen both the nucleated red corpuscles and their colourless predecessors in the process of division, the latter rather more frequently. Zimit finds that both forms multiply by the process of indirect segmentation and Obraszow holds the same opinion. The latter figures red corpuscles in the process of division, the nuclei of the new cells having a rod shape and connected by thin threads of nuclear substance, and forms as a further distinction between the "pale" and ordinary marrow cells that the latter never show division figures. Bizzozero has formed Karyokinetic

Neumann Archiv der Heilkunde 1869 S. 68

figures in the red corpuscles of all animals, the appearance of the red corpuscle in the process of division given by him being practically similar to Obraszow's drawing. Arndt, Fleisch and many others hold the same view.

The nucleated red corpuscles in the adult bone marrow are therefore, I believe, formed from colourless cells of nearly similar structure, and both varieties of cells multiply by division amongst themselves. The any further origin of the colourless cells is doubtful and may be left for the present.

An equal diversity of opinion prevails with regard to the manner in which the non-nucleated corpuscle is formed from the nucleated. The earliest view, - that of Neumann - and that which has obtained most support is that the nucleus gradually decreases in size and finally disappears. The formed the nucleus in some corpuscles becoming dull and homogeneous, and not sharply defined, then indented at one point or dissolved at parts. A lessening of the nuclear mass followed, and in the last stage it consisted merely of a few granules. Along with the disappearance of the nucleus there is a slight diminution in the bulk of the cell. This is the view which also Kölliker held with regard to

[1] Quincke Op. cit.

[1] Byzozero. Op. cit.

[2] Hamburger. Den 15ten Archiv f. klin. Med. Bd XXXV S. 217

the formation of the non-nucleated red corpuscles in adult embryonic life. He saw corpuscles with small granular nuclei and in others he found a marked tendency of the nucleus to break down on the application of acetic acid. This theory has been supported by Obrastzow, Grohe, Osler Gibson & others.

Rindfleisch on the other hand describes the nucleus of a nucleated red corpuscle as surrounded by a small amount of colourless protoplasm which forms small projections into the surrounding yellow-coloured protoplasm, and holds that the nucleus thus enveloped passes out of the cell, by a mode analogous to amoeboid movement. The red corpuscle thus left behind has a bell-shape, which in the circulating blood is converted into the typical bi-concave disc. There would be according to this theory a nucleus with a little protoplasm around it set free in the bone marrow for every red corpuscle formed, with the possibility of its again developing into a nucleated red corpuscle, though he does not speak definitely with regard to its fate. Bizzozero, who at first held the same theory as Neumann, in his later papers tends to support that of Rindfleisch and Hayem agrees also gives it his support. That

Malaspina. Op. cit.

That the nucleus often does pass out of the cell is a matter of common observation. One often sees a corpuscle in which it is half-protruded. There is however a general tendency to regard it as a post-mortem phenomenon due to a contraction of the yellow coloured protoplasm which squeezes out the enclosed nucleus. [illegible] The tendency seen after the death of the cell might be taken in support of a similar occurring in life. The theory of Malassez differs widely from those mentioned inasmuch as he holds that the red corpuscle is formed by the nucleated red corpuscle producing at its periphery a spherical body which becomes detached as a spherical red corpuscle His great objection to a direct transformation of the nucleated into the non-nucleated corpuscle is that the latter is of smaller bulk than the perinuclear portion of the former; and besides he finds no evidence of such a transformation His observations were made chiefly on kids & rabbits by means of dried films fixed with the vapour of osmic acid He makes no mention of corpuscular appearances in the fresh marrow and I have

certainly seen none to support his theory.
I have seen in embryonic blood and organs nucleated red corpuscles in which the nucleus was represented merely by a small collection of granules and the same appearance though rather less frequently in the adult bone marrow. I have also seen comparatively large numbers in which the nucleus had a homogeneous appearance and stained very faintly with methyl-violet, and some in which it was barely visible. In these cases the nucleus appeared to be undergoing a transformation in situ, without breaking down. I think therefore that the nucleus is lost either by its gradually diminishing in size till it ultimately disappears, or by its becoming impregnated with haemoglobin and transformed like the surrounding protoplasm. It must be owned however that the number of such transition forms is not so great as one would expect and I am not convinced that there may be not be some other method of transformation e.g. probably emigration of the nucleus. No one who has studied the matter can doubt the non-nucleated corpuscles of embryonic blood are simply the nucleated ones minus the nucleus and are derived directly

[1] Hoppe. Centralb. f. med Wissenschft. 1869
SS. 249, 257

from them, and that if the red corpuscles of the adult have the same source the method of transformation is the same.

The relation of the circulating blood to the cell-elements of the marrow is a point of great importance, yet about which the facts are not fully known. There can be no doubt that the blood can flow through the marrow without the cell elements of the latter passing into it in any great number. The corpuscular elements of the blood are well defined from those of the marrow through which it flows. I have even cut through the marrow during life and found the blood flowing from it to differ comparatively little from that of the rest of the body. Yet even this fact only shows that the cell elements of the marrow are held in position with considerable firmness and does not prove that there must be a closed capillary system as a similar fact is true with regard to the Spleen & Sol. Hoyer was unable to inject the bone marrow from the arterial side but he found that injected material forced into the medullary cavity passed out by the vein. He also found on microscopic examination that the arteries at the periphery of the marrow substance gave

[1] Rindfleisch L. c. L.

