THE ANAEMIAS OF CHRONIC INFECTIONS.

THESIS

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INTRODUCTION.

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THE ANAEMIAS OF CHRONIC INFECTIONS.

I. INTRODUCTION.

The changes occurring in the red blood cells in infective illnesses are amongst the least carefully studied problems of haematology. In spite of the fact that these anaemias are extremely common, it is difficult to find adequate literature to make possible even a clear description of the blood picture under these circumstances.

Part of the difficulty is due to the fact that anaemias in infections are grouped together with other anaemias under the vague heading "secondary anaemia", in which many mechanisms are undoubtedly active.

Another major source of confusion is the fact that in many of the cases in which "secondary anaemia" occurs, complicating factors such as haemorrhage and liver disease are not adequately dealt with.

Furthermore, very few authors have set out to define the characters of the red cells and the anaemias in infections alone, but have usually included such cases under the heading "miscellaneous anaemias" in studies devoted to other problems.

Three main mechanisms operate in the production of anaemias (Witts, 1932; Vaughan, 1934). These are dyshaemopoiesis, haemolysis and haemorrhage. All three mechanisms undoubtedly contribute to the resulting anaemias in infections. Many infective illnesses are associated with haemorrhage, either large, or small and repeated. Haemolysis may also occur in infections. Treatment given for the infection may also influence the blood picture.

In the present thesis a careful attempt has been made to eliminate all such factors and to study the influence of the infection per se on the blood and blood-forming tissues.

This has been done by studying cases in which all factors other than the infection itself, which are known to affect the red blood cells, could be eliminated by careful clinical and laboratory study of the cases. This programme unfortunately curtailed the number of cases suitable for study, but it was considered that accurate studies on those cases selected in this manner would contribute valuable information about the problem under consideration.

Most textbooks of haematology classify the anaemias of infections under the "toxic

dyshaemopoietic" group. The red blood cells are described as normocytic or microcytic, and in either case the blood picture may be normochromic or hypochromic.

Wintrobe (1930) in early studies with the accurate apparatus designed by him, states that simple microcytic anaemias occur in chronic infections and intoxications such as bronchiectasis, lung abscess, unresolved pneumonia, typhoid, cardio-vascular-renal disease and carcinoma without blood loss.

Britton (1936) agrees with these findings. He found that in chronic colitis, chronic cholecystitis, chronic cystitis, toxic neuritis, subacute rheumatism and rheumatoid arthritis (1 case each) the anaemia was microcytic and normochromic in type. One case of carcinoma of the stomach showed a microcytic hypochromic anaemia.

Cassells (1938) in his miscellaneous group, however, found a somewhat different picture. Calculating macrocytes as those cells with a diameter greater than 7.25 mu. and microcytes as cells smaller than 7.25 mu. in diameter, he found that 1 case of rheumatic carditis, 1 case of aplastic anaemia, 2 cases of malignant disease and 1 case of hydatid disease showed macrocytosis of 55 - 91%. In 1 case of rheumatic carditis the cells were normal in size.

In searching through lists of blood counts in infective conditions, anaemias with a high colour index are encountered not infrequently, e.g. 1.07, 1.12 and 1.21 in cases of lobar pneumonia, 1.33 in a case of bronchopneumonia, 1.13, 1.21, 1.23 and 1.33 in cases of otitis media and 1.08 in a case of congenital syphilis (Kugelmass and Lampe, 1932), 1.1 in a case of rheumatic carditis (Kato, 1940). Ungley (1943) also confirms the occurrence of macrocytosis in the anaemia associated with infection.

The confusion in the type of case studied as discussed above and in the results obtained is apparent.

Equal difficulty attends the search of the literature on the mechanism of production of these anaemias. Only a few studies have been devoted to this problem. The most quoted paper is that of Douglas and Tannebaum (1930). They divided their anaemias according to the icteric index and the reticulocyte counts, the former being taken as an index of blood destruction and the latter as evidence of blood regeneration. They found in a heterogeneous collection of "secondary" anaemias that the icteric index was low and the reticulocytes also were low. From this they concluded that secondary anaemia is the result of diminished

production of blood by the bone marrow.

Kugelmass and Lampe (1932), basing their conclusions also on a study of the icteric index and reticulocytes, say that infection depresses the rate of regeneration of haemoglobin and red blood cells, and quote earlier work in which it was shown that infections interfere with the activity of the intestinal mucous membrane which normally detoxifies or fails to absorb substances injurious to the bone marrow (Dragstedt, 1924; Koessler and Hanke, 1924; Cannon, 1928).

Robscheit-Robbins and Whipple (1936) in a study on haemoglobin regeneration in dogs rendered anaemic by bleeding, found that infection induced in the pleura inhibited the formation of new haemoglobin. Relief of the condition in the pleura restored haemoglobin production to normal.

Vaughan and Saifi (1939) deny that there is depression of the bone marrow in anaemias due to infections, and state also that there is no evidence of excessive haemolysis in these cases, as shown by the normal urobilinogen excretion in the stools. They also found very little evidence of blood regeneration, but on the basis of the finding of excess coproporphyrins I and III in the stools conclude that the anaemias of chronic infections are the result of defective haemoglobin synthesis.

A few other studies of the anaemias of chronic infections contain relevant information.

The fragility of the red blood cells in hypotonic saline was measured by Daland and Worthley (1934) and Cassells (1938) in groups of "secondary" anaemias, and odd cases have been reported in other papers, e.g. Kato (1940). There seems to be agreement that the fragility is normal or decreased in "secondary" anaemias.

The bone marrow was studied by sternal puncture in 5 cases of "secondary" anaemia by Young and Osgood (1935). The cases were as follows:- 1 each of congenital syphilis, tertiary syphilis, Still's disease, acute nephritis and chronic nephritis. In the congenital syphilis and chronic nephritis marrows there was excessive erythropoietic activity of normoblastic type and in the remaining three erythropoiesis was normal in degree and type. Vaughan and Saifi (1939) refer to unpublished studies of their own in which they found at autopsy that the marrow was hyperplastic rather than aplastic in chronic infections. These findings are against the conclusion of bone marrow depression discussed above and more in keeping with the views of Robscheit-Robbins and Whipple (1936) and Vaughan Saifi (1939).

Gross, Sandberg and Helly (1943) estimated the iron and copper content of various organs

including the liver, spleen and stomach in chronic diseases accompanied by secondary anaemia. They found that the iron and copper content is never low, usually it is normal and frequently it is in excess of normal. This finding is in accord with that of Robscheit-Robbins and Whipple (1936) in the experiment quoted above. They found that iron was absorbed normally during the inhibition of haemoglobin formation by the infection in the pleura.

Stiles, Stiles and Kolb (1942) using the bromsulphalein excretion test demonstrated diminished liver function in cases of low grade chronic ill-health. This depression of liver function in infective illnesses has also been noted by me (Berk, 1943), and by many other authors.

Increased urobilin excretion in the urine has been detected in cases of lobar pneumonia (Curphey and Solomon, 1938) and in pneumonia and puerperal sepsis (various authors quoted by Vaughan and Saifi, 1939). This also has been interpreted as evidence of defective liver function in these cases.

The information collected from the literature on the characters and mechanism of the changes in the red blood cells is obviously inadequate and difficult to correlate. The work reported in this

thesis was undertaken to study the anaemias of infections more completely and to attempt to reach a satisfactory explanation of the changes observed.

MATERIALS AND METHODS.

II. MATERIALS AND METHODS.

1. The Cases Studied.

Great care was exercised in the selection of cases for study in order to exclude all factors that might have interfered with the blood picture. In the cases finally studied, the anaemia was attributed solely to the infective process present.

The following sources of confusion were avoided as far as possible:-

1) All cases showing obvious deficiencies of haematinic factors were excluded. Thus no case where deficiency of Castle's factor may have occurred was included. Cases showing evidences of iron deficiency, such as glossitis and koilonychia, were not included. No cases with signs or symptoms suggestive of myxoedema or vitamin deficiency form part of this study.

2) Idiopathic aplastic anaemias or "refractory anaemias" (Bomford and Rhoads, 1941; Davidson, Davis and Innes, 1943) were not included. Leukoerythroblastic anaemias and splenic anaemias, where different mechanisms probably exist, were also avoided.

3) No haemolytic anaemias are included, although it is recognised that certain infective conditions may give rise to a haemolytic process.

4) All anaemias due to blood loss, whether acute or chronic were excluded.

5) Toxic anaemias following irradiation with radio-active substances were not included.

The cases remaining after this selection are those referred to by Wintrobe (1942) as "simple chronic anaemias". Further exclusions had to be made as follows:-

a) Cases shewing obvious hepatic disease, such as hepatitis, cirrhosis or malignant deposits, or cases with jaundice were not included in the infective group, because of the ill-understood relationship between liver disease and anaemia.

b) Cases with gastro-intestinal pathology were excluded because of the many complicating factors introduced by such disease.

c) Cases shewing cardiac failure and cases of oedema due to whatever cause were excluded as well, except for subacute bacterial endocarditis.

d) Any case of toxic anaemia in which blood loss may have been a factor in causing the anaemia was excluded, e.g. ulcerative colitis.

e) No cases of anaemia due to nephritis were investigated, and furthermore all cases shewing clinical evidence of nephritis, either

in lack of concentrating power of the kidneys, albuminuria out of keeping with the pyrexia, or casts in the urine, were excluded, again excepting subacute bacterial endocarditis.

f) No cases in which recent operation had been performed were included.

g) No cases were studied in which drugs which might have affected the blood picture had been used, e.g. the sulphonamide group of drugs.

h) The youngest patient was aged 11.

As far as possible a check on these possibilities of error was made in those cases that came to autopsy.

In spite of this very careful selection of cases, it was impossible to know the patient's blood status before the onset of the illness causing the anaemia, so that this possible source of error has not been excluded satisfactorily.

The cases finally numbered 25, made up as follows:-

Tuberculosis		13 cases.
Pleurisy	5 cases	
Glands	2 cases	
Generalised	1 case	
Glands + Pleurisy	1 case	
Spine, lungs and larynx	1 case	
Spine with lumbar abscess	1 case	

Empyema + Lungs	1 case
Pulmonary alone	1 case
Subacute Bacterial Endocarditis	3 cases.
Rheumatoid arthritis	2 cases.
Pneumonia	1 case.
Empyema	1 case.
Subphrenic abscess	1 case.
Empyema with Broncho-pleural fistula	1 case.
Lung abscess	1 case.
Chronic osteitis	1 case.
Chronic eczema	1 case.
TOTAL	25 cases.

These cases constitute the central theme of this thesis. 3 cases came to autopsy.

The relative smallness of the number of cases studied is attributed largely to the rigid care exercised in selecting the cases.

Other cases are included in this thesis for special reasons which are indicated in the text. A numbered list of all the cases studied, together with the diagnoses is included at the end of the thesis in order to facilitate reference. The case numbers are adhered to throughout.

2. The Methods of Investigation.

The following investigations were carried out in the cases studied. The numbers in brackets indicate the number of cases in which each investigation was performed.

1) Haemoglobin estimation (25).
2) Red cell count (25).
3) White cell count (18).
4) Differential white cell count (18).
5) Reticulocyte count (18).
6) Target cell count (17).
7) Price-Jones curve (23).
8) Haematocrit (25).
9) Platelet count (16).
10) Hypotonic saline fragility test (15).
11) Hippuric acid excretion test (15).
12) v.d. Bergh reaction (18).
13) Plasma bilirubin estimation (18).
14) Urine urobilin (qualitative) (17).
15) Fractional test meal (12).
16) Bone marrow studies (15).

The methods used are described in detail below.

1) Haemoglobin Estimation.

This was performed with a Dare haemoglobinometer. No standardised instruments were available and the Dare was the best of those that

could be used. The probable accuracy of the instrument is referred to later.

2) Red Cell Count.

Two dilutions were made from heparinised venous blood in each instance. Estimations were repeated if the results obtained differed by more than 200,000 cells per cu. mm.. A "Turk" counting chamber was used in almost the entire investigation.

3) White Cell Count.

This was also performed on heparinised venous blood. A single dilution was made and counted in both chambers of the slide.

4) Differential White Cell Count.

Differential counts were performed on Leishman-stained films.

5) Reticulocyte Count.

The reticulocytes were stained with brilliant cresyl blue in the following way. A drop of 1% aqueous solution of brilliant cresyl blue was placed on a slide. To this was added a smaller drop of blood, about half the size of the drop of stain. The two drops were mixed together thoroughly with another slide and a smear was made of the mixture. This was allowed to dry

in air and was then counterstained with Leishman's stain. The slides were again allowed to dryin air. The number of reticulocytes per 1,000 red blood cells was counted and the result expressed as a percentage.

6) Target Cell Count.

The number of target cells seen per 1,000 red cells was counted on either a Leishman- or eosin-stained smear. The count was expressed as a percentage. Counts were made on portions of the slide where the cells were spread thinly, as recommended by Barrett (1938). Three main types of target cells are seen (Barrett, 1938).

a. Cells with a central area of haemoglobin.

b. Cells where the central disc of haemoglobin is continuous at one point with the peripheral stained area of the red cell.

c. Cells in which the central stained area takes the form of a band extending from one side of the peripheral stained area to the other.

Other types of target cells were seen and are described later.

7) Price-Jones Curves.

Thin films of capillary blood were used for the measurement of the red cell diameters. These were stained by one of two methods.

a. In the earlier cases the method recommended by Price-Jones (1933) was used. This consits of staining the smear with Leishman' stain and then counterstaining with a 1% solution of eosin.

b. It was found later in the investigation that the Leishman's stain failed to fix the smear satisfactorily, since the counterstaining washed the smear off the slide. This was attributed to the poor quality of the Leishman's stain, replacements of which could not be obtained owing to wartime supply difficulties. After numerous trials of various methods, the most satisfactory was found to be staining a fresh film of blood with a 1% solution of eosin in methyl alcohol for 3 -5 minutes and washing with tap water. This method embodies the principles of the Price-Jones method in a single rapid operation.

A microscope was arranged with the tube lying horizontally, and the light from an ordinary 150 watt lamp was projected into the microscope through a system of condensers. A prism placed over the eyepiece of the microscope reflected the image of the film on to a glass slab. The whole instrument was enclosed in a wooden box painted black, through one end of

Which the microscope tube passed. The floor of the box was prolonged on that end to hold the glass slab. Doors were arranged in the sides of the box to allow operation of the moving stage, for focussing and for adjusting the lamp.

The magnification of the machine was adjusted by projecting a slide ruled in 0.01 mm. divisions on to a paper scale. The paper scale was prepared from the same transparent ruler as was used for measuring the cells. The tube of the microscope was adjusted so that the 0.01 mm. divisions corresponded to 1 cm. on the paper scale. The magnification was therefore 1,000 times and 1 millimetre was equivalent to 1 mu.

The film was then projected on to paper and the cells were traced. These were measured in two diameters with the transparent ruler mentioned above. The diameters were measured to the nearest 0.5 mm. (0.5 mu) and the mean of the longest and shortest diameters was expressed to the nearest 0.25 mm. (0.25 mu). 500 cells were traced and measured in each case and the results were subjected to a statistical analysis as described by Whitby and Britton (1937).

8) Haematocrit.

The packed red cell volume (VPC) was

measured by the method of Wintrobe (1929) using Wintrobe tubes. Duplicate measurements were made and read to the nearest 0.5 mm.(%).

9) Platelet Count.

This was performed according to the method of Dameshek (1932). It was found that it was satisfactory to use new slides and coverslips cleaned with xylol. The finger is cleaned with ether and allowed to dry. A puncture is made and the first drop of blood wiped off. A drop of the special fluid containing brilliant cresyl blue as stain, sodium citrate as anti-coagulant and glucose to make up the tonicity is placed over the puncture and the finger squeezed gently so that blood enters the drop of stain. The proportion of blood to stain is approximately 1:5. A portion of the mixture is picked up on a coverslip. Thorough mixing of the blood and stain is accompanied by gentle tilting of the coverslip, which is than placed on the slide. About 45 minutes are allowed to elapse before the count is performed. The number of platelets seen in counting 1,000 red cells is recorded. The number of platelets per cu. mm. is calculated by multiplying this number by the red cell count in thousands, e.g. 150 platelets seen in

counting 1,000 red cells. Red cell count 3,000,000 per cu. mm.. Platelet count 150 x 3,000 = 450,000. The normal figures obtained by this method are 400,000 - 900,000 (Dameshek, 1932).

10) Hypotenic Saline Fragility Tests.

Because of the large number of investigations undertaken on many of the cases it was not possible to perform a full quantitative fragility test. A rapid qualitative test was devised in order to overcome this difficulty.

A SIMPLE QUALITATIVE TEST FOR ERYTHROCYTE FRAGILITY.

Dacie and Vaughan (1938) in the paper describing their fragility test state that "the shape of the curve or span of resistance .. .... is without great practical significance". Also, they say that "it is not in practice very useful". The figure that is important is the M.C.F. (median corpuscular fragility, the percentage of saline that causes 50% haemolysis). Normal M.C.F. lies between 0.334 and 0.398% NaCl.

Fragility is increased when the M.C.F. exceeds 0.398 and is decreased when the M.C.F. is less than 0.334.

It appears that in most instances it is sufficient to know whether the fragility lies within normal limits, or whether it is increased or decreased. The degree to which it is increased or decreased does not seem to be of great significance.

The following modification of the method of Dacie and Vaughan (1938) has therefore been devised in order to tell whether the M.C.F. is normal, increased or decreased.

The blood is prepared in the following way. About 10 cc. of blood are withdrawn from a vein and placed in a clean tube containing sufficient heparin to prevent clotting. This sample of blood is oxygenated by placing it in a cylindrical glass separating funnel of about 100 cc. capacity. This is rotated horizontally without a stopper, so that the blood spreads out in a thin film on the sides of the funnel. This rotation is continued for 5 minutes and oxygenates the blood to a standard degree of saturation, i.e. the oxygen tension is brought into equilibrium with that of the atmosphere. A portion of the sample is used to fill Wintrobe haematocrit tubes which are then centrifuged at 3,000 revolutions per minute for 30 minutes. At the end of this time the reading is made of the

percentage of packed red cells.

5 cc. of the exygenated heparinised venous blood are placed in a centrifuge tube and centrifuged lightly for a few minutes and a volume of plasma is removed so that if an haematocrit were performed on the remaining blood the reading would be normal, i.e. correction is made for anaemia. The amount of plasma to be removed from 5 cc. of blood is 5 - 0.11 x VPC%. This corrects the packed cell volume to 45.45%. This figure is a suitable one for a normal reading, and at the same time keeps the calculation of the amount of plasma to be removed reasonably simple.

The solutions are prepared as follows:-
A stock solution is made up containing 20% pure dessicated NaCl. For the test 5 cc. (containing 1 gram of NaCl) is added to 95 cc. triple distilled water. This gives a 1% solution. Two dilutions are prepared from this, one a 0.334% and the other a 0.398% solution. This is done by taking 33.4 cc. of the 1% solution and adding 66.6 cc. of distilled water. This gives a 0.334% solution. The second dilution is made up of 39.8 cc. of 1% NaCl and 60.2 cc. of distilled water. These are the stock solutions for the test.

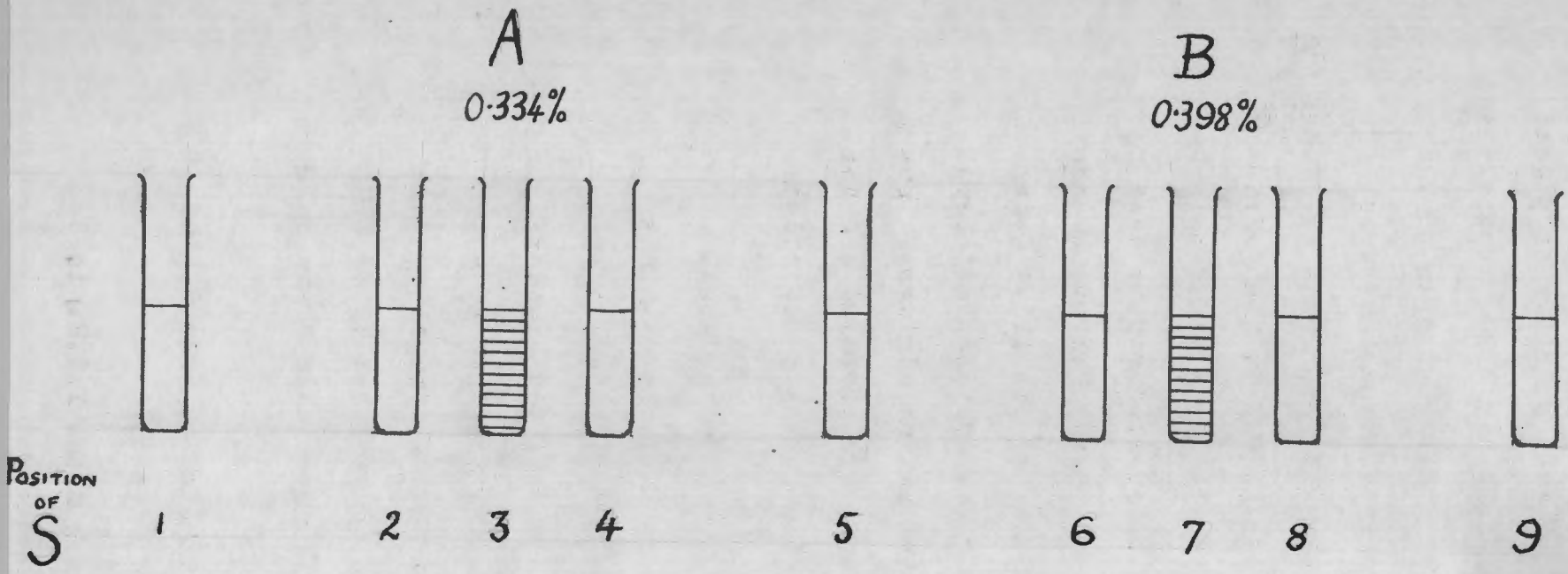


FIG. 1. READING OF THE RAPID FRAGILITY TEST.