[2] Hermann. Archiv. d. Heilk. 1869 S. 68

[3] Beyger Virchows Archiv Bd 52 S 106

off small vessels which did not lead to capillaries, but into open channels between the medullary cells. Accordingly there was a free passage into the blood afforded for the cells in the marrow. He notes also that the arrangement is very similar to that found in the spleen. Rindfleisch succeeded in injecting the bone marrow through the artery and found that the small arteries had exceedingly thin walls but there were not in the capillary system being reached, the capillaries and small veins being mere channels without walls at all. He considered therefore that the young red corpuscles were pushed into the venous channels whilst the colourless cells, being less slippery remained in position. Neumann on the other hand, finds that there are capillaries in the bone marrow which have walls like other capillaries. They are very broad, being in fact of greater width than the smallest arterioles and have also diverticula. Within these are very numerous colourless cells and transition forms to the nucleated red corpuscles. Bizzozero finds that the capillaries have walls composed of spindle-shaped epithelial cells and that the marrow cells lie irregularly in the spaces between. He is doubtful if some of the veins

have walls. Without further research it is impossible to speak definately on the subject. On a section of red marrow, however, and the same can be seen even better in a piece of marrow imperfectly dissociated, the marrow cells are seen lying closely together, whilst the ordinary red corpuscles often variously folded lie in the crevices between. These latter evidently do not lie in Capillaries yet almost certainly they have either passed out of Capillaries or are young corpuscles which have not yet reached the circulation. It is difficult to conceive of these corpuscles being in the position in which they are, if there were not some free channel of communication with the Capillaries. I have also seen in a fresh preparation, in which there were currents, the red corpuscles by their pliability gliding between the other cells whilst the nucleated red corpuscles remained fixed amongst the other cells. I think therefore, as Rindfleisch believes, that there must be some free communication with the Capillaries and that the red corpuscles do not pass into them in ordinary circumstances till they have acquired the characters of the adult non-nucleated stage. Many nucleated red corpuscles may be present in the bone marrow

[1] Litten & Orth. Berlin. Klin. Woch. 1877 S. 743

[2] Seel mayer Op. cit.

without any passing into the blood while, as will be shown below, some do pass in[g] of exactly the same structure as those in the marrow under certain circumstances.

Another important question is, in[g] what numbers are the nucleated red corpuscles to be found in[g] the bone-marrow in[g] the normal condition. In the marrow rabbits and dogs, on which I have made most of my observations, in the healthy ~~marrow~~ condition, they are generally to be found in[g] considerable numbers and are always easily found. When we consider the extent of the red marrow we see at once that by this means an extensive production of red corpuscles is provided for. In the case of the rabbit E.S. I found that the red marrow in[g] the two femora rather exceeded in weight the spleen in[g] weight. In the human subject they are generally also easily found but here some pathological condition is generally present which may influence their number or even in[g] some case cause their absence. Certainly in[g] one or two cases I have failed to find them. Letten and Gibb out of 100 cases missed them only in[g] 8 and Salmuyden[2] failed to find them in[g] 8% of those who had died in[g] a comparatively

healthy condition. The former observers think they are most likely to be absent in old people suffering from chronic disease unaccompanied by marasmus eg. chronic bronchitis. Neumann found them in patients of all ages though ~~&~~ generally fewer in advanced life. — even in patients beyond the age of 80 they were present. In the bone marrow of a man aged 68, who had been killed by a fracture of the skull, I found them pretty numerous whilst in the case of a woman who had died under chloroform and who was in a fairly healthy condition considerable numbers were present. We must also bear in mind that they are very easily decolorised after death and they may appear absent for this reason. I have actually proved this fact in the case of the dog. So far as my experience goes I think that the number is as great in normal conditions as we might expect on the assumption that they ~~are~~ furnish the only means for degeneration of the red corpuscles. We must bear in mind that the rate of transformation is quite unknown though it ~~is~~ probably ~~a~~ very the life of the corpuscle in its nucleated condition is very short in proportion to that in the non-nucleated state

I intend to give a brief Summary of the chief alterations observed in the bone marrow in pathological conditions by other observers and by myself. Considerable obstacles lie in the way of studying its pathology. Its various elements cannot be satisfactorily studied quantitatively as those of the blood, whilst qualitative alterations supervene in the finer structure of the cells a few hours after death so that it is often impossible to distinguish post-mortem effects. The most satisfactory method of examination is that of the marrow pulp by means of some preserving fluid, which stains the elements at the same time eg. Ehrlich's methyl-violet-Salt Solution or Mosso's peptone-Salt solution (v. method)

Results have been obtained in two main directions, firstly in the qualitative and quantitative changes of the cellular elements especially of the red nucleated blood corpuscles and secondly in the macroscopic alterations of the character of the marrow of the long bones which in normal conditions, in the human adult, is composed of fatty marrow. Most interest is naturally attached to its alterations in diseases of the blood, especially

[1] Cohn Lein Virchows Archiv. Bd 68 p. 291

[2] Neumann Berlin klin Wochenschr. 1877 S. 685

[3] Tilton & Goth loc. cit.

pernicious anaemia and leucocythaemia, and in those cases in which there has been severe haemorrhage before death.

Cohnheim describes a case of pernicious anaemia in which all the fatty marrow had been replaced by lymphoid marrow of a bright red colour, and his description has been confirmed by many others. On microscopic examination Cohnheim found many nucleated red corpuscles and many small red corpuscles of spherical form. He regarded the condition as indicating an imperfect formation of red corpuscles, the forms produced being immature and retained in the bone marrow. Neumann found the same change but ascribed the increase in the number of nucleated red corpuscles and the extension of the red marrow to an increased production due to a destruction of red corpuscles. The change he considered not to be peculiar to pernicious anaemia but only indicative of a certain process formed in other conditions also. The same changes were found by Litten & Orth viz the bright red colour of the red marrow its extension into the shafts of the bones and the presence of a great number of nucleated red corpuscles

[1] Osler Centralb. f. med Wiss. 1877 p. 498

[2] Seelmayer op cit

[3] Fiske Berlin klin Woch. 1884 p. 648

X.

and Osler records a similar case Geelmuyden[2] also considers the change to be compensatory in nature. He has collected altogether 43 cases and in 82% of these red marrow filled the shafts of the bones and in 78% nucleated red corpuscles were present generally in large numbers. [3]Yoke gives one case in which the alterations were very well marked and another in which all the shafts of the bones were filled with fatty marrow Quincke and Eichhorst on the other hand record cases in which no alteration could be found. In the red marrow of a typical case of pernicious anaemia (Case X v. Sub ✱) I found the smaller colourless cells (6-7μ) very numerous but these with a distinct band of homogeneous protoplasm not more so than usual. Ordinary red corpuscles were very few in number and shewed irregularities of shape and size as in the blood. Nucleated red corpuscles were much more numerous than usual. Some were about 8μ in diameter but many considerably larger and some were slightly irregular in shape (v. Pl. 35 Fig. 1). The nucleus in some was large and shewed the typical reticulated structure in others smaller and somewhat opaque. A few were in process of division

[1] Reimann Archiv der Heilkunde 1870

[2] Waldeyer Virchows Archiv. Bd 52. S 305

The other elements were little altered. There were no pigment-holding cells and no cells containing blood corpuscles. That an increased formation of red corpuscles was going on I can scarcely doubt.

In leucocythaemia the bone marrow sometimes undergoes important changes which Neumann was one of the first to note. He described a case in 1870 in which along with enlargement of the spleen and lymphatic glands, the spaces both in the long and in the short bones was filled with a purulent-looking material so as to give an appearance like diffuse purulent osteomyelitis. The cells composing it were somewhat similar to those in the blood but somewhat larger. He considered that there was a hyperplasia of the marrow cells with destruction of the marrow capillaries, so that the former passed freely into the circulation in a manner analogous to fat embolism after fractures. He has since recorded other cases. Waldeyer a case in which the marrow was of a greyish pink colour and had caused absorption of the bone in places. There was also enlargement of the spleen and a leukaemic condition of the blood. Though the condition had supervened on some caries he considered that it was not of inflammatory

(Nature

[1] Landois Virchows Archiv. Bd 56 p. 534

[2] Litten Berlin. klin Woch. 1877 S. 257, 278

but a hyperplasia associated with the changes in the blood and analogous to overgrowth of the spleen and lymphatic glands. A somewhat similar case is given by Parfik. There was enlargement of the spleen, and the bone marrow was of dirty yellow colour and contained enormous numbers of lymphoid elements, many of which he says were intermediate between red and white corpuscles. In pseudoleukaemia he found various, apparently unimportant alterations. Litten describes a remarkable case of which pernicious anaemia in which a leukaemic condition of the blood developed, there being numerous white cells up to 16 μ in diameter. There being no enlargement of spleen or lymphatic glands the diagnosis was medullary leukaemia and this was confirmed at the post-mortem examination. The bone-marrow was pus-like in appearance, and contained in enormous numbers cells similar to those in the blood, a few nucleated red corpuscles being also present. He also records a case of pernicious anaemia in which there was a 'hyperplasia' of the bone marrow - increase in the number and size of the marrow cells. This condition in pernicious anaemia is however quite exceptional.

The following is the condition of the bone marrow found which I
in a case of leucocythemia in which there was en-
largement of the spleen and lymphatic glands
The red marrow (in the ribs and vertebrae) was
pale in colour but by no means purple. It was
of nearly normal consistence. It contained enormous
numbers of small granular lymphoid cells 6-8 μ in
diameter, some of which shewed a thin margin of
clear protoplasm but most were without any.
Large cells reaching 16 μ in diameter were also seen
in large numbers but those of the largest size were
comparatively few. The typical marrow cells with
granular protoplasm and large nuclei were
very few in number. Nucleated red corpuscles v. Pl. 34
were rather few in number but many of these
shewed striking peculiarities. Most were large,
some reaching 11 μ in diameter, and so quite
well coloured with haemoglobin. Some had only
one nucleus but others had two, three, four or
even more nuclei, generally of small size. In one
corpuscle the nuclei were arranged round a
centre so as to form a rosette. One or two were
of somewhat irregular shape. After searching
for a long time I saw only one or two red corpuscles
with a single spherical nucleus of typical appear-
ance

Naumann Zeitschrift f. klin Med. Bd III
p. 410

Ordinary red corpuscles were fewer than usual and varied in size, some being 10 μ in diameter. There were a few pigment holding cells and also some granular heaps containing pigment in a diffuse form. There were also a few coarsely granular corpuscles and also irregular heaps of coarse granules. The chief points then were, the great number of the finely granular corpuscles of various sizes, the decrease in the number of the proper medullary cells and the peculiar characters of the nucleated red corpuscles. Those cells which were so numerous were of the same nature as those found in the blood The process might be regarded as a great proliferation of these cells leading to a destruction of the marrow cells and consequently also of the blood forming area. Such a process would explain the rapidly advancing anaemia which was present Of the peculiar appearances shewn by the nucleated red corpuscles I can give no explanation.

The changes found after severe hemorrhage are naturally of great importance and afford strong evidence in support of the haemopoietic function of the bone marrow. Neumann gives a full account of a case in which death had resulted from anaemia due to metrorrhagia. The fatty

marrow had almost completely disappeared from the long bones and had been replaced by red by myeloid marrow. In this, on microscopic examination he found enormous numbers of nucleated red corpuscles — as many as either the non-nucleated corpuscles or as all the colourless cells. They were also present in large numbers in the blood — in the blood of the aorta about one for every twenty non-nucleated corpuscles. They were also found in the Spleen, but not more numerous he thought than could be accounted for by the blood. Similar results though in a less marked degree have been obtained by others. Quelmuyden &c. always found in such cases nucleated red Corpuscles in large numbers. I had the opportunity of examining the marrow from a case in which long continued haemorrhage from an ulcerated tumour of the bladder had been the cause of death. The red marrow was of a dark pink colour and of somewhat cream-like consistence Microscopically it was found to contain nucleated red corpuscles in large numbers. Many could be seen in every field of the microscope, though they were not nearly so numerous as in Neumann's Case. Most were of the typical appearance,

v. Pl. 33

- unnucleated, with nucleus of characteristic reticulated appearance, and about 8μ in diameter some being a little larger, others less. A few had two nuclei and in one or two I saw three small nuclei. In some the appearance was if the nucleus had fragmented into several pieces and in a few only a collection of granules was present representing the nucleus. Some were in process of division and transition forms from the colourless cells were well seen. The ordinary red corpuscles showed considerable variations in size. I could find no marked alteration in any of the other cells. The nucleated red corpuscles in this case were by far the most numerous of all the cases ~~the cases~~ which I have examined. In the case of another patient, whose death was preceded though not caused by severe haemorrhage I found typical nucleated red corpuscles in the bone marrow though not in great numbers than usual. The patient however had also chronic Bright's disease hypertrophy of the heart and aorta and was in such a condition of malnutrition - almost approaching a cachectic state - that signs of increased blood formation could not be reasonably expected.