The test is performed as follows:- Using the same pipette 1 cc. of each dilution is placed in small tubes of equal bore and 1 cc. of distilled water is placed in a third tube. 1 drop of the blood prepared in the way described above is placed in each of the three tubes. The two tubes containing the saline are placed in the refrigerator for 30 minutes, then removed and centrifuged. The distilled water specimen is used to make up a 50% standard by taking 0.5 cc. of the specimen and adding 0.5 cc. of distilled water. This is done in a small tube of identical bore with these containing the saline mixtures. The colours in the three tubes are compared and the tubes arranged in order of intensity of colour.

Nine possible positions of the tubes exist (fig.1). Let A be the tube with the 0.334% saline and B the tube with the 0.398% saline and S the tube with the 50% standard. The result is read from the position of S. The result is expressed as the "fragility test reading" which has been abbreviated to "F.T.R."

1. If A and B are much lighter than S the F.T.R. reading is 1.

2. If A is just slightly lighter than S, the F.T.R. is 2.

3. If A is identical with S the F.T.R. is 3.

4. If S is intermediate in colour between A and B the F.T.R. is 4,5, or 6 depending on whether the colour of S is slightly lighter than that of A, intermediate in colour between A and B or just slightly darker than B.
5. If S is identical with B the F.T.R. is 7.
6. If S is lighter than B the F.T.R. is 8 or 9, depending on how much lighter than B it is.

The advantages of this method are:-

1) Only 3 stock solutions are kept instead of 32.

2) In doing the full test according to Dacie and Vaughan (1938), the same pipette has to be used for measuring out all solutions in order to keep the volume constant. In order to do this the pipette has to be cleaned and dried between each pipetting. In the simple test described only 3 tubes are used as against 15 or more in the full test, i.e. only 2 cleanings are required as compared with 15 or more.

3) The complete test requires the making up of standards ranging from 0 - 100%. In the simple test only 1 standard (50%) is prepared.

4) The information obtained in the simple test is as accurate in every way as the full test, since all variables are controlled in the same way, i.e. the corrections for oxygenation,

temperature and anaemia are used.

5) On completion of the full test, the result is expressed as the M.C.F. and this figure is compared with the normal figures for M.C.F.. In the simple test the information is almost as great, the only difference being that the M.C.F. is not obtained as a figure, but directly as a comparison with the normal.

The fact that the formula for correcting for anaemia works accurately and that the volume of the cells is not altered by the simple removal of plasma is demonstrated in table I. This shows the results of performing haematocrits on the blood sample after the removal of the requisite amount of plasma. 5 cases were studied in this way.

TABLE I.

Correction of the Blood for Anaemia.

Case.	Original VPC.	VPC. after plasma removal.
7	33.0	45.5
8	35.5	46.0
26	41.0	45.5
27	*54.0	45.0
28	40.0	45.0

*For polycythaemic blood the formula is:- to 5 cc. of blood add (0.11 x VPC% - 5) cc. plasma.

The results obtained do not differ from the expected result by more than the 0.5% that is the experimental error in haematocrit determinations. The formulae correct accurately for anaemic bloods and for bloods showing readings above the ideal normal value selected.

THE QUANTITATIVE SALINE FRAGILITY TEST.

When quantitative detail of the behaviour of the red cells in hypotonic saline was required the following method based on the Dacie and Vaughan (1938) modification of the Creed (1938) method was used.

The blood was oxygenated and corrected for anaemia as in the qualitative test.

The dilutions of saline were made up as follows:- From the stock 20% solution of NaCl a 0.5% solution was prepared. Using a 1 cc. pipette graduated in 0.01 cc. divisions, amounts of 0.5% saline were placed into small test tubes of equal bore such as were used in the qualitative test. Using the same pipette after cleaning and drying it, amounts of distilled water were pipetted into the tubes. The quantities of 0.5% saline and of distilled water used are shown in Table II.

TABLE II.

Saline Dilutions.

0.5% Saline (cc.)	Distilled H_2O (cc.)	Final Concentration (%)
.08	.92	.04
.12	.88	.06
.16	.84	.08
.20	.80	.10
.24	.76	.12
.28	.72	.14
.32	.68	.16
.36	.64	.18
.40	.60	.20
.44	.56	.22
.48	.52	.24
.52	.48	.26
.56	.44	.28
.60	.40	.30
.64	.36	.32
.68	.32	.34
.72	.28	.36
.76	.24	.38
.80	.20	.40
.84	.16	.42
.88	.12	.44
.92	.08	.46
.96	.04	.48
1.0	0.0	.50

The final volume of the contents of each tube was 1 cc. The saline and water were mixed by inverting the tubes.

Triple distilled sterile water as prepared for intravenous injection was used throughout all fragility tests to avoid any trace of acid or bacterial contamination.

To each of the dilutions 1 drop of the prepared blood was added by means of a constant drop

pipette which delivered 25 drops per cc.

The blood-saline mixtures were inverted a few times and then the tubes were placed in the refrigerator for 30 minutes. At the end of this time the tubes were removed and centrifuged lightly to deposit the unhaemolysed red cells, the white cells, the platelets and red cell debris. The degree of haemolysis was then read against the standards prepared from the same blood.

The standards were prepared in the following way:- Six cc. of distilled water were measured out into a test tube, using the same pipette as was employed in making up the saline dilutions. Into this, 6 drops of the prepared blood were placed from the same constant drop pipette as was used in adding the blood to the saline dilutions. The test tube was inverted several times and the red cells allowed to haemolyse completely. This solution represents 100% haemolysis.

From this 10%, 20%, 30% etc. solutions were made up as shown in table III, the same pipette being used for all pipettings.

TABLE III.

Preparation of Standard Solutions.

Distilled H_2O (cc.)	100% Solution (cc.)	Final Concentration (%)
.9	.1	10
.8	.2	20
.7	.3	30
.6	.4	40
.5	.5	50
.4	.6	60
.3	.7	70
.2	.8	80
.1	.9	90
.0	1.0	100

In making the readings the following methods were used:-

1) The highest concentration of saline in which haemolysis occurred (minimal saline resistance of American authors) was easily seen by holding the tube up to the light and noting the first tube in which any pink colour at all was present.

2) The point of complete haemolysis (maximum saline resistance) was determined by looking down the tubes as they were held against a well-lit white background. The buttons of deposit are easily seen and these were followed down to the point at which there was no longer any decrease in the size of the deposit and the button was constantly the same. The highest concentration of saline showing this constant deposit was taken to be the point of 100% haemolysis, the deposit

consisting of white cells, platelets and red cell debris.

3) All tubes were then matched against the standards. The readings were made to the nearest 5%.

From the figures so obtained a graph was constructed and the percentage of saline in which 50% haemolysis occurs (M.C.F.) was read from this.

The following features of the test were recorded - the percentage of saline in which haemolysis commenced; the percentage of saline in which haemolysis was complete, and the M.C.F.

When dilutions of saline higher than 0.5% were required, the necessary solutions were made from a 1% solution of saline. The volumes required are shown in table IV.

TABLE IV.

Dilutions of Saline.

1% NaCl (cc.)	Water (cc.)	Concentration (%)
.52	.48	.52
.54	.46	.54
.56	.44	.56
.58	.42	.58
.60	.40	.60
.62	.38	.62
.64	.36	.64
.66	.34	.66
.68	.32	.68
.70	.30	.70
.72	.28	.72
.74	.26	.74

11. Hippuric Acid Excretion Test.

This was performed according to the method of Marrom (1941).

The night before the test the patient is encouraged to take plenty of fluids. On the morning of the test, a breakfast of toast and coffee is given. One hour later the patient is given 6 grammes of sodium benzoate dissolved in 30 cc. of water, followed by a glass of water. The total urine of the next 4 hours is collected accurately.

The volume of the urine is measured and a 100 cc. sample is taken. 1 drop of octyl alcohol is added as a preservative. 35 grammes of ammonium sulphate is added to the urine to saturate it. Then 1 cc. of concentrated sulphuric acid is added drop by drop, stirring the mixture well.

The specimen is filtered, the precipitate is collected, dried and weighed. Hippuric acid excreted is equivalent to (weight of precipitate x volume ofurine specimen, divided by 100, and multiplied by 0.682) gms. benzoic acid.

12) van den Bergh Test.

This was performed according to the method of Haslewood and King (1937).

13) Plasma Bilirubin Estimation.

Plasma bilirubin was estimated according to the method of Haslewood and King (1937).

14) Urine Urobilin.

A specimen of urine was examined spectroscopically and the presence of the absorption band of urobilin noted. When no band was seen, a few drops of iodine were added to the urine to convert any urobilinogen present in urobilin, and the spectroscopic examination was repeated.

15) Fractional Test Meal.

This was carried out in the standard way. After fasting overnight, a Ryle stomach tube was passed and the gastric contents aspirated. 300 mg. of caffeine citrate in 300 cc. of water were given by mouth and specimens withdrawn from the stomach at 15 minute intervals for 3 hours. The sixth specimen was tested for acid and if none was present histamine acid phosphate 0.5 mg. was injected subcutaneously.

The free acid was titrated with N/10 NaOH, using Topfer's reagent as indicator. The results were expressed in the usual units, i.e. ccs. of n/10 NaOH required to neutralise the acid in 100 ccs. of gastric juice. The presence of blood, mucus and bile was noted.

16) Bone Marrow Studies.

Specimens of bone marrow were obtained by sternal puncture, using the technique of Bodley Scott (1939). Puncture was made opposite the second intercostal space and between the midline and the lateral border of the sternum. The marrow was aspirated by gentle suction and films were made from the aspirated material and stained with Leishman's stain. It has been found in this laboratory that aspiration should be attempted as soon as the needle is gripped by the bone and repeated after pushing the needle in a very short distance at a time, until marrow juice is obtained. It has been found that there is less likelihood of hitting a blood vessel and aspirating peripheral blood by using this technique than by entering the marrow cavity directly before aspirating.

The following counts were made on the marrow films:- The red cells precursors were counted according to the classification of Davidson, Davis and Innes (1942,1943). All red cell precursors are called erythroblasts and are divided into types I, II, III and IV, corresponding to the megaloblast, early erythroblast, late erythroblast and normoblast of Whitby and Britton (1937). The rest of the marrow cells were not differentiated, but merely

their total counted. The count was continued until 100 primitive red cells had been counted. The non-red-cell-precursors included all nucleated cells that were not primitive red cell series, and degenerated cells (Osgood and Seaman, 1944).

It was thus possible to state what percentage the red cell precursors formed of the total nucleated marrow cells and also the percentages of the four types of primitive cells recognised.

All the tests were performed personally by the author.

3. Discussion of Technical Methods.

A) Normal Cases.

The probable accuracy of the technical methods used for the estimation of haemeglobin, the enumeration of the red cells and the measurement of the packed cell volume was demonstrated by a series of normal cases investigated at the same time and with the identical techniques, solutions and apparatus used in investigating the anaemias of chronic infections. Counts were performed on 6 nurses, 8 doctors and 6 patients suffering from conditions that do not affect the blood.

The results obtained are shown in table V.

Table VI shows the figures obtained in these normal subjects compared with published figures on normals in various parts of the world.

It can be seen that the results obtained correspond very closely with the results of the other authors quoted.

This study of normal cases serves several other purposes.

1) The results serve as a normal standard of reference for the investigations on the anaemias of infections.

2) It has been established that the normal stand-

TABLE V.

Normal Controls.

Case.	Race.	Sex.	*Hb. %.	R.B.Cs. /cu.mm.	+C.I.	V.P.C. %.	M.C.V. cu.μ.	M.C.H. μμ	M.C.H.C. %.
26	E	♂	85	4.47	1.05	41	92	30	33
28	E	♀	80	4.45	1.0	40	90	29	32
29	E	♂	89	4.93	1.0	42	85	29	34
30	E	♂	94	5.59	0.88	46	84	27	33
31	E	♂	100	6.08	0.9	51	84	26	31
32	E	♂	96	5.53	0.96	49.5	90	28	31
33	E	♂	85	4.92	0.95	44.5	90	28	31
34	E	♂	98	4.95	1.09	46	93	32	34
35	E	♂	98	5.08	1.06	48	94	31	33
36	E	♂	100	5.21	1.06	48	92	31	33
37	E	♀	84	4.64	1.0	43	93	29	31
38	E	♀	84	4.40	1.05	40	91	33	37
39	E	♀	88	4.83	1.0	45.5	94	29	31
40	E	♀	86	4.60	1.03	42	91	30	33
41	E	♀	91	4.70	1.06	42.5	90	31	34
42	E	♀	87	4.84	1.0	43	89	29	31
43	E	♀	83	5.04	0.9	42.5	84	26	31
44	E	♀	95	4.99	1.05	41	82	30	37
45	E	♀	105	5.46	1.06	46	84	31	37
46	E	♀	84	4.34	1.06	41	94	31	33

*100% Hb. = 16.0 gms..
+Calculated to 100% Hb.= 14.5 gms.

TABLE VI.

Normal Range of Haematological Standards.

Authority	Country.	No. of cases.	M.C.V. cu. μ.	M.C.H.C. %.	M.C.H. μμ
McGeorge (1936)	England	50	78-90	31-35	24-29
Price-Jones, Vaughan and Goddard (1935)	England	100	76-96	28-34	24-30
Haden (1932)	America	100	88-96	32-36	29-33
Wintrobe (1932) (1934)	America	187	80-94	32-38	27-32
Linneburg and Schartum-Hansen (1935)	Normay	101	76-93	-- --	27-34
Britton (1936)	New Zealand	50	78-94	32-38	26-32
Symons (1939)	Johannesburg	11	73-91	27-33	21-25
Present Investigation	Cape Town	20	82-94	31-37	26-33

Modified from Whitby and Britton (1937).

ards for Cape Town are the same as those obtaining in other parts of the world.

B) Oxygenation of the Blood Samples.

It has been shown by numerous authors that the red blood cells in venous blood are larger than those in capillary or arterial blood, and also that they are more fragile in hypotonic saline (Whitby and Britton, 1937: Cormick, 1942). A small series of cases was studied in order to find out the extent to which venosity of the blood affects the packed cell volume. In addition comparison was made in a few cases between the volume of packed red cells in specimens in which heparin and oxalate mixtures were used as anti-coagulants. It is said that neither of these affects the cell volume.

The investigation was carried out in the following way:- 20 subjects, both healthy normals and patients with various diseases were used. A specimen of blood was withdrawn from the subject's vein. One portion was added to a tube containing heparin and another sample was added to a tube containing the oxalate mixture.

The heparinised specimen was oxygenated in the way described for the fragility test.

One portion of the oxalated specimen also was oxygenated.

Duplicate haematocrit determinations were made on the heparinised oxygenated specimens from all 20 cases; on all 20 oxalated unoxygenated

specimens, and on 7 specimens of oxygenated oxalated blood.

The results are shown in tables VII, VIII and IX.

TABLE VII.

Haematocrit Determinations on Samples of The Same Blood using Different Anti-coagulants .

Case.	Oxygenated Heparinised Blood V.P.C. % .	Oxygenated Oxalated Blood V.P.C. % .	Difference % .
5	30.5	30.5	0
8	35.5	36	0.5
26	41	41.5	0.5
28	40	41	1.0
32	49.5	50	0.5
33	44.5	44.5	0
37	43	43	0

TABLE VIII.

The Effect of Oxygenation on the Haematocrit.

Case.	Oxalated Venous Blood V.P.C. % .	Oxygenated Oxalated Venous Blood V.P.C. %	Difference %
5	31	30.5	-0.5
8	37	36	-0.5
26	42	41.5	-0.5
28	41	41	0
32	51	50	-1.0
33	45.5	44.5	-1.0
37	44.5	43	-1.5

TABLE IX.

The Effect of Oxygenation on the Haematocrit.

Case.	Heparinised Oxygenated Blood V.P.C. %.	Oxalated Blood V.P.C. %.	Difference
2	43.5	44	0.5
4	35	37	2.0
5	30.5	31	0.5
6	27	30	3.0
7	33	34.5	1.5
8	35.5	37	1.5
26	41	42	1.0
28	40	41	1.0
31	51	52	1.0
32	49.5	51	1.5
33	44.5	45.5	1.0
37	43	44.5	1.5
47	41.5	42	0.5
48	40	41	1.0
49	36	38	2.0
50	38.5	40	1.5
51	46.5	47.5	1.0
52	11.5	12.5	1.0
53	30	31.5	1.5
54	40	41	1.0

The results in table VII show that the anticoagulant used makes no difference to the haematocrit reading. The haematocrit readings on the oxygenated heparinised blood and the oxygenated oxalated blood are identical in 3 cases, no significant difference is seen in 3 cases, and in the seventh case the difference is just outside the range of experimental error.

It will be seen that there is a distinct difference between the readings on the oxygenated heparinised blood and the unoxygenated exsiated blood. This varies from 0.5% to 3%.

This difference between the haematocrit readings on oxygenated and non-oxygenated specimens falls within the range of experimental error in only 3 instances (15%). In the remaining 17 cases (85%) a significant difference is present - 1.0% in 8 cases, 1.5% in 6 cases, 2.0% in 2 cases and 3.0% in 1 case. The reading is always lower in the oxygenated sample.

The differences in the mean corpuscular volume and in the mean corpuscular haemoglobin concentrations that result from the difference produced in the haematocrit reading by oxygenating the blood are shown in table X.

TABLE X.

The Effect of Oxygenating the Blood.

Case.	M.C.V. (cu.mu.)			M.C.H.C. (%)		
	From Oxygenated Haematocrit.	From Unoxygenated Haematocrit.	Difference.	From Oxygenated Haematocrit.	From Unoxygenated Haematocrit.	Difference
2	83	84	I	28	28	0
4	133	141	8	26	24	2
5	78	79	I	27	26	I
6	121	139	18	29	26	3
7	91	96	5	28	26	2
8	93	97	4	29	28	I
26	92	94	2	33	32	I
28	90	92	2	32	31	I
31	84	86	2	31	31	0
32	90	92	2	31	30	I
33	90	92	2	31	30	I
37	93	96	3	31	30	I
47	90	91	I	28	28	0
48	92	95	3	30	29	I
49	108	114	6	30	29	I
50	105	109	4	32	30	2
51	107	109	2	31	30	I
52	141	153	12	26	24	2
53	88	92	4	37	35	2
54	96	99	3	34	33	I

The mean corpuscular haemoglobin concentration is affected to some extent (0 - 3% increase) by oxygenating the blood. The mean corpuscular volume result, on the other hand, is markedly disturbed, the decrease ranging from 1 - 4 cu. mu. in 15 of the 20 cases and reaching on occasio ns as high as 18 cu. mu..

The importance of these results lies in the fact that different degrees of deoxygenation of blood occur in different samples of blood. Thus in comparing results from different groups of cases and in the follow-up studies in the same patient it is necessary to standardise this variable factor as far aspossible. The simplest way of standardising the degree of oxygenation is to bring the blood into equilibrium with the atmospheric oxygen which is virtually constant. This is accomplished in the way described in the fragility test and oxygenated specimens have been used in almost every instance in this thesis.

The results obtained confirm the fact that differences in cell volume in venous and arterial blood are due to a large extent to differences in the oxygen saturation of the blood. Furthermore, since no difference is seen between the readings on oxalated and heparinised blood, it is likely that neither affects the cell volume. This

identity in the behaviour of the oxalate mixture and of heparin is not disturbed by oxygenating the blood.

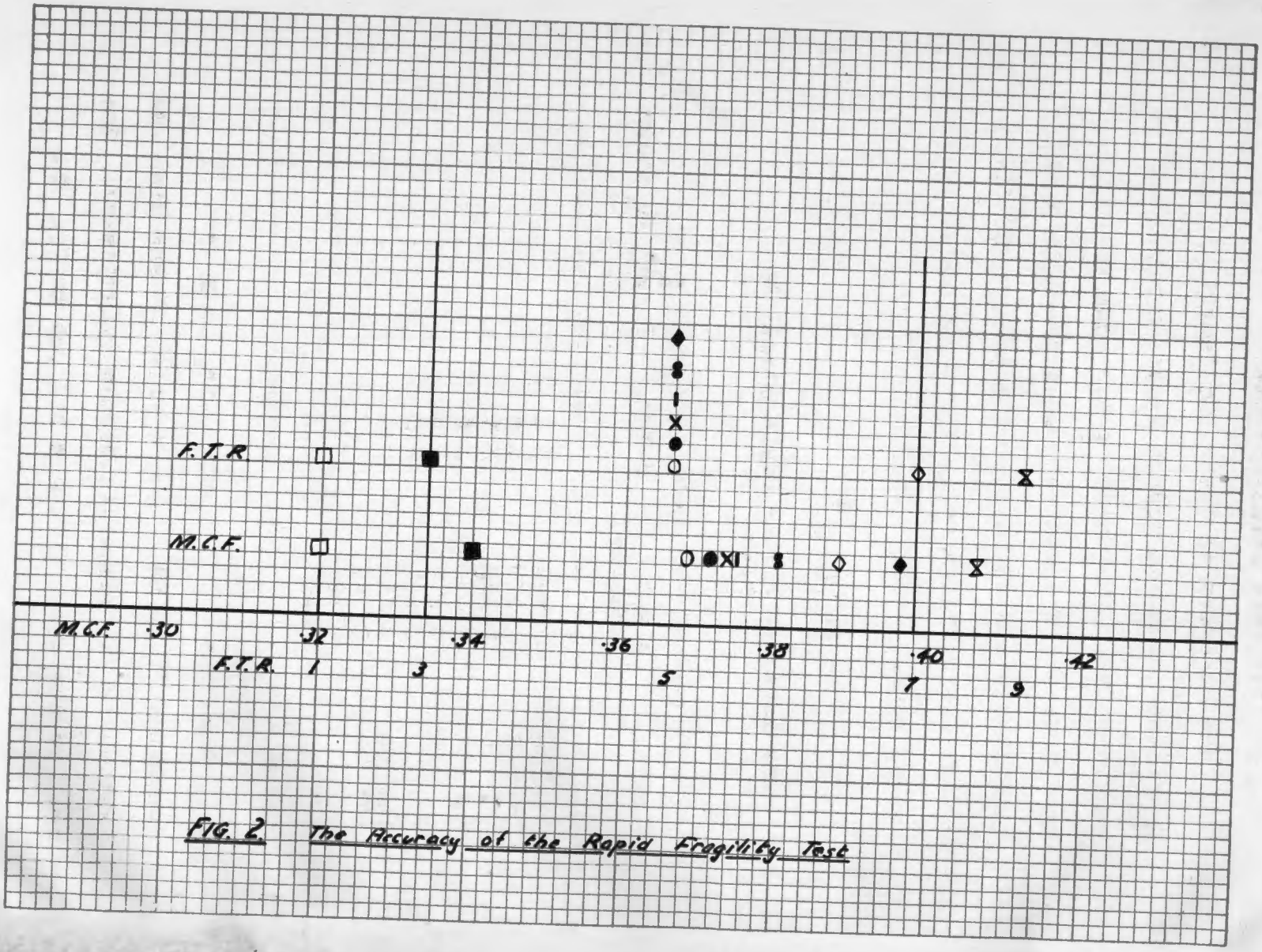


FIG. 2. The Accuracy of the Rapid Fragility Test

C) The Rapid Fragility Test.