In other diseases the changes are less marked

[1] Tilden & Hall Op. cit

[2] Fischer Berlin klin Woch 1882 p. 646

[2] " " 1884 p. 227

[3] Guttmann Op. cit.

yet a considerable agreement exists between the results of various observers. Litten and Orth found nucleated red corpuscles most numerous in wasting diseases such as phthisis and cancer. In fact they lay it down as a General rule that the more marked and rapidly produced the Cachectic State is, the more numerous are the nucleated red corpuscles. In 60% of cases of phthisis and in 53.8 of cancer cases they found ten or more nucleated red corpuscles on an average in a field of the microscope. In such cases also they found the fatty marrow of the Diaphysis of the long bones more or less transformed into red lymphoid marrow. A practically similar has been arrived at by [2]Gohé whose observations extend to 431 Cases. [3]Quelmuyden has made similar observations with like results though she says that the fatty marrow is not transformed into lymphoid marrow but into gelatinous marrow - a marrow in which the fat cells are replaced by a mucoid substance which lies between the remaining marrow cells. In cases of phthisis and malignant disease Ehrlich generally found nucleated red corpuscles in large numbers though not so large as Litten and Orth describe. In acute diseases on the other hand where there has been high

temperature before death I have either formed very few nucleated red corpuscles or have failed altogether to find them. Such cases are acute general tuberculosis, acute pulmonary phthisis, septicaemic pneumonia &c. It is noteworthy that cases of pneumonia are amongst those in which they were found to be fewest both by Gulliver and by Litten & Orth. In one case of acute tuberculosis in which the temperature reached 107° before death I found the red marrow much congested there being a great number of ordinary red corpuscles present but I quite failed to find a nucleated red corpuscle. In cases of chronic syphilitic disease the nucleated red corpuscles have also formed to be few in number. The changes in cachectic conditions are by those above-mentioned observers considered to be compensatory in nature and to be somewhat similar to what is found in many cases of pernicious anaemia - as before described. The changes also resemble those found after haemorrhage and it is interesting to note in this connection that as I shall afterwards shew the changes in the blood in the cachectic and in the post-haemorrhagic state are very similar to one another

Observations have also been made with regard to some

of the other marrow elements chiefly the pigment-cells and the red corpuscle holding cells. It is noteworthy that in cases of typhus pigment cells were always found in large numbers both by Zeelmuyden and by Parfick. Neumann thought that in this condition the capillary walls of the marrow gave way and the red corpuscles which passed out broke down and were taken up by marrow-cells Litten and Orth found them in all sorts of conditions in 37% of their cases. In some cases of phthisis I have seen them very numerous and a similar observation has been made by others. I found them by far most numerous, however, in a case of alcoholic cirrhosis of the liver with slight jaundice. In this case the marrow was very soft and pulpy and there was evidently some reticulum of the medullary spaces, the bones being very easily broken. On microscopic examination I found great quantities of yellow or brown pigment for the most part contained within cells, some however lying free (v. Plate 36) The formation of this pigment could be easily traced from red corpuscles included within cells. In some cells the outlines of the red corpuscles could be seen with great distinctness whilst yellow amorphous pigment was also present, and intermediate

v. Pl. 36

stages

were seen. Other cells of somewhat irregular outline were filled only with amorphous pigment, and a further stage seemed to be that in which the pigment was seen lying free amongst broken down granular masses. Occasionally few nucleated red corpuscles could be seen. A possible explanation appears briefly viz that owing to the impaired function of the liver the decrepid red corpuscles were not removed in the usual way but were taken up within mononuclear cells and disintegrated. Further observations in like cases would however be necessary. The whole appearance would lend support to Gaskell's theory which is that blood-corpuscle-holding cells always become pigment cells and indicate regressive changes in the blood corpuscles. The corpuscle-holding cells are formed in three ways viz. by a simple 'agglutination' of red corpuscles by the taking up of single red corpuscles within cells, by a process of 'invagination,' and by a combination of these two methods, the red corpuscles first being taken up and afterwards ~~[illegible]~~ agglutinated. He considers also that the giant cells indicate a fat corresponding process in the case of the colourless cells. The débris from all these cells is utilised in the production of new cells as Rind fleisch also believed.

Regarding the grosser pathological Lesions E.g. Watery diseases, tubercle, inflammation &c. It is unnecessary to Speak here

Thesis for the Degree of M.D.

Contributions to the Physiology and Pathology of the Blood

VOL II

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Chapter VII
Clinical Cases

I. Traumatic Anaemia

Under this head I have placed cases of anaemia due to haemorrhage from various causes – cases of haematemesis, metrorrhagia, expistaxis, haematuria and post partum haemorrhage. In such cases we must always consider the possibility of there being some pre-existing change in the blood predisposing to haemorrhage, but I have excluded this possibility as far as possible and believe from the manner of recovery and for other reasons that they simply cases of traumatic anaemia

Case I. This was a case of anaemia due to violent epistaxis ~~aged 27~~ in a very strong and healthy man aged 28. The haemorrhage lasted several days and nearly baffled all surgical endeavours to stop it. The patient was not subject to epistaxes, this being the first attack. The case is of great interest on account of the intense anaemia reached

On Sept 23rd the red corpuscles showed considerable variations in size 6-8 μ. A few oval forms were present but none of irregular shape They formed small coherent & irregular heaps. The leucocytes were evidently very much increased in number, most being of the large variety 8-9.5 μ. The blood plates formed large flocks in the field

Table I Traumatic Anaemia (a)