The accuracy of the rapid fragility test described above was tested by doing rapid and complete fragility tests on the same blood specimens. 10 cases were studied in this way. The results are shown in table XI, and in fig.2. A close correspondence in the results obtained can be seen.

TABLE XI.

The Accuracy of the Rapid Fragility Test.

Case No.	M.C.F. (% Saline)	F.T.R.	Symbol in graph (Fig.2)
Within normal range of M.C.F. 10	0.320	1.1	□
8	0.340	3.3	■
7	0.368	5.5	○
11	0.371	5.5	●
58	0.373	5.5	×
1	0.374	5.5	|
60	0.380	5.5	‡
53	0.388	7.7	◇
9	0.396	5.5	◆
62	0.408	8.8	X

The value of the rapid fragility test is demonstrated in 22 cases in which it has been used (excluding the cases of chronic infections). The results are shown in table XII and in fig. 3.

TABLE XII.

The Use of the Rapid Fragility Test.

Case	Diagnosis.	F.T.R.	Target Cells %.
26	Normal	5.5	0.5
28	Normal	5.5	0.0
30	Normal	*5.5	0.0
31	Normal	5.5	0.0
32	Normal	5.5	0.0
33	Normal	5.5	1.0
37	Normal	5.5	0.0
27	Gastric Ulcer	5.5	0.0
29	Duodenal Ulcer	6.6	0.0
48	Sciatica	6.7	0.0
58	Acute Monocytic Leukaemia	5.5	0.0
60	Chronic Lymphatic Leukaemia	5.5	0.6
61	Chronic Myeloid Leukaemia	8.8	1.1
47	Polycythaemia	9.9	0.0
56	Polycythaemia	1.1	0.4
49	Cirrhosis of the Liver	1.1	34.2
50	Cirrhosis of the Liver	5.5	1.2
51	Syphilitic Hepatitis	1.1	9.8
36	Acholuric Jaundice	9.9	0.0
53	Myxoedema	7.7	0.0
55	Sickle-cell Anaemia	1.1	8.1
57	Hodgkin's Disease	5.5	0.4

*No correction for anaemia made.

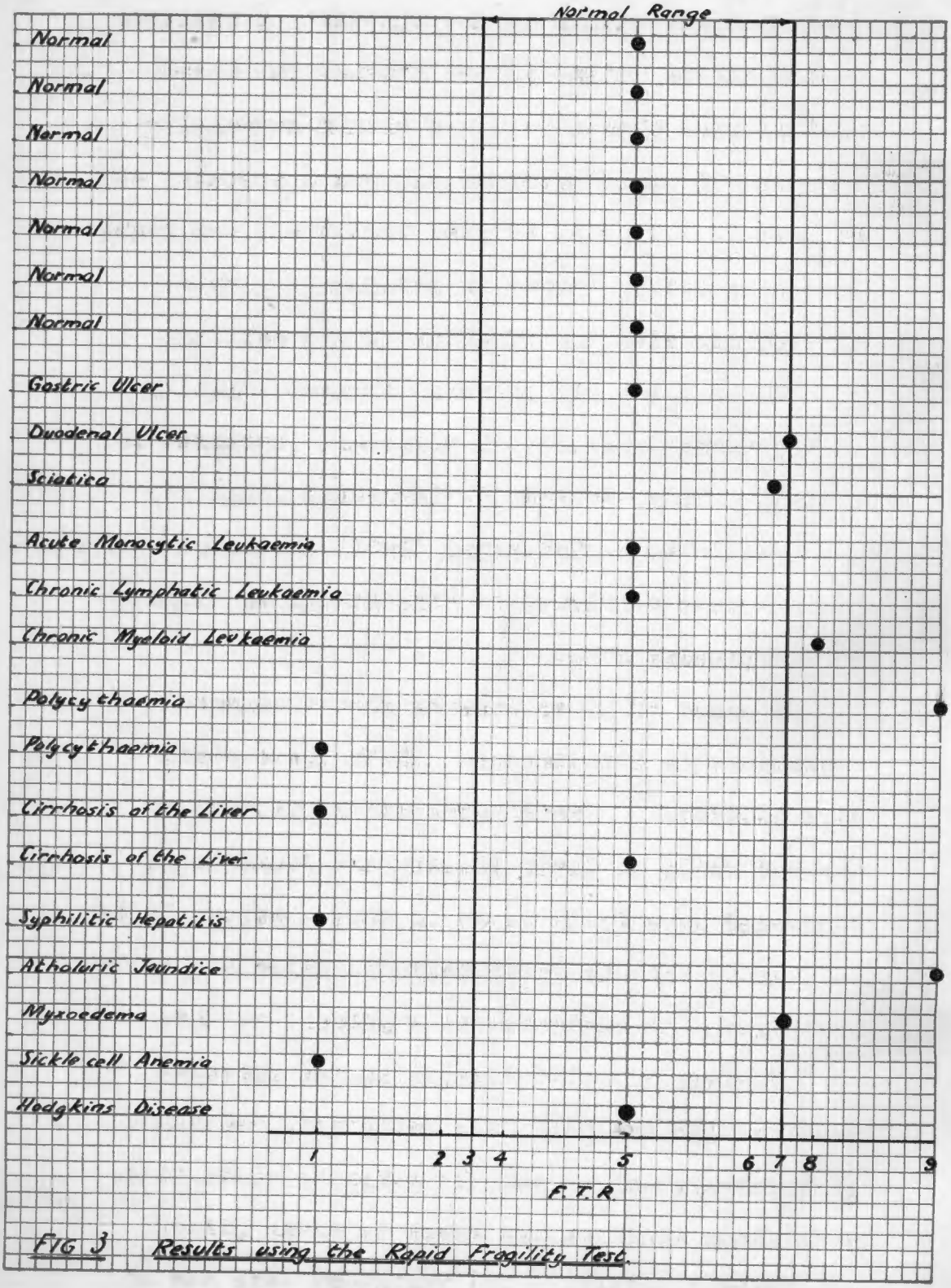


FIG 3 Results using the Rapid Fragility Test.

The 7 normal cases all showed fragility test readings within normal limits. 3 patients with diseases which did not disturb the blood picture also showed readings within normal limits.

The 15 toxic cases tested are discussed later in detail. It will be seen that they gave results that were either normal or resistant. This corresponds with the findings quoted earlier.

Three cases of leukaemia showed fragility within normal limits in 2 cases and increased in 1 case. This tendency to increased fragility has been noted by Heilmeyer (1936) and Singer (1940), and has been confirmed in more extensive studies by the present author.

3 cases of liver disease showed abnormal resistance in 2 instances. Both these cases were jaundiced. This increased resistance of the red cells in liver disease and jaundice is discussed more fully later in this thesis. It is well known that in obstructive jaundice the cells are abnormally resistant to hypotonic saline.

2 cases of polycythaemia showed opposite results with the test, one being very resistant to hypotonic saline and the other being fragile. Both these findings are known to occur (Minot and Buckman, 1923). The resistant case was of recent onset. The fragile case was one of 10

years duration in whom anaemia was now present. This case has been reported in detail by Ziady (1943).

One case of Hodgkin's disease and 1 case of myxoedema showed normal fragility.

One case of sickle cell anaemia was studied. This case showed the characteristic resistance to hypotonic saline. This case also has been reported in detail (Berk and Bull, 1943).

It can be seen that the rapid fragility test gives accurate and valuable information. The results both in the normal cases and in the abnormal cases studied correspond closely with results reported in the literature and in many of the cases the results are of diagnostic significance, e.g. the case of sickle cell anaemia.

The relative ease and rapidity of the test taken in conjunction with the above facts make this simple test a valuable addition to haematological technique.

D) Target Cells.

Target cells were first described by Haden and Evans (1937) in the blood of patients with sickle cellanaemia. They stated that these cells are never present in significant numbers except in cases of sickle cell anaemia.

Shortly after this Barrett (1938) gave an account of the best study to date of target cells. The main features demonstrated by him were the following:-

1) Target cells occur in many conditions, notably in obstructive jaundice, hypochromic anaemias, after splenectomy and in steaterrhoea.

2) Target cells cannot be seen in wet preparations of blood and hence are artifacts produced in the preparation of films. Greenblatt and Kaplan (1943) in studies of the blood in postvaccinal claim that they were able to recognise target cells in wet preparations by dark ground illumination, but their photomicrograph is very unconvincing.

3) Target cells are formed from bowl shaped corpuscles which can be seen in wet preparations.

4) Target cells, because of their shape, are resistant to haemolysis by hypotonic saline solutions. Furthermore,in the bloods in which target cells are found, the non-target cells also are more resistant to hypotonic saline haemolysis

than normal cells.

Bohrod (1941a) found target cells in the blood of patients, appearing 3 -4 days after haemorrhage. He expressed the view that target cells are formed in response to bloodloss, this being the cause of target cell production in all cases. In trying to account for the occurence of bowl shaped corpuscles from which target cells are formed, Bohrod (1941a) accepts the view of Weidenreich and of Radash that the red cells normally circulate in a bowl-shaped form and not as biconcave discs. Bohrod suggests that as these cells are "resistant", they retain their bowl shape when shed, and in smears become target cells instead of biconcave discs.

Bohrod (1941b) also records the occurence of target cells in a blood that resisted lysis by 1% acetic acid used in counting white blood cells and that required a 3% acetic acid solution to enable a white cell count to be made.

Barker (1940) has recorded the occurrence of target cells in a case of microcytic hypochromic anaemia occurring in an early general paretic.

Greenblatt and Kaplan (1943) found target cells in large numbers in cases of post-vaccinal jaundice.

Dameshek and his co-workers (1940, 1941, 1942, 1943a) have advanced the knowledge about target cells very considerably and their work has provided a clue to the concept of the target cell that will be outlined later in this thesis.

In the first paper on the subject, Dameshek (1940) described the case of a patient of Mediterranean extraction who showed an anaemia characterised by the occurence of large numbers of target cells. In a later paper (Dameshek, 1943a) it was shown that a group of conditions exist which Dameshek called the "Familial Mediterranean Oval-Target Cell Syndromes". The best known of this group is Cooley's anaemia. The underlying basis was considered to be a congenital abnormality in the formation of erythrocytes, whereby target and oval cells were produced. This work confirmed earlier studies by Wintrobe, Matthews, Pollack and Dobyns (1940) on the same group of conditions.

More notable in connection with target cells in general is Dameshek's work on the relationship between the spleen and target cells. Singer, Miller and Dameshek (1941) showed in cases of splenectomy for various diseases other than acholuric family jaundice (cirrhosis of the liver, leukaemia, traumatic rupture, Gaucher's disease, Werlhoff's purpura, acute haemolytic anaemia) that

one of the most constant findings present in the blood was an abnormally high percentage of target cells. Miller, Singer and Dameshek (1942) were able later to prove the relationship further by producing target cells experimentally in animals by splenectomy or simply by interfering with the splenic circulation. In this last paper they point out that the target cells occur either because of the loss of the control over the bone marrow normally exerted by the spleen, or because of the changes in the cells in the peripheral blood.

Relevant to this is the finding of Stephens (1941) who demonstrated in electrophoretic studies that target cells behave in the same way as immature red cells such as reticulocytes and normoblasts.

The following observations may be added to those described above.

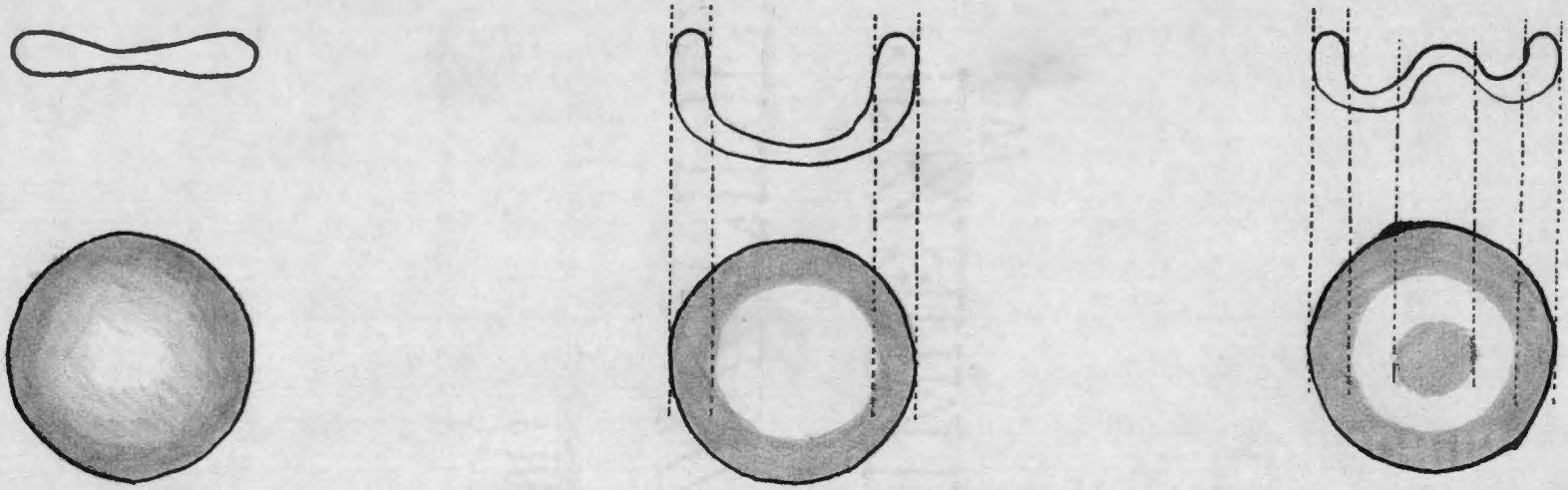
1) It was noticed that considerable variation in the appearance of the central stained dot of the target cell can be seen. The central dot varies considerably in size. It may be any size from very small with faint staining, to a dot with a diameter half that of the red cell and dark staining. In some instances the dots are not circular, but triangular or irregular in

shape. In others there may be 2 or even three dots within the cell, the appearance then being unlike a target, but undoubtedly of the same nature.

2) The outer ring of haemoglobin may also vary considerably in thickness in different target cells.

3) In view of the fact the target cells are artifacts produced during smearing, it was not unexpectedly found that very marked differences in the numbers of target cells were present in different parts of the smear. This has already been pointed out by Barrett (1938) who suggests that more target cells are seen in thin portions of the smear. This last observation was confirmed on the whole, although exceptions were encountered, e.g. case 49, in which the target cells showed a higher percentage in thick portions of the smear. This unsatisfactory distribution of target cells makes all target cell counts unreliable.

4) Striking confirmation of Barrett's statement that target cells are formed from bowl shaped corpuscles was seen in the case of sickle cell anaemia. Here at one end of the film numerous cells were seen in which the peripheral eosinophilic portion of the red cells was sharply outlined on its inner aspect. In this part of the film no target cells were seen. In other parts of the



NORMAL CELL

BOWL-SHAPED CORPUSCLE

TARGET CELL

FIG. 4. THE APPEARANCES OF RED CELLS IN SMEARS.

film no such cells were seen, but target cells were plentiful (8.1%). It is believed that the cells with the sharp inner outlines to the peripheral stained portion are bowlshaped corpuscles and the explanation of the phenomenon is suggested in fig. 4.

A similar sharp outline is usually seen in the inner border of the peripheral stained portion of target cells (see fig. 4) and the central dot or dots are sharply outlined as well.

5) It will be seen from table XII that significant rises in the target cell count occur only in those cases that show increased resistance of the red blood cells to hypotonic saline. Conversely, almost all cases that show resistant corpuscles also show increased numbers of target cells. Identical results were obtained in the cases of the anaemias of chronic infection (see later). Cassells (1938) has shown that all anaemias apart from acholuric jaundice and pregnancy anaemias show resistance to hypotonic saline.

From the above data it seems reasonable to conclude that the presence of target cells are of no diagnostic value, except that they indicate that the blood cells are probably resistant to hypotonic saline. Further suggestions as to the significance of target cells in blood films are made later.

E) Factors that affect blood counts.

Wintrobe (1930) has given an account of the various factors that affect the red cell count, and other characters of the red cell picture. Of these the following are the most important:-

1) Age. The normal blood picture varies with age. This factor has been excluded by studying only adult cases.

2) Sex. This produces very little difference in the characters of the red cells apart from total number.

3) Diurnal variation. This factor was standardised in the present work by collecting all specimens for investigation at a fixed time, i.e. 11 o'clock in the morning.

4) Exercise. All the patients were bed patients.

5) Reaction of the blood. This could be standardised only as far as the carbon dioxide tension was concerned. This is accomplished during the oxygenation of the specimens.

6) Barometric pressure. All the cases were studied at the Groote Schuur Hospital.

7) Dehydration. This was not present in any of the cases studied.

8) Weight, stature and surface area. The influence of this is nil according to Wintrobe.

9) Race and climate. Europeans, Cape coloureds and Bantu patients were studied. There is no reason to believe that this factor per se makes any difference to the red cells, although other factors such as nutritional differences may be introduced. This is considered later. The changes in climate with the different seasons was not controlled.

In order to standardise the investigation as far as possible, the following programme was adopted.

1. Sternal puncture was performed.

2. Blood was drawn from a vein into a heparinised tube. This specimen was used for haemoglobin, red cell, haematocrit, white cell estimations, fragility test, v.d. Bergh reaction, and bilirubin estimation.

3. From a fingerprick the following preparations were made:-

a) The preparation for platelet count was made first.

b) Thin smears for target cell counts and Price-Jones curves.

c) Brilliant cresyl blue smears.

4. A specimen of urine was collected for the urobilin test.

All these specimens were collected within 15 - 20 minutes.

On the following day the hippuric acid test was performed and on the third day a fractional test meal was done.

All wet specimens were dealt with within 4 hours, the time during which no change occurs in the red blood cells with regard to their behaviour in hypotonic saline (Dacie and Vaughan, 1938). Counts on films and Price-Jones curves were done later at a convenient time.

It was considered desirable that all specimens should be taken at the same time as outlined above, so that the results would show the features investigated as they existed at the same time. This makes permissible, and much more valuable, correlations between various data. Unfortunately it was not possible to do all the tests simultaneously, owing to the nature of some of the investigations.

F) Expression of results.

Haemoglobin was expressed as a percentage. 100% Dare equals 16.0 grammes. In addition the reading was standardised for working out the colour index by converting the reading to a scale of 100% = 14.5 grammes, as recommended by Wintrobe (1933) and Whitby and Britton (1937). The figures recorded in the tables and text are the original Dare readings.

Red Cell counts were expressed in millions per cubic millimetre.

Colour Index was calculated in the usual way, using the haemoglobin percentage corrected to 100% haemoglobin = 14.5 grammes.

White Cell counts were expressed as the number per cubic millimetre.

Differential white cell counts were recorded as percentage of each type of white cell present.

Volume of packed cells was expressed as a percentage.

Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were calculated in the usual way from the haemoglobin percentage, the haemoglobin in grammes per cent, the red cell count and the haematocrit reading.

Reticulocyte and Target cell counts were expressed as percentages of the total red cells.

The rapid fragility test results were referred to as F.T.R. (fragility test reading).

When the full fragility test was performed, the percentage of saline in which haemolysis commenced, the percentage of saline in which haemolysis was complete and the percentage of saline giving 50% haemolysis (MCF) were recorded.

The hippuric acid test results were expressed as "hippuric acid excreted equivalent to x gms. benzoic acid".

v.d. Bergh reaction results were expressed as direct prompt positive with one or more plus signs to indicate the intensity of the reaction, or as feeble prompt positive, or as negative.

Where the direct v.d. Bergh reaction is negative, the result of the indirect reaction is reflected in the bilirubin estimation.

Bilirubin was recorded as mgs. % unless too low to be read accurately.

No separate estimation of indirect-reacting and direct-reacting bilirubin was made.

Urine urobilin was recorded as negative, faint trace, + or++ .

The fractional test meal. The highest figure for free acid is recorded in the tables.

Price-Jones curves. The mean corpuscular diameter, sigma and v were calculated from the

data according to the method described by Whitby and Britton (1937). Mean corpuscular average thickness was calculated from the mean corpuscular volume and the mean corpuscular diameter. The diameter:thickness ratio was easily calculated. Macrocytosis and microcytosis were calculated after the drawing of a graph including the case studied and the two curves giving the limits of normality.

RESULTS.

TABLE XIII.

The Anaemias of Chronic Infections - The Red Cell Size and Haemoglobin.

Case.	Hb. %Dare	R.B.Cs. m/cu.mm.	CI	VPC %	MCV cu.mm.	MCH yy	MCHC %
1	75	3.81	1.09	44.0	121	31	27
2	77	5.27	0.80	43.5	83	23	28
3	60	3.83	0.90	35.5	90	25	27
4	56	2.63	1.18	35.0	133	34	26
5	51	3.92	0.70	30.5	78	21	27
6	48	2.15	1.20	27.0	125	38	29
7	57	3.61	0.87	33.0	91	25	28
8	65	3.80	0.95	35.5	93	27	29
9	48	2.67	0.99	30.5	114	29	25
10	60	4.19	0.80	35.5	85	23	27
11	63	4.22	0.83	35.0	83	24	29
12	48	2.71	0.98	25.0	92	28	31
13	57	3.74	0.84	32.5	90	24	28
14	66	4.59	0.80	39.5	86	23	27
15	67	5.74	0.64	40.0	70	20	27
16	66	4.70	0.70	37.0	80	23	29
17	63	3.89	0.90	36.0	93	26	28
18	46	2.40	1.06	31.0	127	31	34
19	56	2.55	1.20	32.0	126	35	28
20	70	4.09	0.94	41.0	100	27	27
21	61	4.20	0.80	37.0	88	23	27
22	62	3.21	1.06	35.5	111	31	28
23	60	3.45	0.96	32.0	93	28	30
24	47	3.34	0.78	31.5	94	22	24
25	81	4.14	1.07	42.0	101	31	31

TABLE XIV.

The Anaemias of Chronic Infections - Red Cell Diameters.

Case.	MCD mu.	σ mu.	v %	MCAT mu.	D:T ratio :1	Macrocytosis %	Microcytosis %
1	8.567	0.639	7.5	2.100	4.08	58.6	0.0
2	7.323	0.608	8.25	1.971	3.70	0.0	0.0
3	7.430	0.691	9.3	2.075	3.58	0.6	0.4
4	8.887	0.751	8.45	2.145	4.14	70.8	0.0
5	7.068	0.802	11.3	1.988	3.60	0.2	2.4
6	8.068	0.802	10.0	2.400	3.40	27.2	1.0
7	8.480	0.561	6.6	1.611	5.26	55.8	0.0
8	7.967	0.491	6.2	1.866	4.27	19.0	0.0
9	7.920	0.763	9.6	2.314	3.42	21.0	0.4
10	8.147	0.803	9.9	1.631	5.00	34.8	0.2
11	7.493	0.555	7.4	1.882	3.98	0.0	0.0
12	7.694	0.694	9.0	1.979	3.89	7.2	0.2
13	7.606	0.661	8.7	1.981	3.84	3.2	0.6
14	6.573	0.617	9.4	2.534	2.59	0.0	11.0
15	6.949	0.692	9.95	1.846	3.77	0.0	0.8
16	6.596	0.811	12.3	2.341	2.82	0.4	16.0
17	6.951	0.615	8.8	2.450	2.84	0.0	0.8
18	7.611	0.614	8.0	2.175	3.50	2.2	0.0
19	7.826	0.646	8.25	2.620	2.98	12.4	0.2
20	7.951	0.655	8.9	2.014	3.95	18.4	0.0
21	6.976	0.594	8.5	2.302	3.03	0.0	0.0
22	7.425	0.709	9.8	2.565	2.96	3.4	0.0
23	7.336	0.687	9.35	2.202	3.33	0.2	0.2

TABLE XV.

The Anaemias of Chronic Infections - The Bone Marrow.

Case.	W.B.Cs. /cu.mm.	Platelet /cu.mm.	Bone Marrow				
			Total Erythro-blasts %	Type I %.	Type II %.	Type III %.	Type IV %.
1.	6,500	678,200	8.3	1	3	18	78
2	6,500	463,800	-	-	-	--	--
3	13,250	651,100	4.8	0	2	23	75
4.	8,850	920,800	-	-	-	--	--
5	9,150	548,800	14.3	2	9	22	67
6	24,200	585,200	4.2	2	5	17	76
7	7,200	617,300	3.3	1	7	17	75
8	10,400	570,000	5.7	1	4	12	83
9	10,400	512,600	9.1	1	6	23	70
10	11,800	1,508,400	5.6	0	6	25	69
11	5,900	611,900	14.3	1	3	12	84
12	9,150	----	11.1	1	2	12	85
13	14,300	542,300	8.1	3	7	23	67
14	6,300	950,100	5.6	3	6	19	72
15	10,850	872,500	16.7	2	11	29	58
16	----	586,700	14.9	0	5	30	65
17	21,300	----	-	-	-	--	--
18	17,750	836,600	6.5	1	4	17	78

TABLE XVI.

The Anaemias of Chronic Infections - Miscellaneous Investigations.

Case.	Target Cells %	Fragility Test F.T.R.	Reticulocytes %	v.d.Bergh Direct	Plasma Bilirubin mgs.%	Urine Urobilin	Hippuric Acid Test gms.	Gastric HCl. Highest Reading.
1	0.0	5	0.2	- ve	0	Trace	2.3	-
2	0.3	5	0.4	- ve	ft.tr.*	0	3.3	-
3	2.3	2	1.4	- ve	0	0	1.9	44
4	14.0	1	0.8	- ve	ft.tr.	Trace	1.5	49
5	2.1	3	1.0	- ve	0	+	2.5	30
6	6.7	1	0.8	- ve	ft.tr.	+	1.2	0
7	0.0	5	0.3	- ve	0	+	-	8
8	1.2	3	0.5	- ve	0	+	3.9	32
9	0.1	5	3.4	- ve	0	+	2.7	82
10	1.2	1	2.4	- ve	ft.tr.	0	-	43
11	0.7	5	0.6	- ve	ft.tr.	0	1.9	36
12	1.0	5	1.0	- ve	ft.tr.	+	2.8	-
13	2.4	5	0.2	- ve	0	+	1.6	20
14	0.2	5	0.6	- ve	0	+	3.3	41
15	0.0	-	0.2	- ve	0	0	3.3	-
16	0.0	-	1.1	- ve	0	+	2.7	-
17	0.4	1	3.7	-	-	-	-	-
18	-	-	1.0	- ve	0	Trace	-	25
22	-	-	-	-	-	-	2.8	-

*ft.tr. = faint trace.

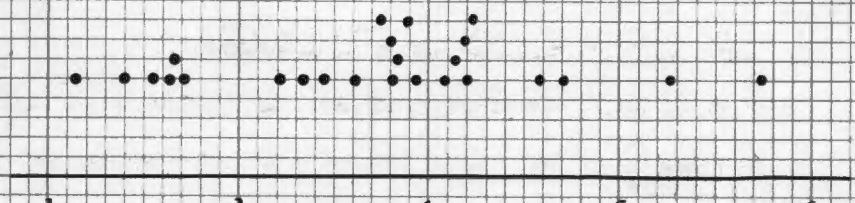


FIG. 5 Red Cell Counts (millions / cu. mm)

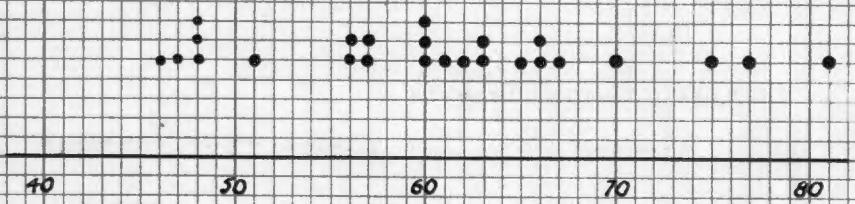


FIG 6 Haemoglobin Estimation Results (% Dare)

III. RESULTS.

The results of the investigations on the cases on chronic infections are shown in tables XIII to XVI. A detailed analysis follows.

a) THE NUMBER OF ERYTHROCYTES.

The red cell counts varied between 2.15 and 5.71 millions per cu. mm.. The distribution of the red cell counts is shown in fig. 5.

In over half the cases (60%) the red cell count lay between 3.21 and 4.22 millions, i.e. the counts are mostly reduced by less than 1 million. 24% of the cases fall below 2.71 millions and the remainder (16%) showed normal red cell counts.

b) THE HAEMOGLOGIN OF THE CELLS.

1. Haemoglobin readings.

These varied from 46% Dare = 7.4 grammes to 81% Dare = 13.0 grammes in the cases studied. The distribution of the readings is shown in fig. 6.

84% of cases fall between 46 and 67% i.e. 10-30% below normal. The cases are fairly evenly distributed over this range, which thus seems to be the common level of haemoglobin in

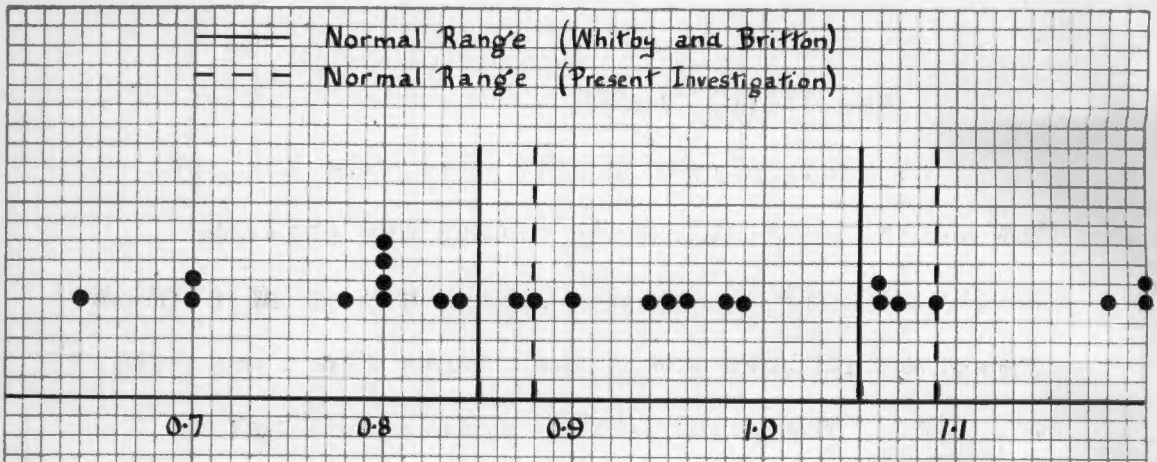


Fig. 7. Colour Index Results.

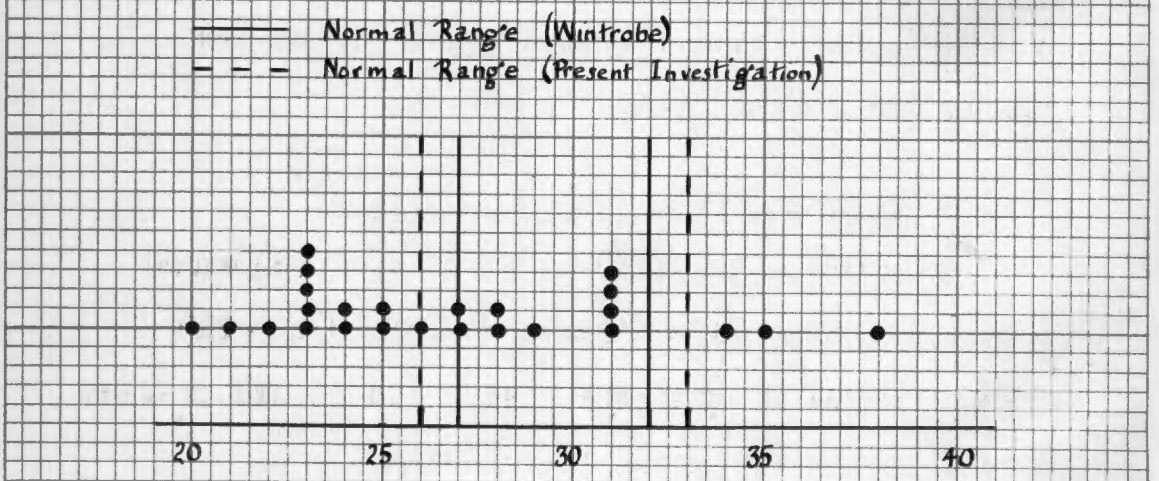


Fig. 8. Mean Corpuscular Haemoglobin (γγ)

in these anaemias. Lower readings do undoubtedly occur.

2. The Colour Index.

The colour index ranged from 0.64 to 1.20. The normal range given by Whitby and Britton (1937) is 0.85 to 1.05. In the series of normals presented earlier the normal range found was 0.88 to 1.09. The distribution of the readings is shown in fig. 7.

Thus 11 cases (44%) show a low colour index. Another 11 cases (44%) show a normal colour index. The remaining 3 cases (12%) show a high colour index. To put it simply, a few cases show a high colour index. Of the majority of cases, half show a normal colour index and half a low colour index.

3. Mean Corpuscular Haemoglobin.

This ranged from 20 to 38 micromicrogrammes with the following distribution (fig.8). The normal ranges of Wintrobe (1930) and of the present investigation are shown in vertical lines.

The results here are essentially the same as in the case of the colour index. In 12% the M.C.H. is raised. In 40% it is normal and in 48% it is reduced. In only one case is there a discrepancy between the colour index and the MCH, C.I. 0.90, MCH 25 micromicrogrammes, both

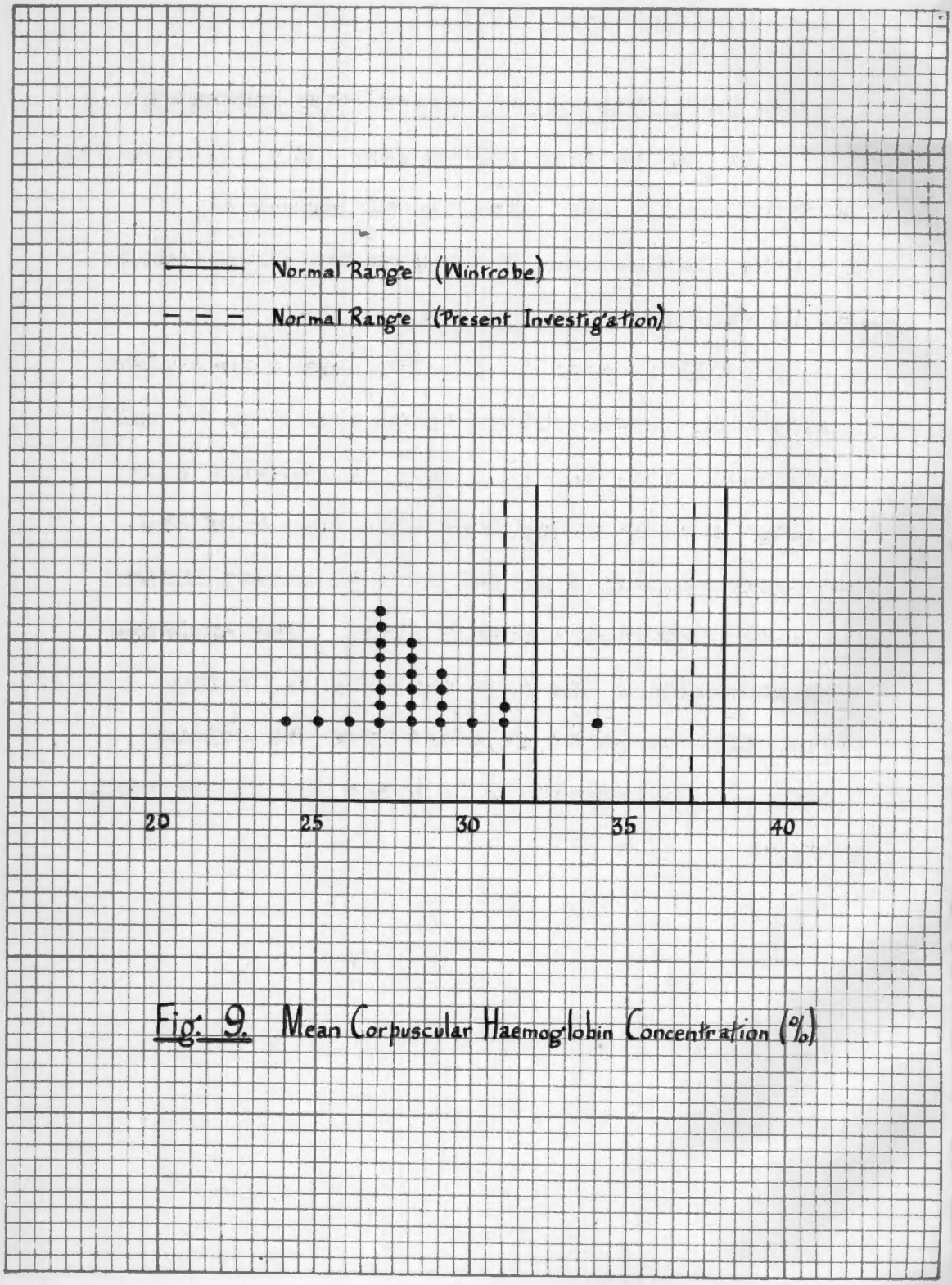


Fig. 9. Mean Corpuscular Haemoglobin Concentration (%)

these readings being very close to the lower limit of normality.

4. Mean Corpuscular Haemoglobin Concentration.

This varies from 24% to 34%. The distribution is shown in fig. 9. The normal ranges of Wintrobe (32-38) and of the present investigation (31-37) are shown in the dotted lines.

It will be seen that a low mean corpuscular haemoglobin concentration is an almost universal feature of these cases. This is somewhat surprising in view of the fact that over half the cases show either a normal or high colour index and mean corpuscular haemoglobin. The explanation must lie in the fact that the cells are larger than would be expected from the colour index. This suggestion is borne out in the next section.

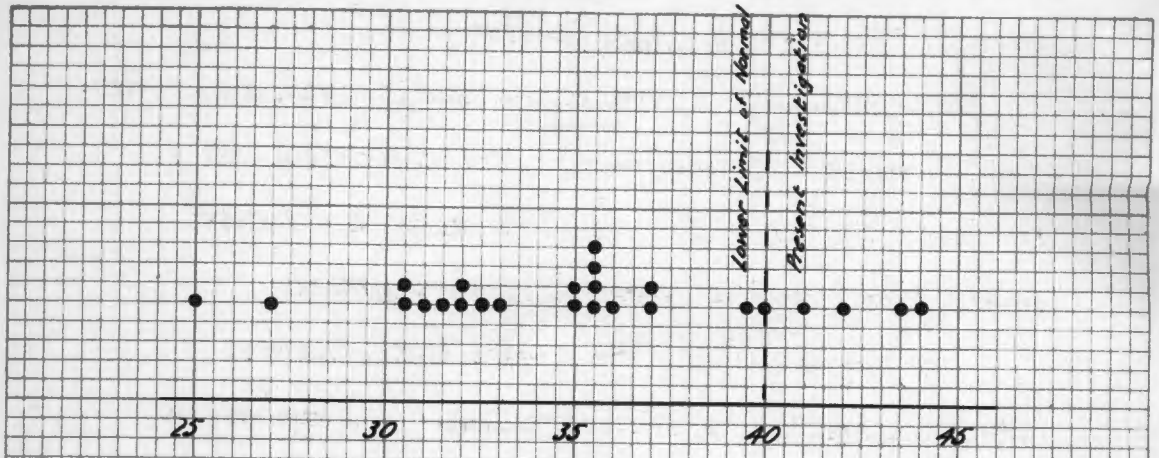


FIG 10 Packed Cell Volume (VPC %)

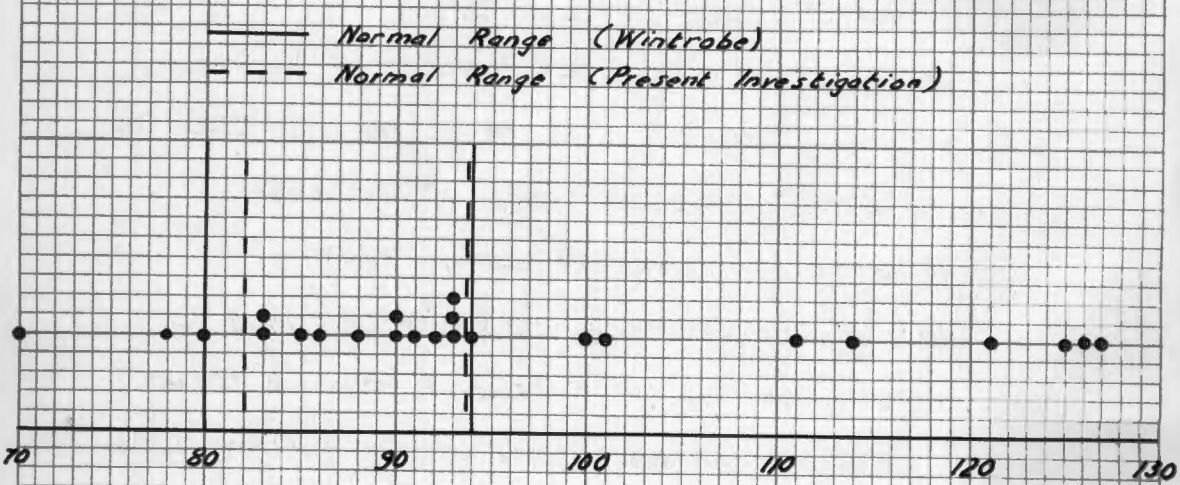


FIG 11 Mean Corpuscular Volume (cu. μ)

c) THE RED CELL DIMENSIONS

1. The Packed Cell Volume.

The results of the haematocrit determinations fall between 25% and 44%. The distribution is shown in fig. 10. together with the normal range found in the present investigation.

As is to be expected most cases showed VPC readings below normal values. Most cases show a relatively small reduction in VPC, only 2 cases (8%) falling below 30%. This and the fact that 5 cases show normal values is also attributable to the high mean corpuscular volume results.

2. Mean Corpuscular Volume.

The range of mean cell volumes is 70 to 133 cu. mu.. The distribution is seen in fig. 11. together with the normal range of Wintrobe (80-94) and of the present study (82-98).

As suggested above a definite tendency to macrocytosis as regards volume is seen in these cases. 36% of the cases show an MCV greater than normal. In only 3 cases (12%) is the MCV below normal and then to a marked extent only in 1 case. Amongst the 13 cases (52%) showing a normal MCV, 8 are in the upper half of the normal range.

A tendency to large cell volume with MCV as high as 133 cu. mu. is thus a striking feature of the anaemia in the cases of chronic infections investigated.

The results correspond very closely with those of Calder, Steen and Baker (1939). In 286 cases of brucellosis they found macrocytosis in 108 instances. Hyperchromia (high M.C.H.) was frequent. Only 6 cases showed microcytosis and hypochromia (low M.C.H.). The M.C.H.C. was low in 68 instances.

Haden (1932) refers to the results of investigations on 110 cases of "anaemia due to qualitative defect of blood formation" and gives details of 5 cases of chronic infection. The M.C.V. readings were 80,90,90,94 and 102. The M.C.H.C. was low in 4 of these cases.

Tauber and Goldman (1935) describe a case of tertiary syphilis with macrocytic anaemia, with improvement in the anaemia and dimunition in the macrocytosis under antisyphilitic treatment.