	Date	Red Corpuscles	Leucocytes	Blood-plates	Prep. 1	Prep. 2	Prep. 3	Poikilocytes
Case I J.M. 28. M. Anaemia due to Exposure	Sept 23	1,786,000	25000	286,000	1:66	1:6·3		None
	„ 28	967,500	21000	370 000	1:47	1:2·6	1:4·2	Very few
	„ 29	977,500	21000	392,000	1:46	1:3·0		„
	Oct. 2	1201,000	13000	368000	1:92	1:3·2		„
	„ 3	1,420,000	11000	416000	1:129	1:3·4		None
	10	1932000	12000	284000	1:161	1:6·9		„
Case II AR. 33. F Anaemia following Placenta Praevia	Jan 2	1,427,000	26,000	414,000	1:54	1:3·4	1:3	None
	3	1,511,000	23,000	411,000	1:65	1:3·6	1:2·4	„
	4	1,425,000	18,000	380,000	1:79	1:4·0	1:2·7	„
	5	1,285,000	15,000	348000	1:85	1:3·7	1:1·9	
	6	1,246,000	16000	430000	1:77	1:2·9	1:2	Very few
	8	1,215,000	13,000	328000	1:93	1:3·7	1:2·3	„
	10	1,450,000	11,500	361000	1:131	1:4·0	1:2	None
	14	1,376,000	9,000	418000	1:153	1:3·3		None
	19	1,945000	13000	423000	1:149	1:4·7	1:1·9	„
	23	2,041,000	10,500	343000	1:194	1:5·9	1:1·7	„
	25	2,100,000	9,000	341,000	1:233	1:6·1		„
Case III M.W. 37 Anaemia due to Metrorrhagia	Sept. 8	1921000	9,000	288,000	1:213	1:6·6		A few
	„ 13	2,447,000	8,000	289,000	1:306	1:8·4	1:1·8	„
	„ 28	3,208,000	4,000	280,000	1:802	1:11·4		„

but were rather smaller than usual most being about 1·5 μ in diameter

On Sept 23rd few polychromatophilic red corpuscles were seen but only after searching several fields. The same variations in size as before were present - but more marked The blood plates were more numerous and larger than before.

On 6th Oct 10th The red corpuscles still showed some variation in size but these were less marked now

Stained specimens on Sept 28th showed that the proportion of multinucleated leucocytes was very high (1:4·2) Many intermediate forms were also seen. The multinucleated forms generally had many nuclei & generally nucleoli could be seen. The point of by far the greatest interest is the presence of nucleated red corpuscles. Of this there could be no doubt. They were present in larger numbers than in any other case which I have examined. They were of typical structure (normoblasts of Ehrlich) containing a single nucleus equal to half the corpuscle in diameter and showing a well marked reticular structure. The perinuclear portion was stained by methyl-blue a little more deeply than the ordinary red corpuscles but a few red corpuscles took the stain in the same way. These latter I

W. Pl. 19 fig 1+2

believed to be those which had most recently lost their nucleus. Unfortunately I made dry films only on the one occasion.

I may add that I had the opportunity of examining this patient's blood about six weeks later and it was then in a practically normal condition

The chief points noteworthy are.

(1) The extreme degree of anaemia reached.

(2) The comparatively little alteration in the characters of the red corpuscles

(3) The presence of hyperchromic nucleated red corpuscles

(4) The increase in the number of the blood plates but without increase in size.

(5) The leucocytosis with the increase in the proportion of multinucleated forms.

Case II This was a case of placenta praevia in a patient aged 35. There were large haemorrhages on three occasions before the delivery, none after it. The first examination of the blood was about a week after the last haemorrhage.

On Jan 2nd Many of the red corpuscles were undersized - 6-7μ. They formed small rouleaux or lay in heaps. ~~The colourless~~ No true poikilocytes were present though a few showed slight irregularities

The colourless elements were much increased in

number of the leucocytes chiefly the large forms. The blood-plates did not exceed 2·5 μ in diameter.

On Jan 6th a few micrococci were seen but otherwise the blood had the same characters. I last examined it on Jan 25th when the red corpuscles were rather better coloured and showed great regularity in size. The leucocytes were much fewer, the blood-plates about the same number.

Stained specimens showed that the proportion of multinucleated forms had increased at first and afterwards fell as the leucocytes disappeared. Intermediate forms were numerous at first. On Jan 2nd and Jan 3rd one or two nucleated red corpuscles were found after diligent search.

The chief points in this case are.

(1) The anaemia rather increased for some time after the last haemorrhage (fourteen days) and afterwards the process of regeneration was very slow

(2) Beyond slight variations in size & less in shape the red corpuscles were little altered. Nucleated red corpuscles though present were not nearly so numerous as in the preceding case

(3) The blood plates were increased in number nearly to the same extent throughout.

(4) There was a leucocytosis at first which afterwards disappeared

Case III M. W. aged 27. The patient was a female who had had several uterine haemorrhages of great severity with the preceding eighteen months. The last haemorrhage was six months before admission. She had been very pale and weak since.

Sept^r 8^th The red corpuscles were pale and varied in size 5-9 μ, most were under size. A good number were of decidedly irregular shape. The blood-plates were increased in number and many were of large size 3·5-5 μ. The leucocytes were not increased in number - they had the usual appearances.

Sept^r 13^th practically the same conditions were present, though the red corpuscles were increasing in number

Sept^r 28^th Still irregular red corpuscles present. Blood plates as before of large size. Patient is rapidly improving. Stained specimens showed that the number of multinucleated corpuscles were not in so high a proportion as is usual in such cases. They leucocytes had the usual characters

This case shews that anaemia due to haemorrhage may persist for a long time afterwards but may rapidly improve under proper treatment. It is to be noted that although the degree of anaemia was not so great as in the two previous cases irregular corpuscles were much more numerous. This I believe

Table II Traumatic Anaemia (b)