The tendency to macrocytosis is demonstrated further by fig. 12. Here the MCV is plotted against the colour index. The anaemias of chronic infections are represented and the results from other cases are included. The details of these

TABLE XVII.

The Size of the Red Cells Relative to their Haemoglobin Content.

Case	CI	MCV cu.mu.	Case	CI	MCV cu.mu.
	Liver Disease			**Iron Deficiency**	
65	1.21	111	70	0.61	61
64	1.09	108	72	0.57	64
66	1.28	113	71	0.91	85
79	1.10	104	73	0.82	83
90	1.32	105	84	0.82	80
67	1.28	128		**Reticulosis**	
50	1.14	105	107	1.06	83
68	1.33	114	57	1.03	93
	Leukaemias		76	0.63	82
58	1.16	99	88	0.83	78
59	1.20	100		**Haemolytic Anaemias**	
61	1.07	100	86	1.00	77
108	1.10	96	78	1.04	77
92	0.95	85	111	1.06	83
110	1.02	90	115	1.15	84
60	1.34	102		**Myxoedema**	
	Pernicious Anaemia		53	1.13	88
91	1.17	91	75	0.94	92
83	1.15	102		**Scurvy**	
94	1.47	121	82	1.04	94
74	1.30	123		**Refractory Anaemia**	
52	1.30	141	85	1.10	96
89	0.98	85		**Secondary Carcinoma**	
	Carcinoma of the Bronchus.		80	1.14	95
69	0.87	77	**TOTAL**	**40 Cases.**	

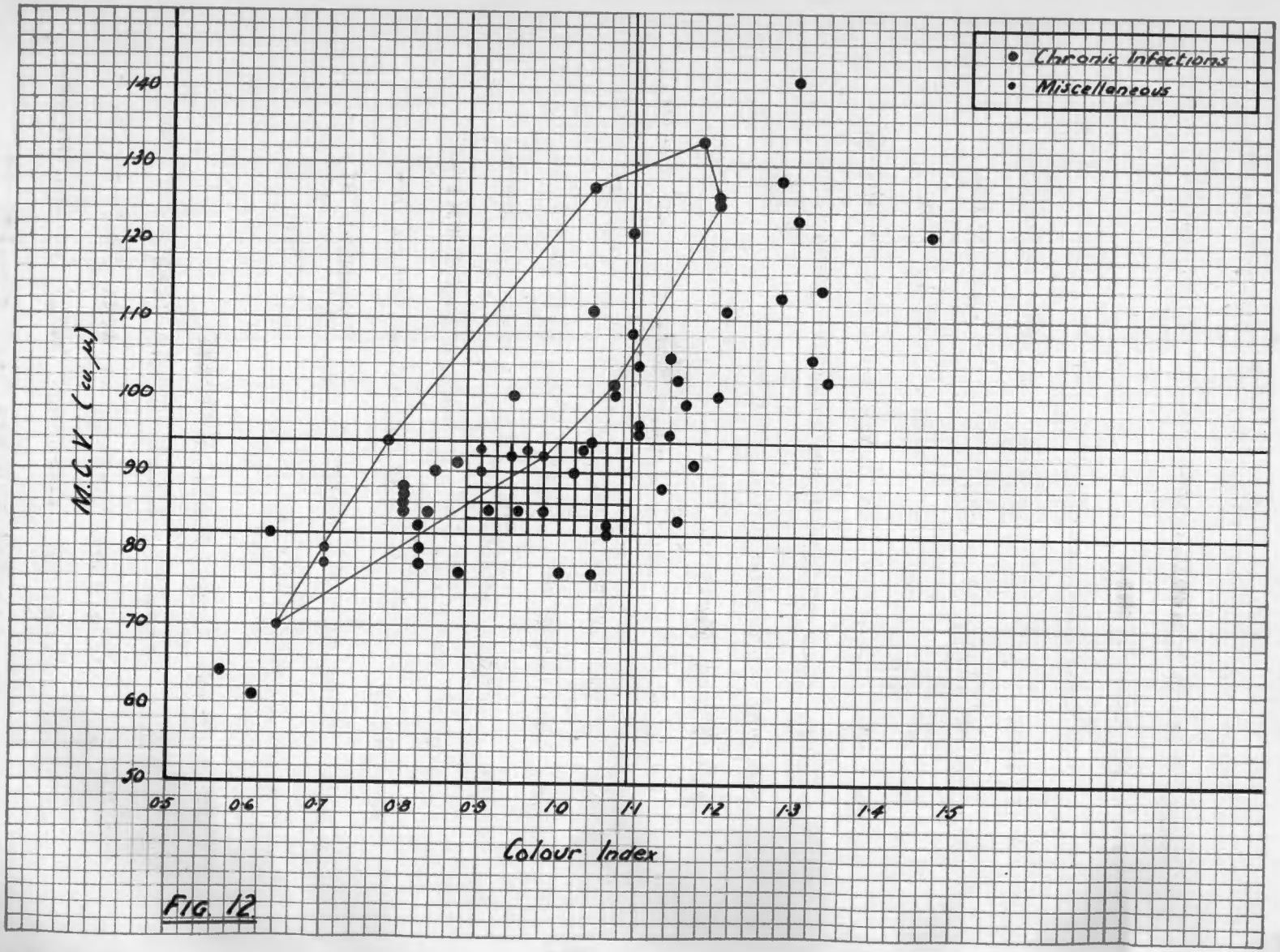

Chronic Infections
Miscellaneous
M.C.V. (cu. μ)
140
130
120
110
100
90
80
70
60
50
0·5
0·6
0·7
0·8
0·9
1·0
1·1
1·2
1·3
1·4
1·5
Colour Index

FIG. 12

cases are shown in table XVII.

The graph shows that the anaemias of chronic infections are placed at a higher level than the miscellaneous anaemias and normal cases, with very few exceptions.

Thus the anaemias of chronic infections are an exception to the rule that in most anaemias the colour index is a guide to the cell size. The latter point is well illustrated by the graph, where most of the cases not due to chronic infections show either a macrocytic hyperchromic, a normocytic normochromic or a microcytic hypochromic blood picture.

From this it would appear that for the degree of haemoglobinisation as shown by the colour index, the cells are larger than one would expect. This finding goes with the low mean corpuscular haemoglobin concentration found in these cases. The colour index is therefore a poor guide to cell size in the anaemias of chronic infections studied.

3. Mean Corpuscular Diameter.

The figures for M.C.D. range from 6.573 to 8.887 mu. The distribution is seen in fig. 13. The normal range (Price-Jones, 1933) is indicated.

The results for MCD are very similar to those for MCV. 9 cases show large MCD. 2 show a

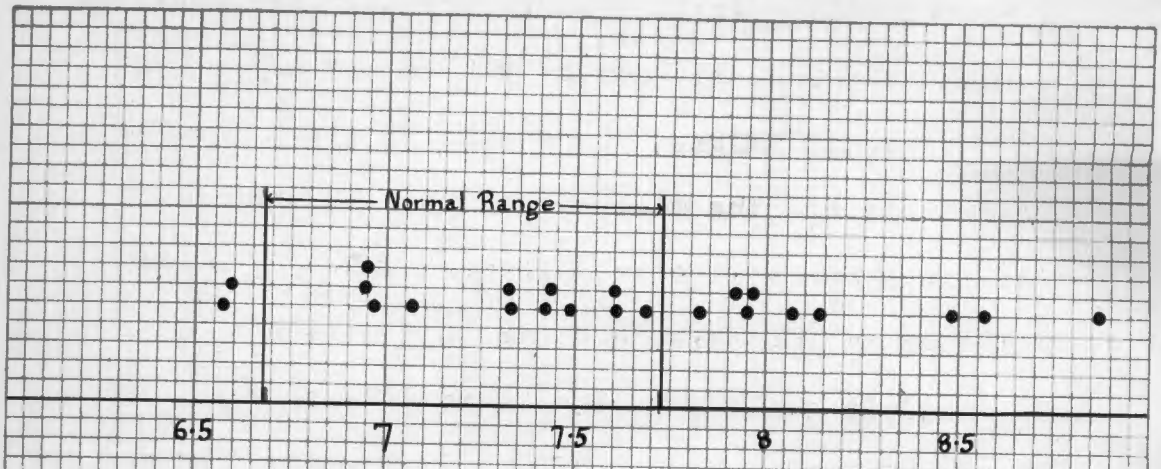


Fig. 13. Mean Corpuscular Diameter (μ)

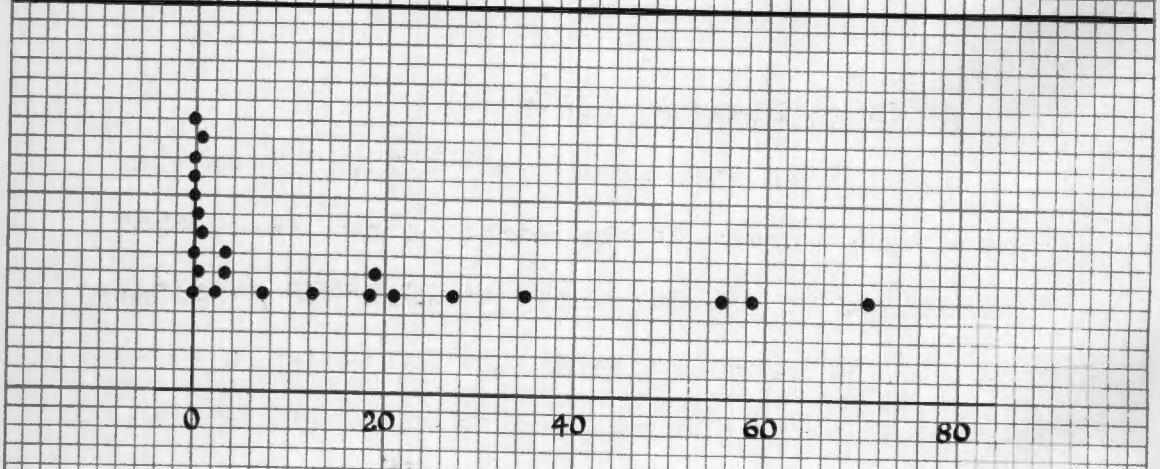


Fig. 14. Macrocytosis (%)

MCD smaller than normal and 12 of the cases show a normal MCD. Again amongst the 12 cases showing normal MCD, 9 cases have an MCD in the upper half of the normal range. It should be noted however, that it is not the same cases that show an increased MCV that show increased MCD. The combination of increased MCV and increased MCD occurred in 6 cases. In 2 cases there is a raised MCV and a normal MCD. In 3 cases there is a normal MCV and a raised MCD.

The relationship between MCV and MCD is shown in the mean corpuscular average thickness figures and the figures for diameter-thickness ratio.

4) Large Diameter Cells.

It will be seen from table XIV and from the Price-Jones curves of the cases (figs. 18-40) that in addition to the frequency of high MCD readings, 17 of the 23 cases on which the Price-Jones curves were done show cells falling to the right of the normal range. This macrocytosis as regards diameter varies from 0.2% to 70.8% and is greater than 12% in 9 of the cases. The distribution of large diameter cell percentages is seen in fig. 14.

5) Small Diameter Cells.

13 cases show some cells falling below the lower limits of normality (table XIV and figs. 18-40).

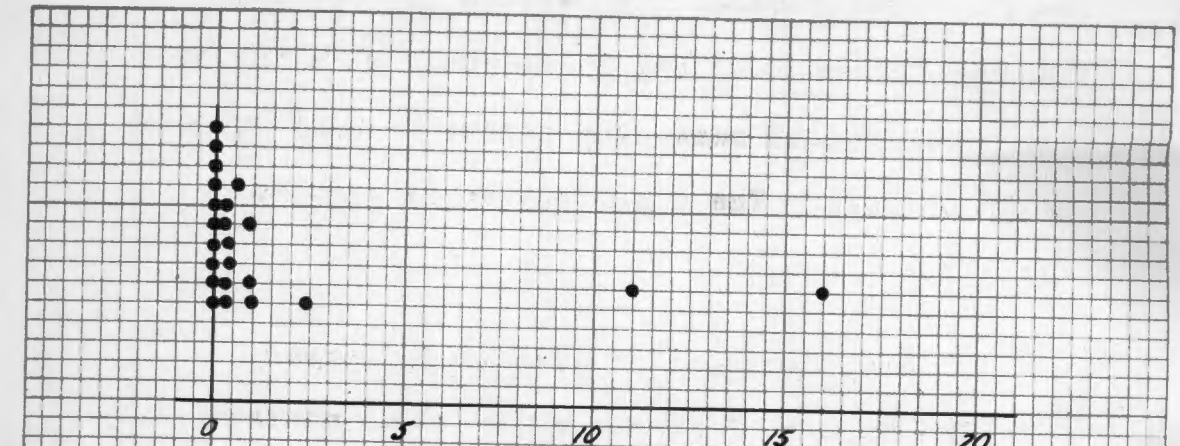


FIG 15 Microcytosis %

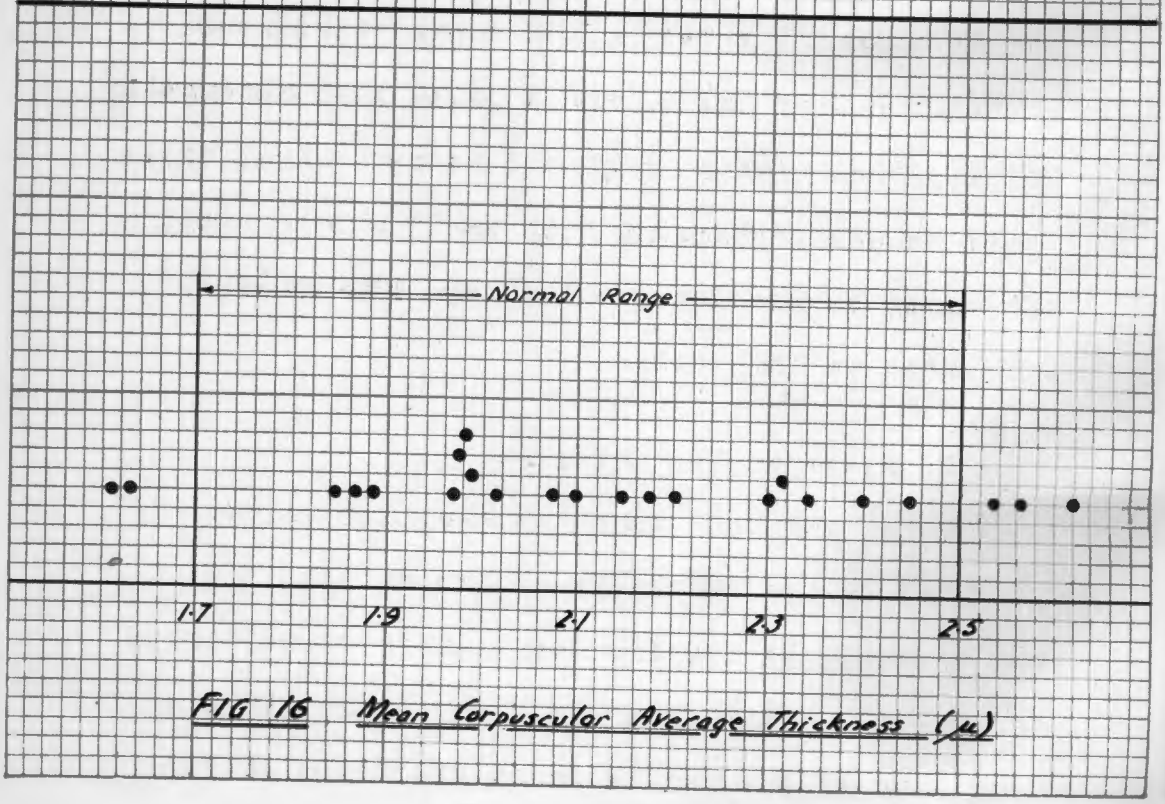


FIG 16 Mean Corpuscular Average Thickness (μ)

However the percentages of such cells is very small. The highest figures are 11% and 16%. One case shows 2.4% small diameter cells and in the remaining 10 cases the degree of microcytosis is 1% or less. The distribution is seen in fig. 15.

6) Mean Corpuscular Average Thickness.

The figures for mean corpuscular average thickness range from 1.611 mu. to 2.62 mu. The normal range is 1.7 to 2.5 mu. (Price-Jones, Vaughan and Goddard, 1935). The distribution is shown in fig. 16.

In 18 of the 23 cases studied the MCAT lies within normal limits. In 2 cases there were abnormally thin cells, and 3 cases show abnormally thick cells. Again it should be noted that these cases do not correspond with small MCD or small MCV cases in the former, or large MCD or large MCV cases in the latter.

7) Diameter-Thickness Ratio.

More important than the absolute values for MCAT is the thickness of the red cell in relationship to its diameter. This relationship is especially important in connection with the fragility of the red cells in hypotonic saline solutions (Haden, 1940) and is discussed more

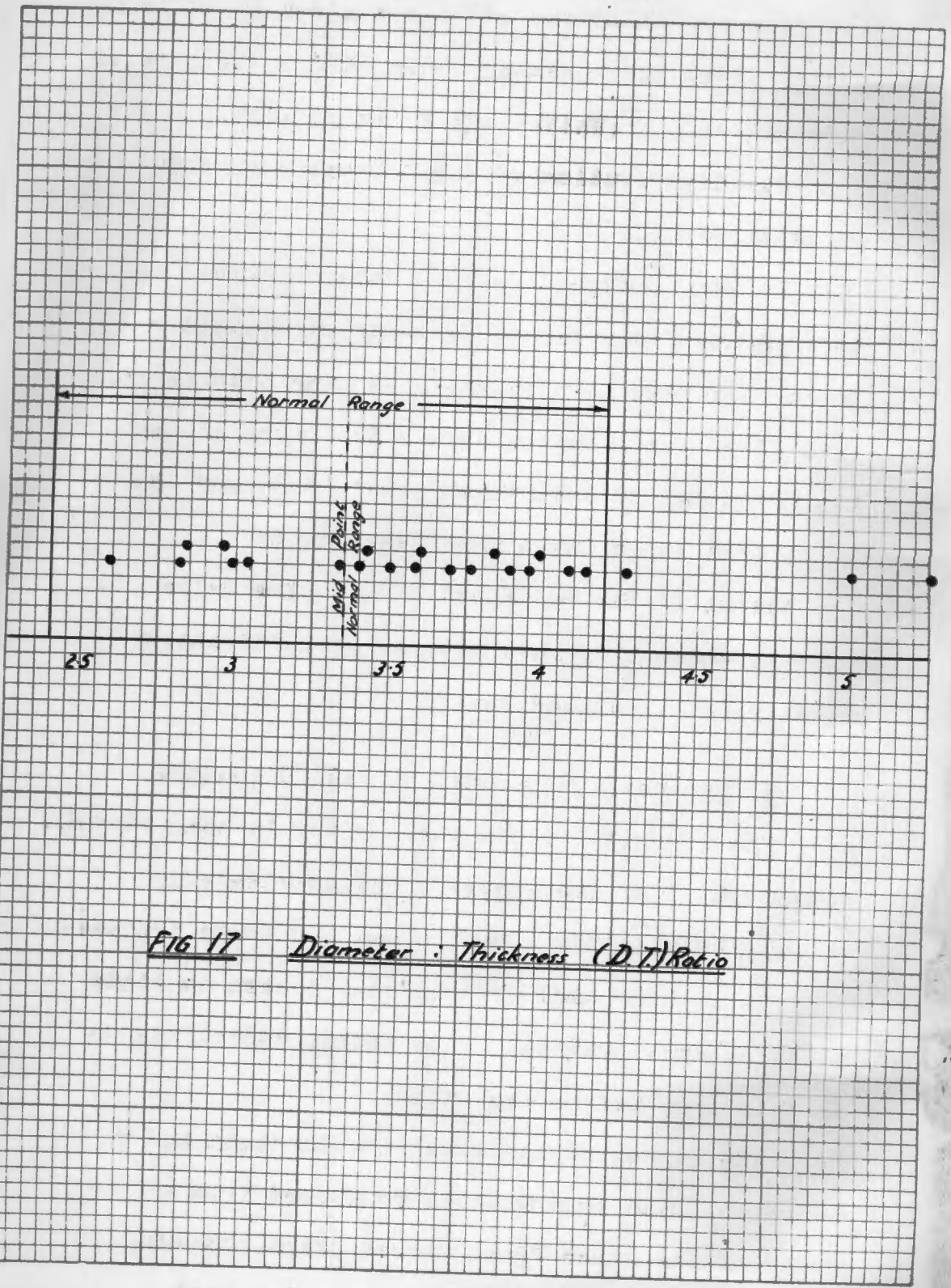


FIG 17 Diameter : Thickness (D.T.) Ratio

fully in that connection. The figures for the diameter-thickness ratio are given in table XIV. Normal diameter-thickness ratio (D:T ratio) varies from 2.4 to 4.2 (Whitby and Britton, 1937).

The distribution of the D:T ratio results is shown in fig. 17.

It can be seen that 20 of the 23 cases in which Price-Jones curves were performed show a normal D:T ratio. The graph however also reveals that 17 of the 23 cases show a D:T ratio above the mid-point of the normal range (3.3) and the group as a whole shows some tendency to flattening of the red blood cells. The cells thus appear not to have altered proportionately equally in all dimensions.

8) Variability in the Red Cell Diameters.

The figures for the variability in the red cell diameters, i.e. the degree of anisocytosis is shown in table XIV. Both sigma and v are included.

It will be seen that in only 1 case is sigma within normal limits. v is within normal limits in this case and in one other.

The degree of increase in both sigma and v is slight, and this fact is confirmed from a study of the Price-Jones curves (figs. 18-40). From these curves it will be seen also that

1) The base of the curve is usually only

slightly increased in width.

2) The height of the curves is more or less normal in most of the cases.

3) A few cases show considerable widening and flattening of the curve.

4) In most of the curves with an increased MCD the whole curve is shifted bodily to the right with very little change in contour.

From all this it can be concluded that there is only slight anisocytosis in most cases.

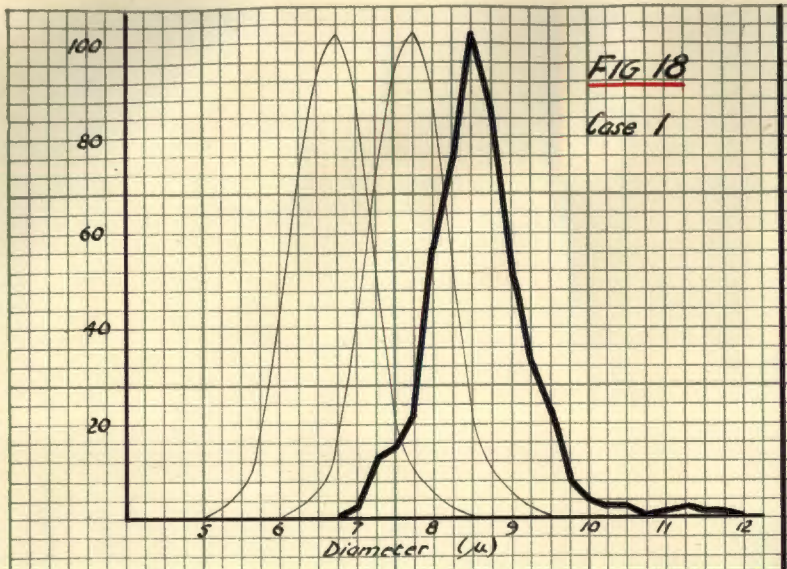


FIG 18

Case 1

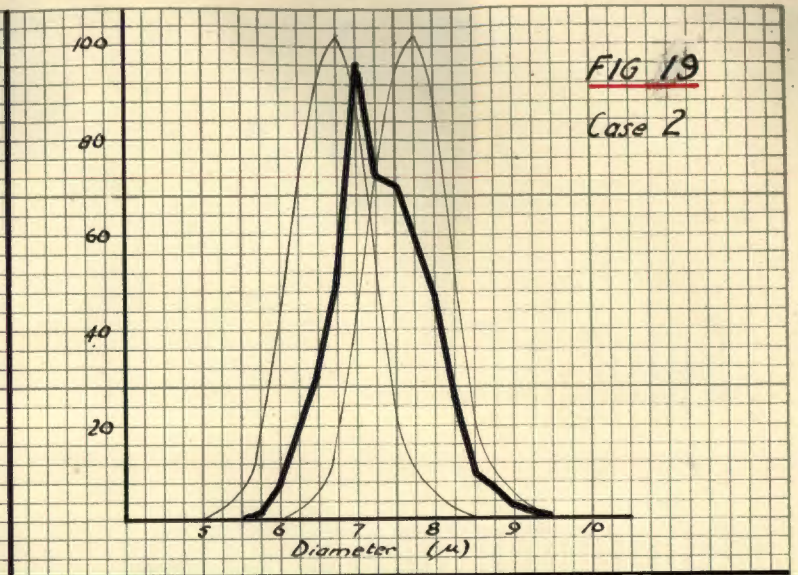


FIG 19

Case 2

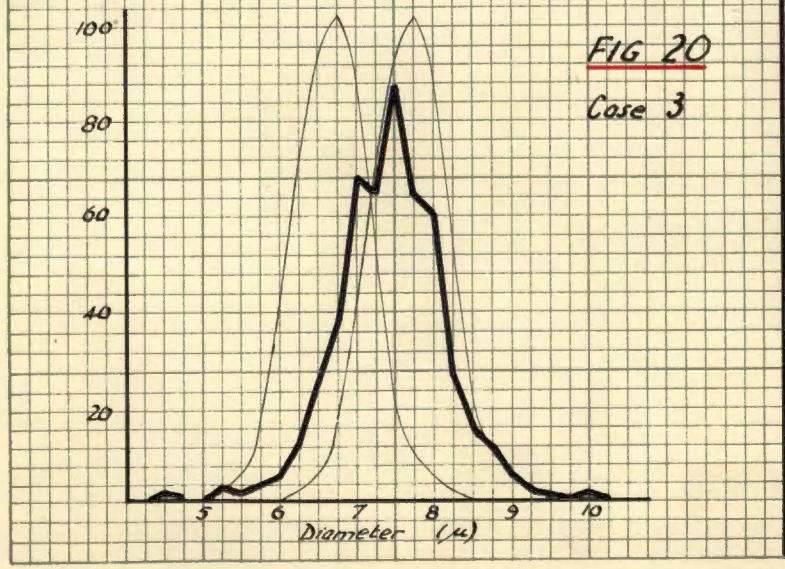


FIG 20

Case 3

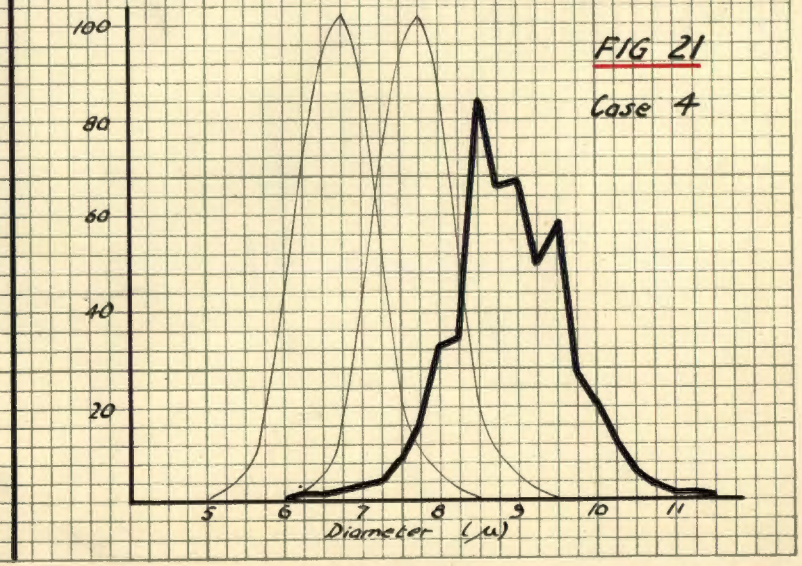


FIG 21

Case 4

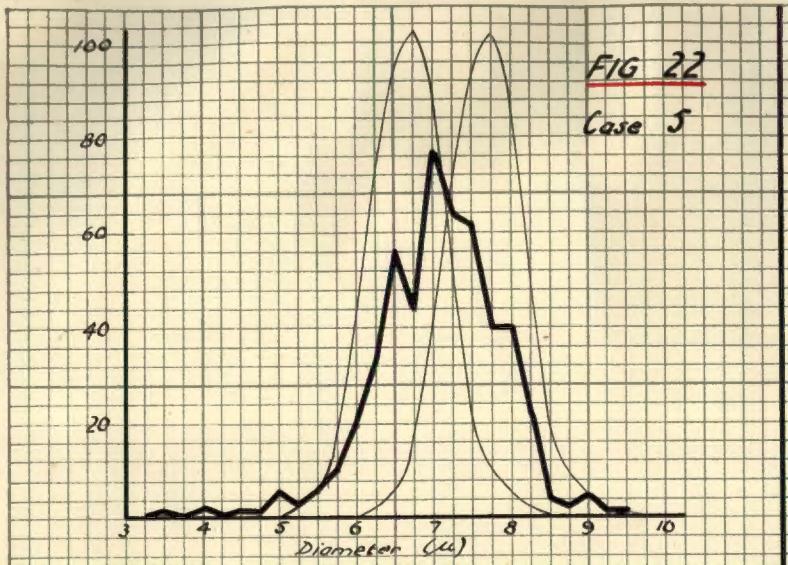


FIG 22
Case 5

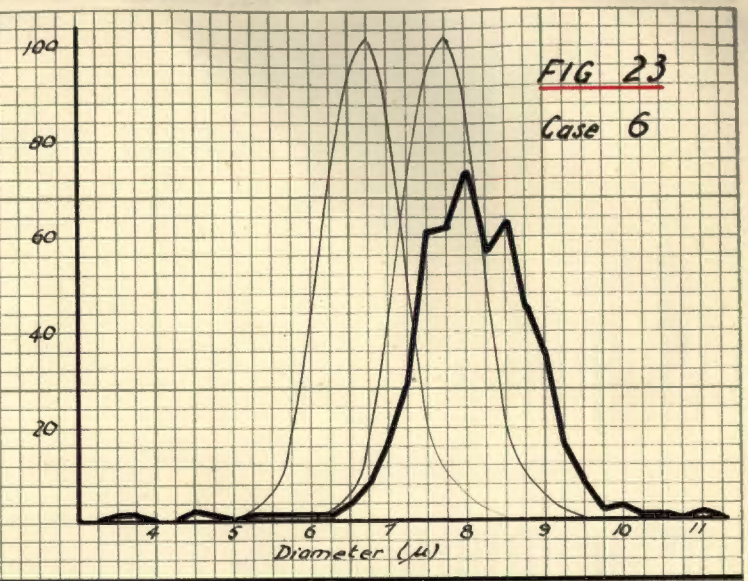


FIG 23
Case 6

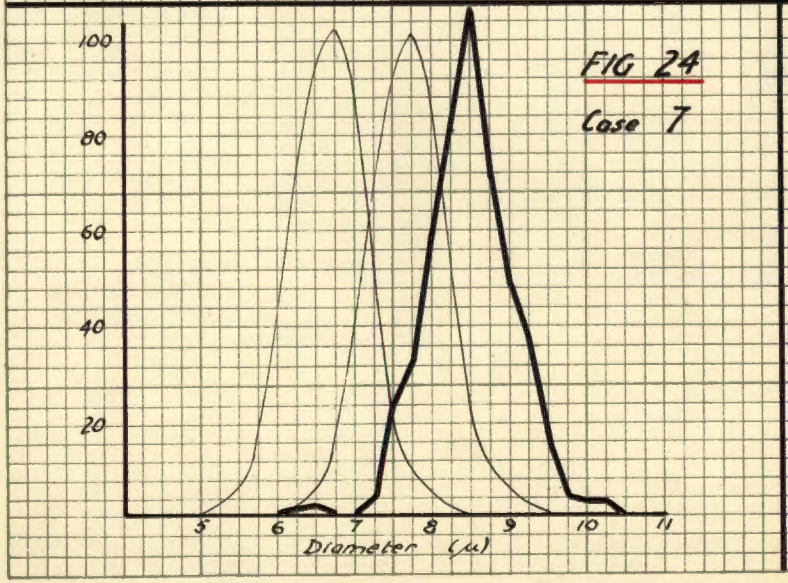


FIG 24
Case 7

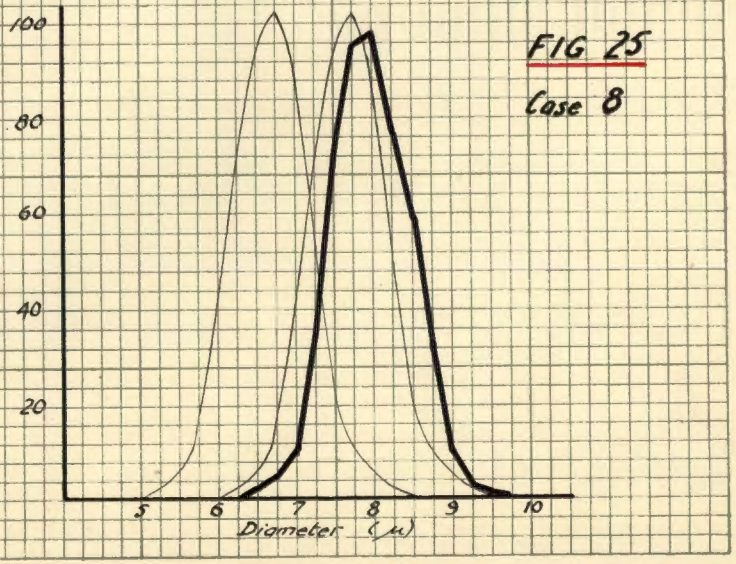


FIG 25
Case 8

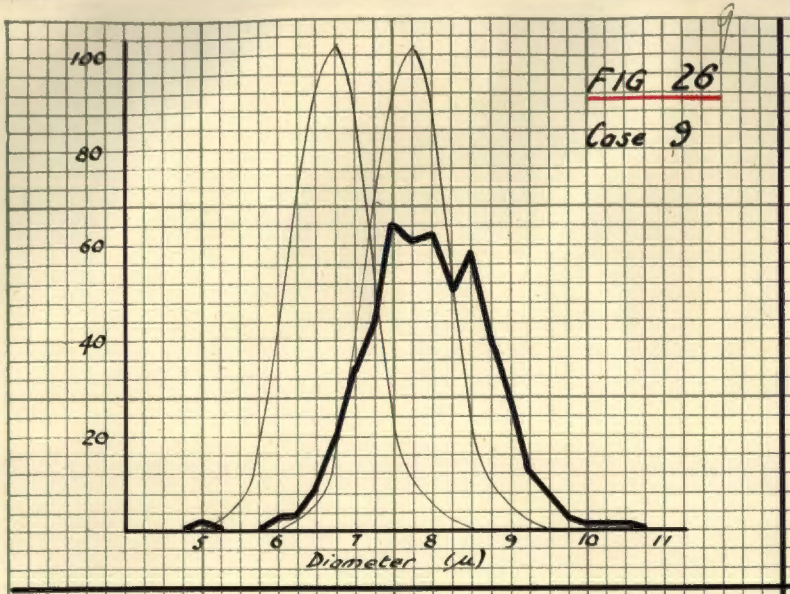


FIG 26
Case 9

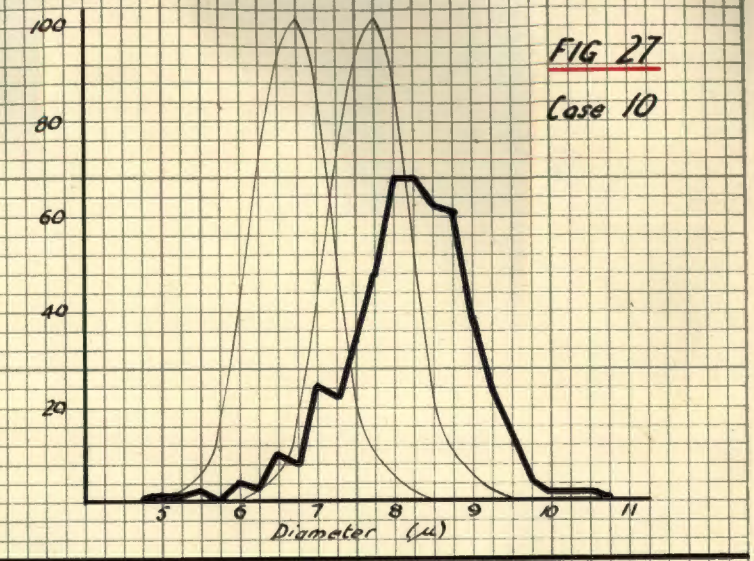


FIG 27
Case 10

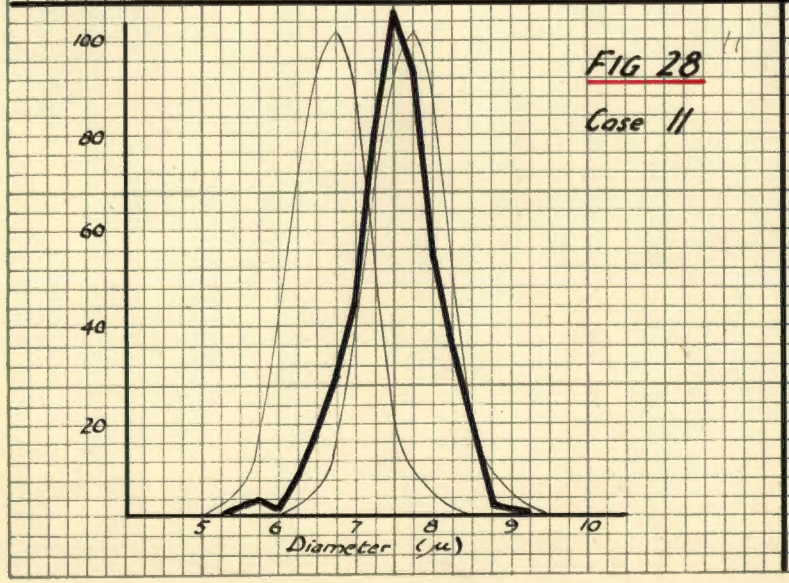


FIG 28
Case 11

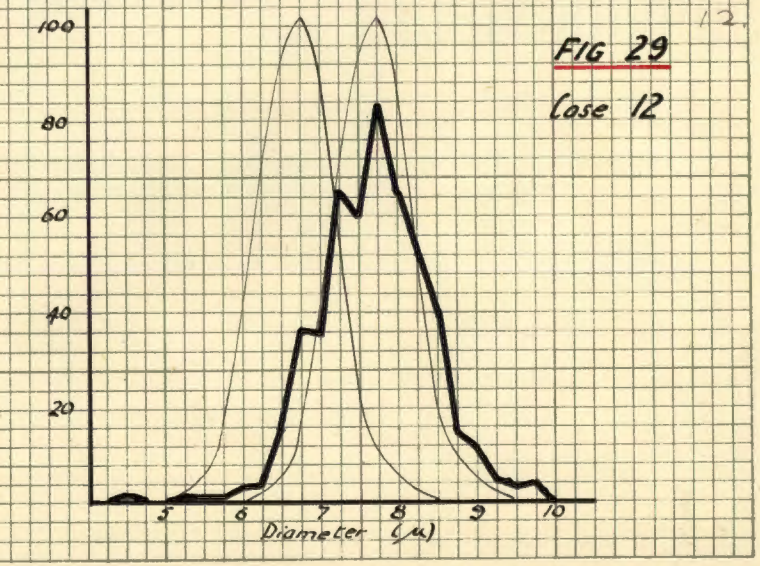


FIG 29
Case 12

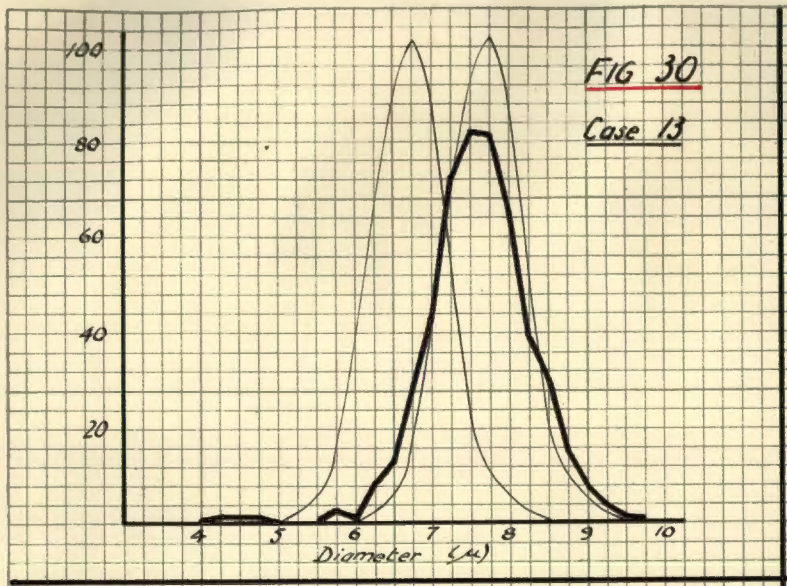


FIG 30

Case 13

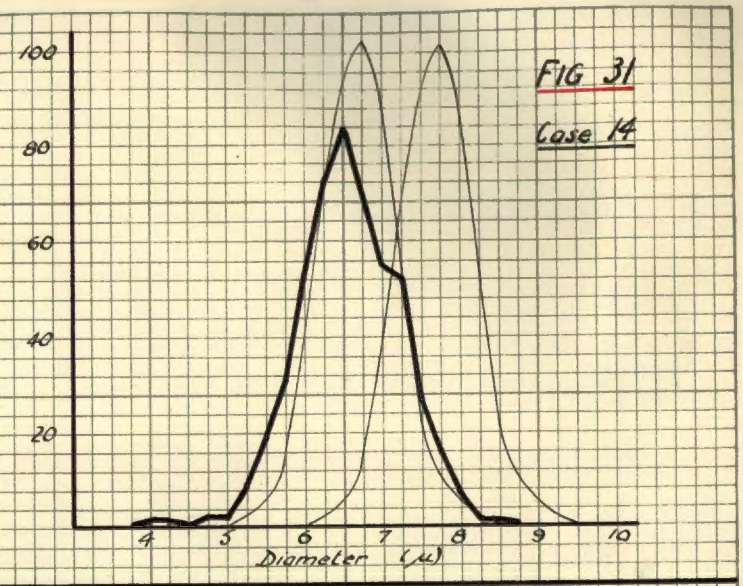


FIG 31

Case 14

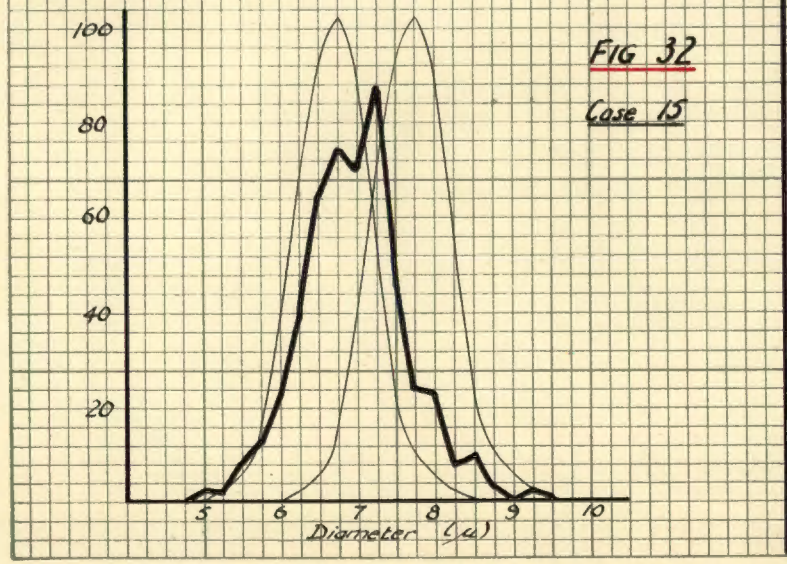


FIG 32

Case 15

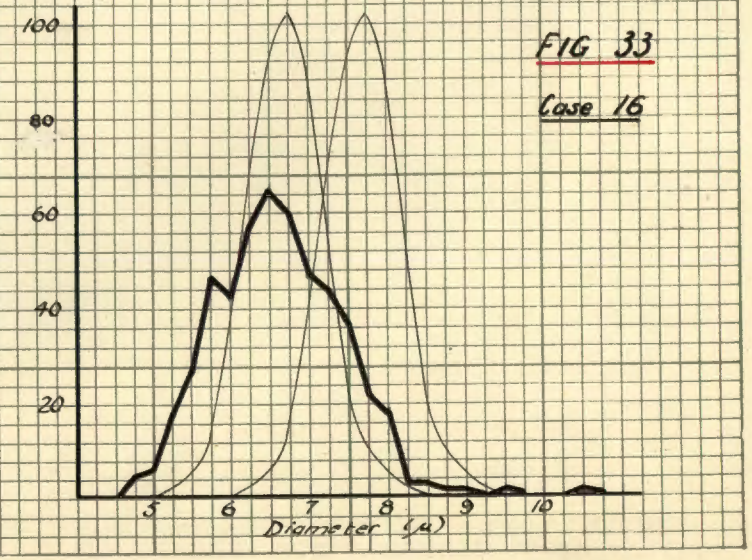


FIG 33

Case 16

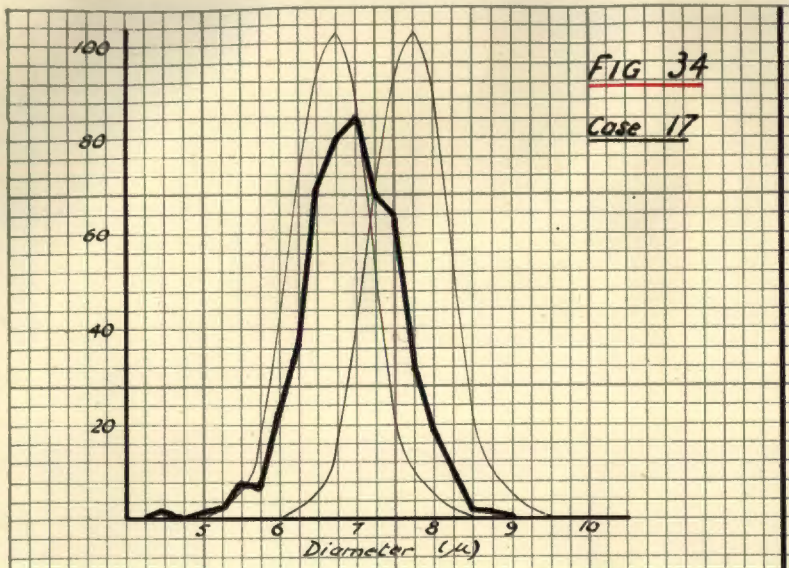


FIG 34

Case 17

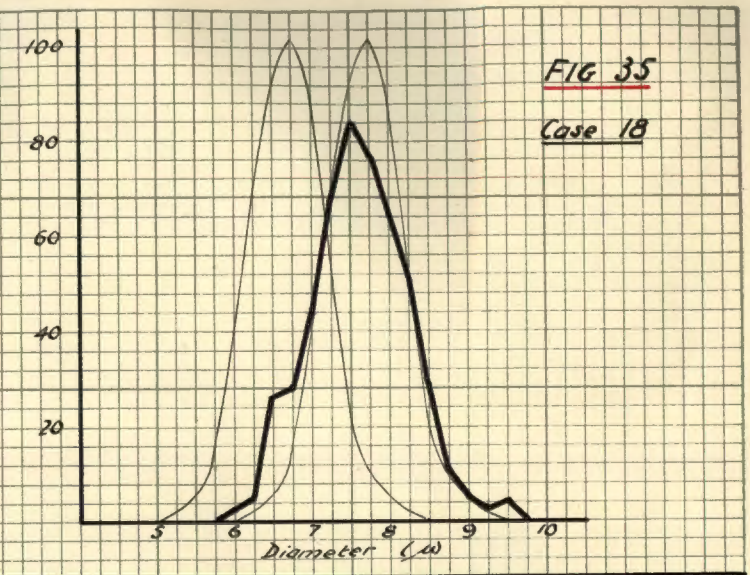


FIG 35

Case 18

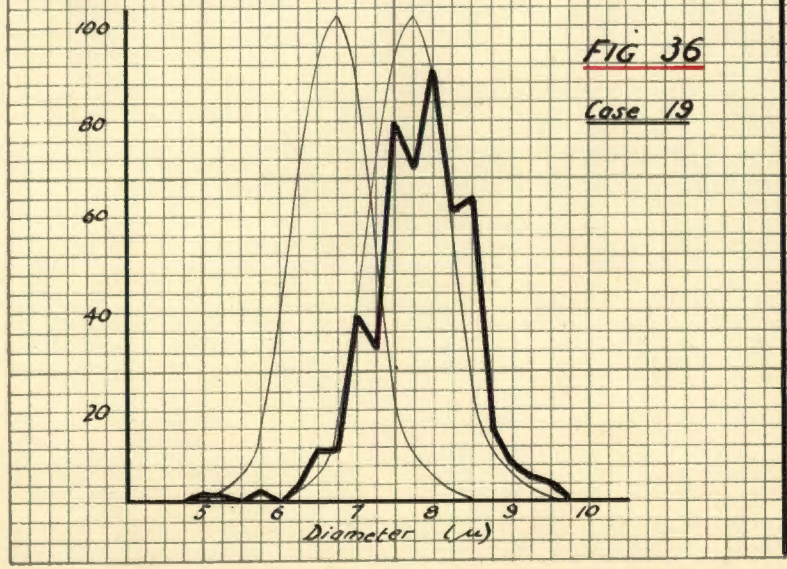


FIG 36

Case 19

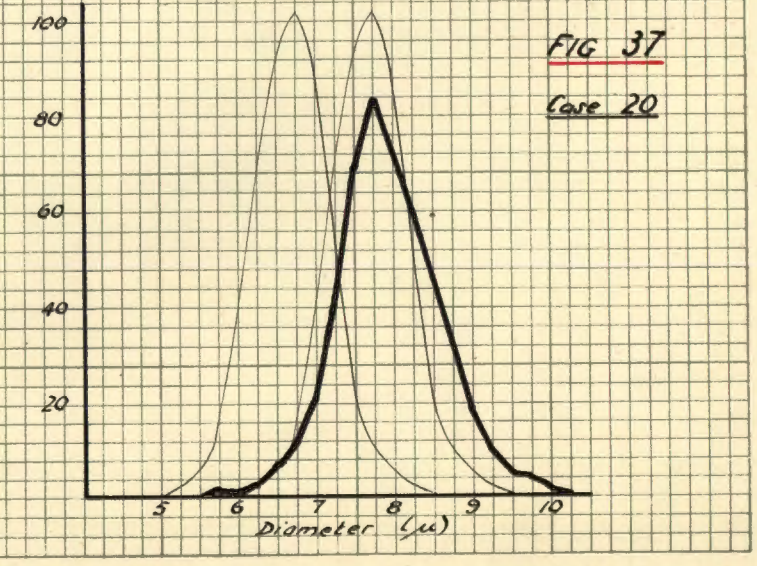


FIG 37

Case 20

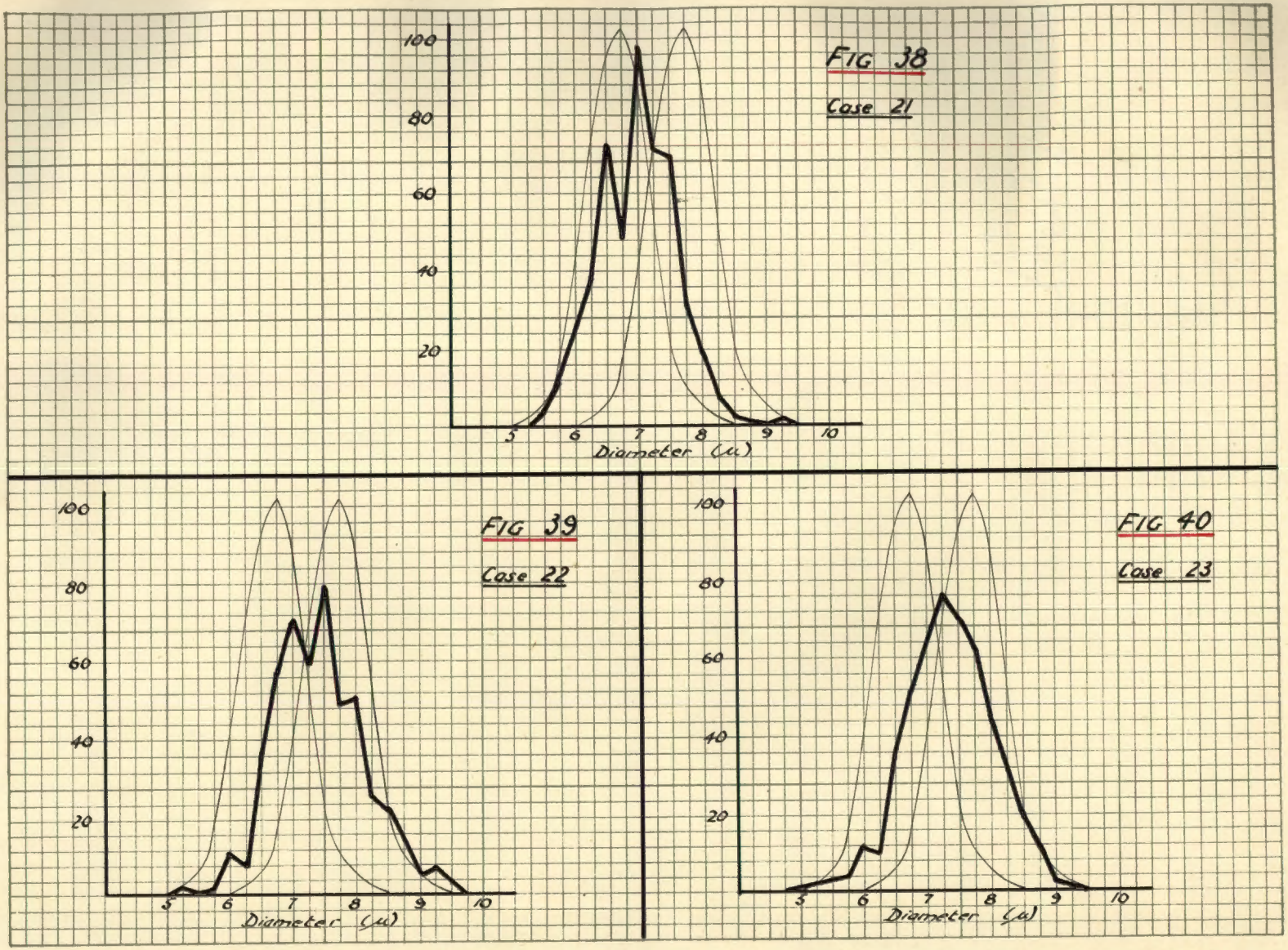


FIG 38

Case 21

FIG 39

Case 22

FIG 40

Case 23

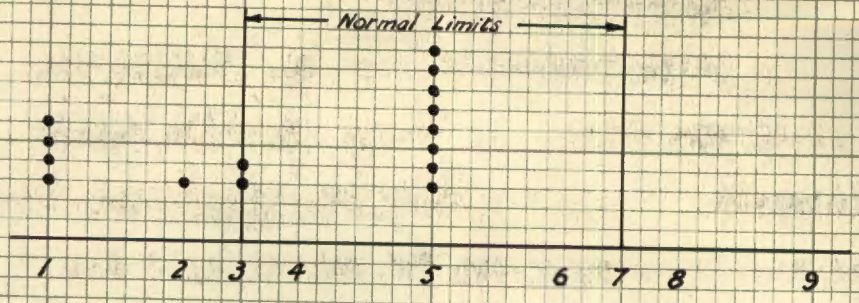


FIG 41 Fragility of the Red Cells

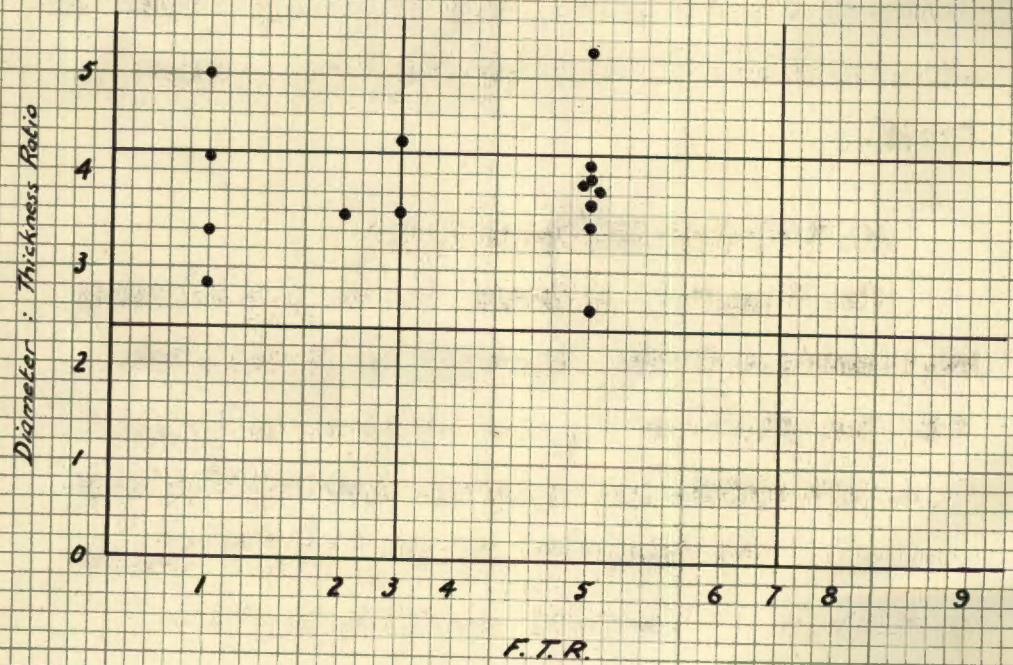


FIG 42 Correlation between Diameter : Thickness Ratio and Fragility

d) THE FRAGILITY OF THE RED CELLS.

1. Hypotonic Saline Tests.

The rapid fragility test was carried out in 15 of the cases. Of these 8 showed median corpuscular fragility (MCF) readings within normal limits. In 2 cases the MCF was at the lower limit of normal and in the remaining 5 cases there was increased resistance to hypotonic saline (fig. 41).

It is apparent therefore that these cases show a marked tendency to have increased resistance of the red blood cells to hypotonic saline. This is in accord with the findings of Cassells (1938).

2. Diameter-Thickness Ratio.

The diameter-thickness ratios are repeated here because of the suggestion of Haden (1940) that the fragility of the red blood cells in hypotonic saline is dependent upon the shape of the cells, the important factor being thickness or thinness of the cells relative to their diameters.

Fig. 42 shows the result of plotting D:T ratios against the F.T.R. (Data from table XVIII).

TABLE XVIII.

The Relationship between the Diameter-Thickness Ratio (D:T) and the Fragility (F.T.R.) of the Red Blood Cells.

Case	D:T	F.T.R.
1	4.08	5
2	3.70	5
3	3.58	2
4	4.14	1
5	3.60	3
6	3.40	1
7	5.26	5
8	4.27	3
9	3.42	5
10	5.00	1
11	3.98	5
12	3.89	5
13	3.84	5
14	2.59	5
17	2.84	1