	Date	Red Corpuscles	Leucocytes	Blood-plates	Prep. 1	Prep 2	Prep 3	Poikilocytes
Case 4 M. J. 35 Anaemia due to Melanthaemia	Nov 7	1,675,000		580,000			1:3·5	Very numerous
	„ 13	2,210,000		625,000				„
	17	2,360,000	9200	360 000	1:250	1:250	1:2·3	Numerous
	24	1920,000	6000	734 000	1:316	1:316	1:1·9	Very Numerous
	Dec 1	2160 000	12,000	656,000	1:180	1:180		„
	„ 8	3190 000	11000	506 000	1:290	1:290	1:1·4	Numerous
	„ 15	3756 000	8000	522,000	1:469	1:469		„
	„ 21	3844000	7000	384,000	1:549	1:549	1:·9	„
	„ 29	4816000	7,000	316,000	1:688	1:688		Few
	Jan 17	2,865,000	7,500	518,000	1:382	1,382	1:1·05	None
	„ 30	2858,000	12,000	462,000	1:238	1:238	1:1·8	None
Case 5 J. M. 27 Haematemesis	Mar 14	2636,000	8,000	396,000	1:329	1:6·6	1:2·3	Few
	„ 16	2,502,000	7,500	341,000	1:333	1:7·3	1:2·4	„
	„ 21	2754,000	9,000	402,000	1:306	1:6·8	—	„
	„ 27	2664,000	7,500	424,000	1:355	1:6·2	1:1·7	Very few
	Apr. 1	3121,000	7,000	312,000	1:446	1:10·0	1:1·9	„
Case 6 J. B. 55 M. Haematuresis	July 27	2815,000	10,500	314,000	1:268	1:8·9	1:2·3	None
Case 7 B. M. 62. M. Bleeding Haemorrhoids	Mar 4	2,801,000	6,500	322,000	1:431	1:6·5	1:1·8	None
Case 8. J. M. 43. M. Haematemesis	Mar 15	4,030,000	7,500	268,000	1:537	1:15	1:3	None
Case 9. J. B. 20. M. Haematemesis		2058,000	18,000	Numerous	1:114	—	1:10	A few

is to be associated with the long duration of the anaemic condition. Though the blood-plates were rather larger than usual, no intermediate forms either in size or in quality could be found between them and the smallest red corpuscles present.

Case IX M. G. aged 35. The patient was a woman who had been suffering for a long time from a bleeding fibroid the amount of blood lost, chiefly in the form of clots being very great. Bleeding had been taken place for several months.

Nov.ᵇᵉʳ 7ᵗʰ 10ᵃᵐ. Of fresh blood. The red corpuscles were profoundly altered. Most were of the most irregular shapes whilst those which were of circular shape were 4·9 μ in diameter. The irregular forms were of the most varied description, some were oval, some oval & flat-sided tailed some pear shaped &c. and others too irregular to be described. They did not form rouleaux but lay irregularly in heaps. Fingerprinting was well marked. They were very pale in colour. The blood plates were in enormous numbers forming large flocules in the areas between the red corpuscles. The leucocytes appeared numerous. Most measured 18·6 μ but a few were smaller.

On Nov.ᵇᵉʳ 9ᵗʰ 10ᵃᵐ & 13ᵗʰ 10ᵃᵐ. Showed the same condition.

On Nov.ᵇᵉʳ 17ᵗʰ Poikilocytes were rather less numerous.

W. Pl. 18

Nov. 24th After renewed severe bleeding. The blood was not more altered in appearance than ever Scarcely a regular normal red corpuscle was to be seen and the irregular forms were of the most fantastic types. The most striking feature was the presence of irregular forms of very small size and extreme transparency (as shown by focussing) and scarcely visible as they floated in the serum. Some had very delicate processes which waved in the currents around They seemed however to be perfectly elastic. Some few filamentous forms were also seen; these were probably detached processes of larger forms but the small corpuscles which I have just described could scarcely have such an origin as they were practically ordinary poikilocytes in miniature The blood plates were even more numerous than before they differed markedly in character from the smallest red corpuscles present. The leucocytes were in practically the same condition as before.

Dec. 1st Condition again improved somewhat

Dec. 8th Irregular red corpuscles less numerous. Some are of circular shape but vary greatly in size = 5-10 μ. Blood plates are still fewer than ever

Dec. 21st A considerable number of red corpuscles are of normal appearance and form Rouleaux well

Irregularities in size and shape still occur but they are less numerous.

Dec 24th Though the red corpuscles are practically normal in number now and most are of normal size, corpuscles 3·3-9 μ in diameter can be found also a few typical singular forms.

Haemorrhage returned again and on Jan 17th-24th the blood showed a partial return to its former condition but no poikilocytes appeared.

Stained films showed the characters of the red corpuscles well and the enormous numbers of blood-plates. At first the proportion of multinuclear leucocytes was high and large corpuscles showing differentiation - intermediate between the two varieties were common. As repair took place the latter became much scarcer and the mononuclear leucocytes relatively increased in number.

Summary. The following were the chief facts noted.

(a) The red corpuscles showed more marked poikilocytosis at the height of the anaemia than in any case I have seen

(b) The blood-plates were increased in number to a great degree - absolutely three fold, relatively to the red corpuscles twenty fold. They were not increased in size.

(c) The leucocytes were slightly increased in number especially after the haemorrhages

In the (d) no nucleated red corpuscles were present.

In the progress of the case the following changes were seen

(a) The irregularities in shape and size of the red corpuscles gradually disappeared as the number increased, but poikilocytes were still present though the number had returned to normal

(b) The blood plates diminished in number but increased again after the later haemorrhages

(c) The number of leucocytes decreased a little and the proportion of multinucleated forms also decreased.

The case also illustrates the great rapidity with which the blood may be restored when the blood forming apparatus is in a healthy (or more probably hyperplastic) condition, there being an addition in a fortnight of over a million and a half corpuscles per c.m.m.

Case V Anaemia due to severe haemorrhages. Patient was a female aged 27.

Mar. 14th Red corpuscles were pale in appearance and showed considerable variations in size many being 6·7μ. Poikilocytes were present but numerous in number. The blood-plates though very numerous were not increased in size. The leucocytes showed the usual varieties. Mitosis was seen in

stained specimens a large number of leucocytes intermediate in character between the uni- and the multinucleated forms.

The blood therefore showed changes of the same nature as, though less marked than, in the previous cases

Cases VI & VII The one was a case of anaemia due to haematuria the other of anaemia due to bleeding haemorrhoids. In both similar changes were seen, the red corpuscles being of smaller size than usual and of paler colour. The number of the blood plates was increased a little.

Case VIII Slight anaemia due to haemoptysis; the patient was a healthy man aged 43

The anaemia was slight and the red corpuscles showed no qualitative change.

Case IX Anaemia due to haemoptysis in a boy aged 21. This was however not a simple case as there was also chronic Bright's disease and marked malnutrition almost scorbutic in nature.

The red corpuscles were undersized as a rule and pale. Blood plates were increased in number but to a small degree. The leucocytes were much increased in number

Summary with regard to Traumatic Anaemia.

From the preceding cases the following facts may be gathered. The amount of blood which may be lost without a fatal result in the human subject is very great if the bleeding be extended over some time, so that there is time to allow a passage of fluid into the vessels and restitution of the volume of the circulating blood. Death is caused in sudden haemorrhage not by the amount of red corpuscles lost but by the emptying of the vessels of fluid. Thus in case I more than four-fifths of the blood (i.e. of the red corpuscles) were lost in the course of a week i.e. on the assumption that the volume of blood at the height of the anaemia was no greater than in the normal state. So far as I know this is ~~one of the~~ a case of the greatest anaemia due to haemorrhage on record. The rate of recovery after haemorrhage in favourable conditions is very great. An increase of nearly a million corpuscles per c.m.m. per week may be got and this as will be shown below is rather less even than what is sometimes found in artificial anaemia in dogs and rabbits. Case II shews that after severe haemorrhage the anaemia may increase for some time and afterwards slowly diminish (This increase of the anaemia is to be distinguished from that which

(this place within the first few days after haem(orhage
due to absorption of fluid into the vessels and con-
sequent dilution of the blood) Cases are seen on re
cord where the anaemia has gone on increasing and
become pernicious in character Cantlie shews that
post-haemorrhagic anaemia when persistent for some
time owing to unfavourable conditions is sometimes
very amenable to suitable treatment. The continuance
of the anaemia does not imply that it is intractable.

The following are the chief alterations in the corpuscular elements.
(1) If the anaemia be acute, even though very pronounced
the red corpuscles may be little altered in character.
There are more undersized corpuscles (6-7 μ) and they
are paler and of less firm consistence. Otherwise there may
be no change. If the anaemia be severe and of long
continuance marked changes occur. The size of the cor-
puscles varies widely, small bodies being sometimes
found as low as 3 μ in diameter whilst megalocytes
(up to 10 15/16 μ) are formed though in comparatively few
numbers. The corpuscles also may become exceedingly
irregular in shape, all sorts of forms being formed. They
are also very pale in colour. As the blood returns to
normal these poikilocytes gradually disappear
yet some may be formed even when the number of
the corpuscles has reached 4,000,000. The number of

poikilocytes seems to depend more upon the duration of the anaemia than upon its intensity

Nucleated red corpuscles may appear in the blood if the anaemia be intense They were found in large numbers in Case I and they were also present in Case II. They are of typical structure and generally about 8 μ in diameter with a single round nucleus of reticulated appearance. Their presence would seem to depend upon the degree of anaemia. I cannot as yet say whether they afford any aid in prognosis.

The leucocytes after acute haemorrhage generally shew a marked increase in number. This increase in number is chiefly on the part of the large multinucleated forms - the proportion of the multinucleated forms becomes increased and many intermediate forms are also seen which are generally of large size. The number of nuclei in the multinucleated corpuscles is generally high and a nucleolus is generally visible in the nucleus. With a return to normal the above proportion falls and intermediate forms become less numerous

The number of the blood-plates is increased often very markedly and diminishes as recovery takes place The number would appear to be greater in cases of long standing anaemia due to repeated haemorrhages

Table II. Idiopathic Anaemia (a)

	Date	Red Corpuscles	Leucocytes	Blood-Plates	Prep 1	Prep 2	Prep 3	Peculiarities
Case 10 J.C. 54.M Pernicious anaemia	Feb 21	1,404,000	3,500	31,000	1:401	1:45	1:81	Very numerous
	" 28	1,560,000	3,500	74,000	1:445	1:21	1:·74	"
	Mar 6	1,636,000	5,000	92,000	1:327	1:18	1:·8	"
	" 14	1,614,000	3,500	117,000	1:448	1:14	1:·79	"
	" 27	1,601,000	2,500	124,000	1:640	1:13	1:·8	Numerous
Case 11 J.G. 60.M Pernicious Anaem	~~Jul 14~~							
	July 21	790,000	3,000	18,000	1:263	1:43	1:·59	Very numerous
	" 28	717,500	5,000	15,000	1:143	1:47		"
	" 28	701,000	2,000	8,000	1:350	1:87	1:·59	"
Case 12 D. McG. M. aged 62 Pernicious Anaem.	Feb 2	812,500	2500	11,000	1:324	1:73	1:1·1	Very numerous
	" 17	961,000	1000	13,000	1:961	1:74	1:1·2	"
	Mar 6	1465000	1,000	23,000	1:1465	1:63	1:1·3	"
Case 13 D.R. M. aged 55 Pernicious Anaem	Feb 8	909,280	2,000	44,000	1:227	1:20	1:·25	Numerous
	20	1,522,500	1,500	68 000	1:1013	1:22	1:·4	"
	25	1,923,000	2,000	76,000	1:961	1:25	1:·5	"
	Mar 13	2,359,000	3,000	173,000	1:786	1:13	1:·21	Few
	Ap. 9	2,436,000	2500	262,400	1:972	1:9·3	1:1·2	Very few
Case 14 J.B aged 51 M Pernicious Anaem	Mar 13	785,000	1,500	9,000	1:523	1:87	1:·7	Very numerous
	" 24	481,250	1,000	7,000	1:481	1:68	1:·4	"
	" 29	575,000	2,500	33,000	1:228	1:17	1:·54	"
	Ap. 8	940 000	3,000	54,000	1:313	1:17	1:1·2	"

than in cases of equal degree of anaemia due to one or few large haemorrhages. In only one case were they markedly enlarged but even in that case the largest forms did not exceed 3·5 μ in diameter.