In general the cells tend to be flat and to show increased resistance to hypotonic saline. However, in the detailed analysis in fig. 42 it will be seen that there is no close correlation between the D:T ratio and the MCF. In the cases with normal fragility the D:T ratio is actually high in 2 cases out of 9. In the cases with resistant blood 4 show normal D:T ratios and in only one of the cases are the cells flat.

Therefore while the general correlation holds good, namely that the tendency to resistance of the corpuscles is associated with a

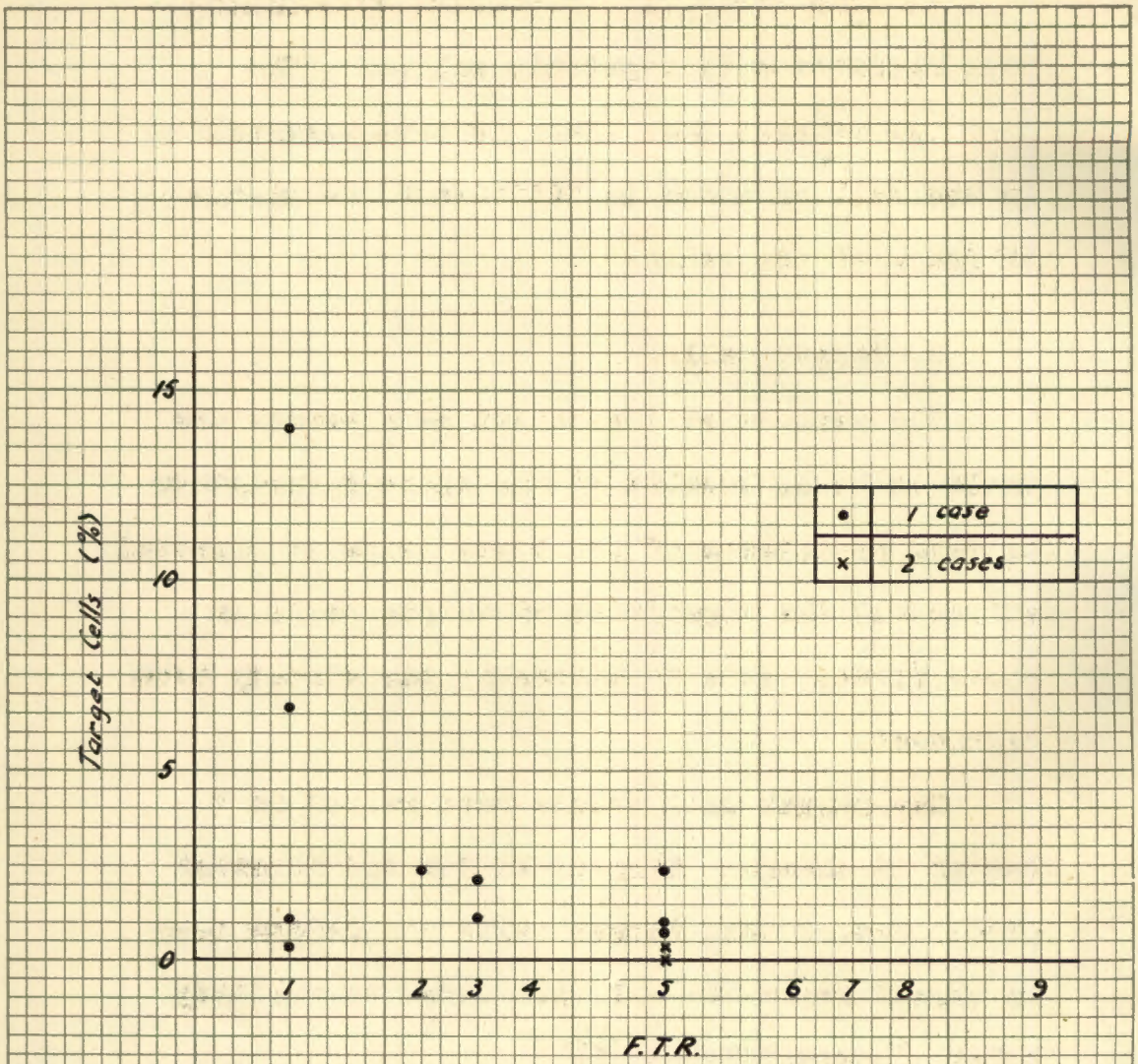


FIG 43 Target Cell - Fragility Correlation

tendency to flatness of the cells, there must be some other factor present determining this resistance to hypotonic saline. The findings suggest that there is some abnormality in the cell envelope in addition to the change in shape of the cells.

3. Target Cells.

The results of the target cell counts are included here, because of the close correlation between resistance of red blood cells to hypotonic saline and the appearance of target cells in blood films. This relationship has already been discussed.

The target cell counts were raised in 7 out of 17 cases. Singer, Miller and Dameshek (1941) remark that target cells are seldom seen in normal smears at all, but fixed an arbitary limit of normality at 1%.

It will be seen from table XIX and fig. 43 that the target cell counts are raised only in those cases that show MCF readings below the normal limits and that the highest target cell counts are found in the most resistant bloods.

TABLE XIX.

Correlation between the Number of Target Cells and the Fragility of the Red Cells.

Case	Target Cells (%)	F.T.R.
1	0.0	5
2	0.3	5
3	2.3	2
4	14.0	1
5	2.1	3
6	6.7	1
7	0.0	5
8	1.2	3
9	0.1	5
10	1.2	1
11	0.7	5
12	1.0	5
13	2.4	5
14	0.2	5
17	0.4	1

While the target cell counts are not high except in 1 case, the presence of target cells form quite a striking feature of the blood picture in these cases, the frequency of target cells here contrasting markedly with the paucity of target cells in normal blood films.

e) EVIDENCES OF HAEMOLYSIS.

1. Reticulocyte Counts.

In 18 cases tested, 5 cases showed reticulocyte counts over 1%. In one of the cases 3 normoblasts were seen in addition in the peripheral blood. In case 10 the reticulocyte was normal 1 week later. In case 17 the reticulocytes were persistently raised until the patient's death 4 weeks later, but the normoblasts disappeared from the peripheral blood within a few days. The other cases could not be followed up.

Thus it may be concluded that in most cases the reticulocyte count is not raised. This corresponds with the findings quoted earlier. Transient or more persistent raising of the reticulocyte count may be seen sometimes. Also, associated with reticulocytosis a few normoblasts may appear in the peripheral blood. Vaughan and Saifi (1939) also record the presence of raised reticulocyte counts in some cases of chronic infections.

Calder, Steen and Baker (1939) found reticulocyte counts of 0.3 - 3.3% with a mean of 1% in cases of brucellosis.