I obtained the impression that the increase in the number of leucocytes was a more pressing phenomenon than that of the blood plates - more related to the alterations in the volume of the blood caused by the haemorrhage. The increase of the blood-plates was a later phenomenon to be associated rather with the process of regeneration of the blood or with the impoverished condition. This impression was confirmed by the results of experiments (v. sub C)

II Idiopathic Anaemia

I have arranged under this head cases both cases of pernicious anaemia and also others of somewhat doubtful nature, but unassociated with any malignant disease. The characters of the blood also in these cases were wanting in distinctness.

Since last summer I have been fortunate enough to examine the blood of no fewer than five patients suffering from pernicious anaemia in which the number of corpuscles fell below a million per c.m.m.

Case X Pernicious anaemia. J. C. aged 54. Male. The patient had been ill for about a year before the

first examination. He dated his illness from the death of his wife. I made examinations in the Spring of 1889 and once in the Summer of the same year immediately before his death.

Feb 21. (Shortly after admission to the Hospital) Red corpuscles were greatly altered. Poikilocytes were exceedingly numerous of great variety. The corpuscles also showed great variations in size. Some were as small as 3μ in diameter but were not deeply coloured. Many were much over normal size. Some oval forms reached 15μ in their long axis. The corpuscles did not form rouleaux. They were well coloured. The blood plates were very scanty and of small size. The leucocytes were also very few & many were of the small variety 6-7μ in diameter. It was curious to see the leucocytes lying amongst red corpuscles many of which were of larger size.

Stained specimens showed that the leucocytes were practically of two varieties viz small uninucleated corpuscles & fully developed multinucleated corpuscles, the former being more numerous than the latter. No nucleated red corpuscles were found.

Examinations were made on Feb 28, Mar 6 & 14 with the same results. On March 27th I saw more megalocytes were present, some being 18·5μ in diameter. I may add that the haemoglobin was less decreased than the number of the red corpuscles.

the general condition had improved considerably though the number of Red corpuscles was but little altered, shewing a slight increase. The number of the blood plates had increased considerably. At the time he left the Hospital he felt fairly well.

He was re-admitted on Aug. 16th evidently in a dying condition. I examined his blood on Aug. 13th (the day before he died. The red corpuscles shewed practically the same alterations as before the large number of Megalocytes being a very noticeable feature. Poikilocytes were rather less numerous than formerly. The number of red corpuscles was now under half a million per c. mm. (the enumeration was performed twice with separate specimens of blood). The blood plates were exceedingly scanty whole fields being without any visible. The Leucocytes were evidently much decreased especially the large varieties. Nothing else was noticed. Dried films shewed the number of Multinucleated Leucocytes to be proportionately smaller than before. The small mononucleated forms being more than double the Multinucleated in number. The latter were well developed and contained as a rule massive nuclei, very distinct from one another. The two classes of Leucocytes were very well separated from each other. No nucleated red corpuscles were found (though they were found in large numbers

(over

v. pl. 35

v. Pl. 22

v. Pl. 22

v. Pl. 22

large and of oval shape - many measuring 14μ in their long axis and 10μ in their short. Also circular forms very much above normal size were numerous. Small forms down to 4μ in diameter were also seen.

The colour of the red corpuscles was rather deeper than normal. There was no attempt at the formation of roulaux.

The colourless elements were very scanty. The plaques were exceedingly few in number measuring all about 2μ in diameter. The leucocytes were also few in number especially those of larger size.

Stained films showed that the leucocytes were few in number. By far the greater number were of the small mononuclear variety showing no protoplasm around the nucleus. Multinuclear corpuscles were little more than half as numerous as the mononuclear variety. They showed as a rule many nuclei - in one as many as seven were seen. Many forms intermediate between the mi- & the multinuclear forms were seen. One or two nucleated red corpuscles were found after careful seeking. These were not of the typical erythroblastic as in the cases of traumatic anaemia. The nucleus was more homogeneous as a rule, the characteristic reticular appearance being absent. The shape of some was distinctly irregular.

Scanning many blood films could be found in the

Stained Specimens.

Similar examinations on shewed practically the same conditions, the blood plates being found still.

In the general character of the red corpuscles this case resembled the previous. In the extreme paucity of the blood plates and the diminished number of the leucocytes with preponderance of the small mononuclear forms it also offered points of resemblance.

The presence of nucleated red corpuscles in this case is a point of difference between the two.

Case XII Pernicious Anaemia D. M'G. aged 62.

The patient was a man whose general nutrition was good.

Feb 1 The red corpuscles shewed alterations very like those in the previous case. They did not form rouleaux but were scattered over the field singly or in small heaps. All the corpuscles were well coloured. Figures many were of very irregular shape as well as of very various sizes. Some of the circular forms were megalocytes of 10 μ in diameter. Some of the oval forms were 16 μ in their long axis and some of the tailed forms measured 24 μ in length. Small forms down to 4 μ in diameter were also seen. Some of the forms appeared like thin films variously folded, the contrast between these and the large oval forms being very striking

The blood plates were very scanty and scarcely any exceeded 2 μ in diameter. The leucocytes were also few - the large & small varieties appeared to be in nearly equal nos. Stained films showed the uni- & multi-nucleated corpuscles to be practically equal in number. They had the normal appearance. No nucleated red corpuscles

On Feb 17th a practically similar condition was found In the dried films a few nucleated red corpuscles were found of similar characters to those in the previous case Mrs B. Considerable improvement was noted. The red corpuscles had increased in number as had also the blood plates.

In almost every respect this case resembled the previous one. The leucocytes were slightly more numerous and the multinucleated forms were in higher proportion. The condition of the red corpuscles and blood-plates in the two cases was practically identical

Case XIII D.R. aged 65. Pernicious Anaemia.

Had classical symptoms & characters for about a year. The case was of great interest because with all the symptoms of pernicious anaemia present a very marked improvement was effected if not a complete cure.

Feb 8th Haem. of pale blood. Red corpuscles are markedly altered. Apart many poikilocytes are present but the most noticeable feature is the