2. v.d. Bergh Reaction.

The direct reaction was uniformly negative in all 17 cases in which the test was carried out.

This is easily explained by the findings in the next section.

3. Plasma Bilirubin.

An attempt was made to estimate this in 17 cases. In 11 instances no colour at all developed on adding the alcohol and ammonium sulphate to the solution obtained in the v.d. Bergh test and thus no bilirubin was detected in these 11 cases. In the remaining 6 cases the colour was so pale that attempts to match this with a standard that represented 1.6 mgs. bilirubin per 100 cc. were futile. Thus in these 6 cases the merest trace of bilirubin was present.

Plasma bilirubin therefore is very low in these cases. This is in agreement with all previous reports on this aspect. Bernheim (1934) described 33 cases of secondary anaemia with hypobilirubinaemia. St. George and Brown (1925) found the icteric index to range between 1 and 3. Perkin (1927) found a serum bilirubin ranging from 1 - 3.5 mgs. per 1,000 cc. in secondary anaemias. Calder, Steen and Baker (1939) in brucellosis found serum bilirubin values below 0.5 mgs. % in 61% of cases, most of the rest of the cases showing less than 1.0mg. %.

4. Urine Urobilin.

The qualitative test for urobilin in the urine showed that it was present in 12 of the 17 cases in which it was looked for. In 3 cases the quantity was small in amount but definitely more than normal. Strong bands appeared in the other 9 positive cases and in one instance it could still be detected after diluting the urine 8 times with distilled water.

Thus urobilin is frequently found in the urine of cases with chronic infections. This is in keeping with the findings of the authors quoted by Vaughan and Saifi (1939) in more acute cases of infections

The results recorded in this section indicate definitely that no haemolytic process is present. The chief points against the possible presence of haemolysis are as follows:-

1) There is a striking absence of bilirubin from the plasma. This in itself is against haemolysis, but does not absolutely exclude the presence of this process, since a normally functioning liver may be able to handle very large amount of bilirubin presented to it by the blood and not allow abnormal amounts to accumulate in the blood stream (Hench, 1940). However, in these cases the latter possibility

is excluded since liver function is not normal (see below).

2) The frequent absence of reticulocytosis is against a haemolytic process. It is true that in some haemolytic cases e.g. blackwater fever in the early stages (Blackie, 1943) and haemolytic disease of the new-born (Kariher and Spindler, 1943) there may be absence of reticulocytosis on occasion. Also it may not be surprising to find no reticulocytosis in severely toxic and ill patients. However, the cases studied here were not severely ill for the most part and the frequency of the absence of raised reticulocyte counts argues strongly against haemolysis.

Two features have to be explained:-

a) Urobilinuria is frequently present. This finding can be due to causes other than haemolysis. In these cases, in view of the absence of other features of haemolysis, it is probably explained by the depression of liver function that is present, and thus cannot be used as an argument in favour of haemolysis.

b) Reticulocytosis and even normoblastosis does occur in some cases. No satisfactory explanation can be given for this. One can only mention the fact that reticulocytosis, transient or permanent does occur in other non-haemolytic

disturbances and even in cases of profound bone marrow depression such as occurs in some refractory anaemias (Bomford and Rhoads, 1941). A possible explanation for the normoblastosis is discussed later.

It seems reasonable to conclude that haemolysis is not a feature of these cases. Quantitative pigment studies have not been made in the present investigation, but have been carried out by Vaughan and Saifi (1939). They also concluded that there is no evidence of a haemolytic process in these cases.

f) OTHER ELEMENTS OF THE BLOOD.

1. White Blood Cells.

The white blood corpuscles and the platelets were studied in order to get some idea of the state of function of the bone marrow in regard to its ability to form leukocytes and platelets. White cell counts are also important in interpreting the degree of erythropoietic activity of the marrow.

The total white cell counts are given in table XV. These varied from 5,900 to 24,200 and appeared to be dependent on the primary disease present. Differential white cell counts also appear to be dependent upon the primary condition causing the anaemia. No special studies were made apart from the enumeration of the white cells.

It is obvious from the fact that 8 of the 16 cases showed a leucocytosis, that the bone marrow is capable of increasing its output of white blood cells and thus there appears to be no depression of leukopoietic activity. This is in accord with what one would expect in the type of case studied.

2. Platelets.

The platelets were plentiful in all cases in which the count was performed (16 cases). This

again indicates no depression of the ability of the bone marrow to produce platelets.

g) THE BONE MARROW.

The marrow counts are shown in fig. 44.

A study of the literature reveals considerable differences of opinion as to the normal percentage of erythroblasts in bone marrow smears. Young and Osgood (1935) included disintegrated cells in their differential counts and found the erythroblasts to range from 5.4 - 24.2%.

Hynes (1939), whose standards are the ones usually quoted by English authors, arrived at his normal range by using the results of Young and Osgood (1935) on 28 cases and adding 13 cases of his own. He excluded disintegrated cells and gave the normal range as 9 - 30%.

Kandel and Le Roy (1939) also reviewed normal bone marrow differential counts and stress the extensive studies of Segerdahl (1935). Her results show an average erythroblast percentage of 12.88 with a standard deviation of 4.39. Kandel and Le Roy (1939) state that their experience agrees well with that of Segerdahl.

Osgood in the most recent review (Osgood and Seamen, 1944) again includes disintegrated cells, which form 12.8 - 31.8% of the cells in marrow smears. Osgood and Seamen (1944) subjected

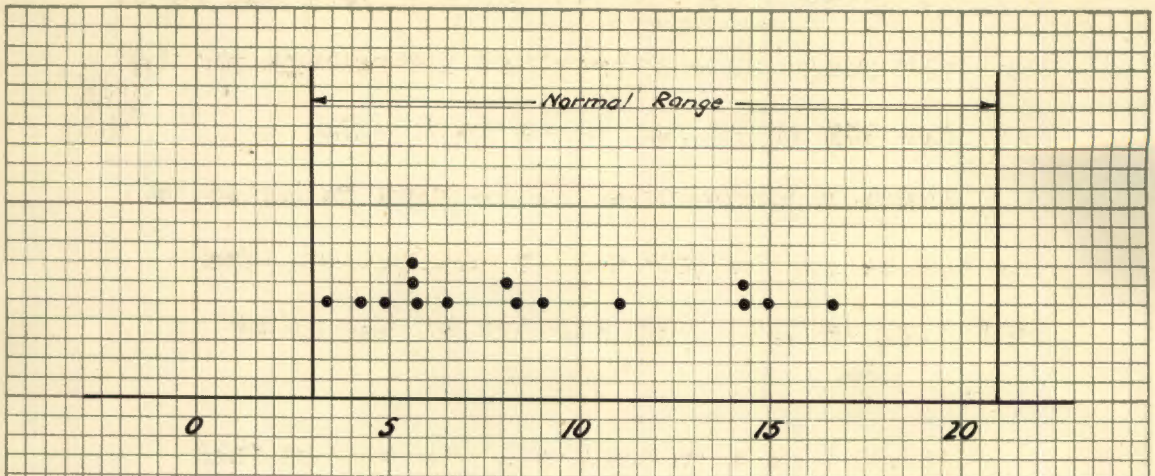


FIG 44 Erythroblasts in Marrow (%)

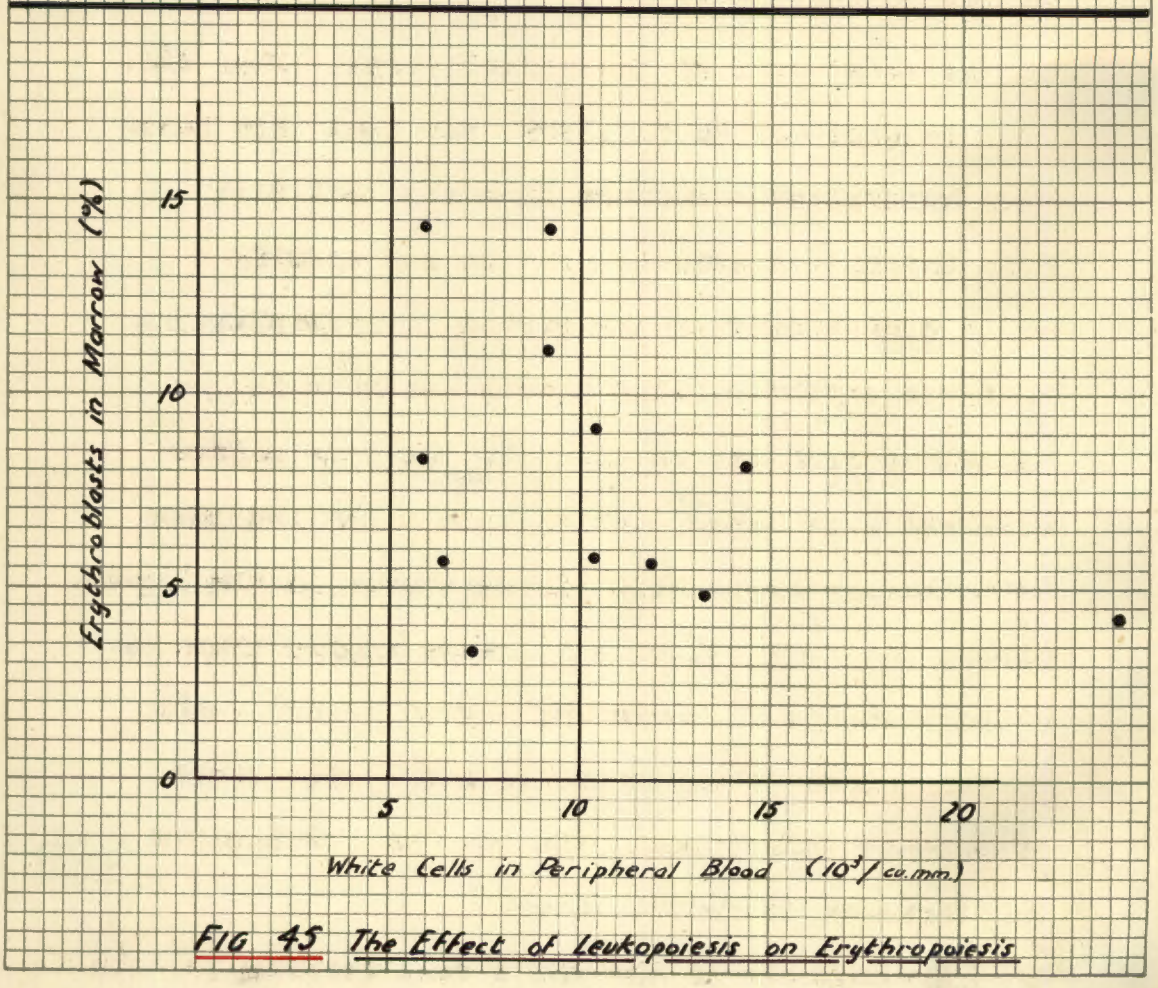


FIG 45 The Effect of Leukopoiesis on Erythropoiesis

the available marrow differential counts of various authors to statistical analysis and found that the range of erythroblast percentage which includes 95% of normal cases is 3 - 21.

Since disintegrated cells were included in the counts in the present investigation, the last figures have been adopted as the normal range.

It will be seen that all the counts fall within the normal range of 3 to 21%. Eleven of the 15 cases show an erythroblast percentage in the lower half of the normal range. (Fig. 44).

Vaughan and Saifi (1939) say that they have seen hyperplastic marrows in cases that died from chronic infections, but do not specify which constituent of the marrow is hyperplastic. Here the erythropoietic tissue tends to be low normal in relative amount. If the bone marrow as a whole is hyperplastic, this might indicate that the erythropoietic tissue shares in the hyperplasia but to a relatively lesser degree than the other elements. Young and Osgood (1935) found no depression of erythropoiesis in their cases of chronic infection. The bone marrow picture is discussed in detail later.

Hyperplasia of the white cell forming portion of the marrow could give a false impression of underactivity of erythropoiesis and wice versa.

TABLE XX.

The Influence of Leukopoiesis on the Erythroblasts of the Bone Marrow.

Case	% Erythroblasts	W.B.Cs.
1	8.3	6,500
5	14.3	9,150
7	3.3	7,200
11	14.3	5,900
12	11.1	9,150
14	5.6	6,300
3	4.8	13,250
6	4.2	24,200
8	5.7	10,400
9	9.1	10,400
10	5.6	11,800
13	8.1	14,300
15	16.7	10,850

Taking the total count of the white cells in the peripheral blood as an index of the activity of the white cell forming portion of the marrow, it appears that neither of these alternatives plays a part in the bone marrow picture. This aspect of the marrow function is analysed below. (Table XX and fig. 45).

1) In cases with leucocytosis ranging from 10,400 to 24,200 the red cell percentage ranged from 4.2 to 16.7.

2) In cases with white cell counts between 5,900 and 9,150 the red cell percentage ranged from 3.3 to 14.3.

3) The absence of an inverse proportion between the white cell count and the percentage of red cell precursors in the bone marrow is shown in fig. 45.

The type of erythropoiesis is shown by the differential count of the erythroblasts. It will be seen that the predominant type of erythroblast is the type IV which constitutes between 58% and 85% of the red cell precursors. Thus the erythropoiesis is normoblastic in type in all the cases in which the marrow was studied. The erythroblasts all appeared normal in character and size in the smears. No actual measurements of the red cell precursors were made.

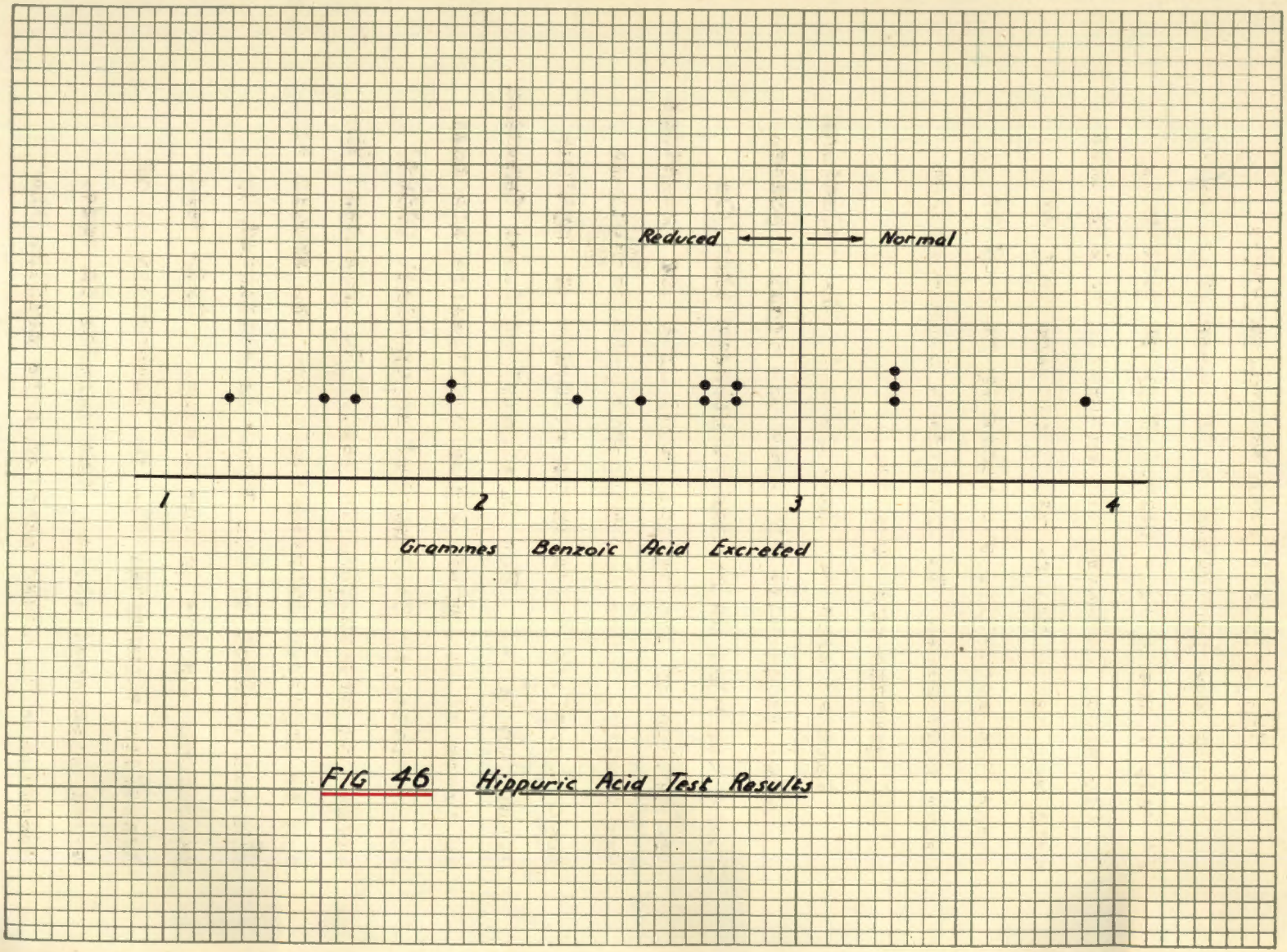


FIG 46 Hippuric Acid Test Results

h) LIVER FUNCTION.

1. The Hippuric acid excretion test.

This was performed in 15 cases (fig. 46). In only 4 cases were normal figures obtained. In the other 11 cases the excretion of hippuric acid was below the normal limit and in 5 of these the excretion was markedly reduced to below 2 grammes. In one case a figure as low as 1.2 grammes was obtained.

Special care was exercised in the selection of cases to exclude all possible renal disease. There was also no reason to believe that the absorption of the benzoic acid from the intestine was faulty. The low hippuric acid excretions must therefore be taken as evidence of impaired liver function. The impairment of liver function in cases of low-grade chronic illnesses has been demonstrated by Stiles, Stiles and Kolb (1942) using the bromsulphalein test. In this test the dye is injected intravenously and the amount of dye not handled by the liver is estimated by measuring the dye in the plasma. This test therefore eliminates the two possibilities of error discussed above. The results in cases comparable to those studied here confirm the depression of liver function.

2. Urine Urobilin.

Urobilin appears in the urine under three circumstances:-

1) Very slight traces normally.

2) After haemolysis.

3) When the liver function is disturbed.

The amounts of urobilin present were in excess of normal. No evidence of haemolysis was present in the cases. The presence of urobilin in the urine must therefore be interpreted as evidence of impairment of the function of the liver in cases of chronic infection.

The results also demonstrate the well-known fact that all liver functions are not necessarily affected in any given case. (Table XVI). Thus in 2 cases there is a very low hippuric acid excretion and no urobilin is present in the urine, while in one case with a normal hippuric acid test, urobilin was found in the urine.

Only two cases out of 17 in which one or both tests were performed showed normal results in both investigations.

3. Clinical and Autopsy Evidence.

Clinically, there is no record of the state of the liver in cases 13 and 15. Of the remaining 23 cases, the liver was not palpable in 17 instances.

In 2 cases the liver was 1 fingersbreadth below the costal margin, in 2 cases, 2 fingers down, in 1 case 3 fingers down. In all the liver was smooth and soft and the edge was not sharp.

In the remaining case the liver was 4 fingersbreadth below the costal margin, firm and tender. This was case 17 with subacute bacterial endocarditis, in which some cardiac failure supervened. No hippuric acid test was done in this case, nor was urobilin tested for in the urine.

Of the cases that died, 3 were autopsied. In two of these the liver showed fatty changes only, there being a moderate amount of deposit of fat in the periportal zones. In the third case (subacute bacterial endocarditis) the main change was passive congestion.

I have already pointed out (Berk, 1943) the fact that the liver function may be disturbed in cases of generalised tuberculous adenopathy without anything more than fatty changes being demonstrable in the liver. Here too it appears that in chronic infections the liver function may be grossly disturbed without actual involvement of the liver by the infection, but merely as a toxic affect. It is known, too, that even

more severe liver involvement can occur, sufficient to be termed "hepatitis", in cases of infection, e.g. it has been found to be very common in cases of pneumonia in natives in Southern Rhodesia (Gelfand and Lewis, 1942). In such cases too, nothing more than fatty changes in the liver need be found.

The exact significance of fatty change in the liver is in doubt. Much controversy has taken place as to whether the fatty change is degenerative or merely infiltrative and not indicative of any disturbance in the cells.

Some cases of fatty liver are called "hepatitis", e.g. the alcoholic liver is typically a fatty one. Also Himsworth and Glynn (1944) note that in animals starved of carbohydrates a fatty liver results and this goes on to a fine diffuse cirrhosis.

Thus one can only state the fact that in chronic infections fatty changes occur in the liver and liver function can be shown to be defective.

i) GASTRIC FUNCTION.

Only the secretory activity of the stomach was investigated. This was done in 12 cases. Only one case showed complete absence of free hydrochloric acid in the gastric contents. No response followed histamine injection in this case. In the remaining 11 cases hydrochloric acid appeared in the gastric contents. 3 cases showed some depression of acid secretion and in the rest the secretion was normal with one case showing hyperchlorhydria.

The distribution of the types of acid secretion is in keeping with what is found in groups of perfectly healthy people and thus there is no evidence of depression of hydrochloric acid secretion in these cases. Calder, Steen and Baker (1939) also found no achlorhydria in cases of brucellosis.

1) FOLLOW-UP AND TREATMENT STUDIES.

Only two cases were followed up, one of them having been placed on iron therapy. The position with regard to therapy is summarised by Vaughan and Saifi (1939) and needs no amplification. "Transfusions, iron and liver therapy are without permanent effect in anaemias associated with sepsis". This is illustrated by case 5 in whom iron therapy was given for 10 weeks. During this time she also had treatment for the rheumatoid type of arthritis from which she was suffering. The blood was counted at intervals and the results are shown in fig. 47.

In 11 weeks the haemoglobin rose 7% and the red cells 670,000. There was no increase in the reticulocytes during the first three weeks of treatment.

Case 4 was followed without treatment directed against the anaemia itself for 7 weeks. During this time the haemoglobin rose 6% and the red cell count 190,000. (Fig. 48).

Thus these two cases remained virtually stationary despite iron administration in the one case and treatment for the primary condition in both cases. From the literature this appears to be characteristic of this type of anaemia.

FIG 47 Case 5 Follow-up Study

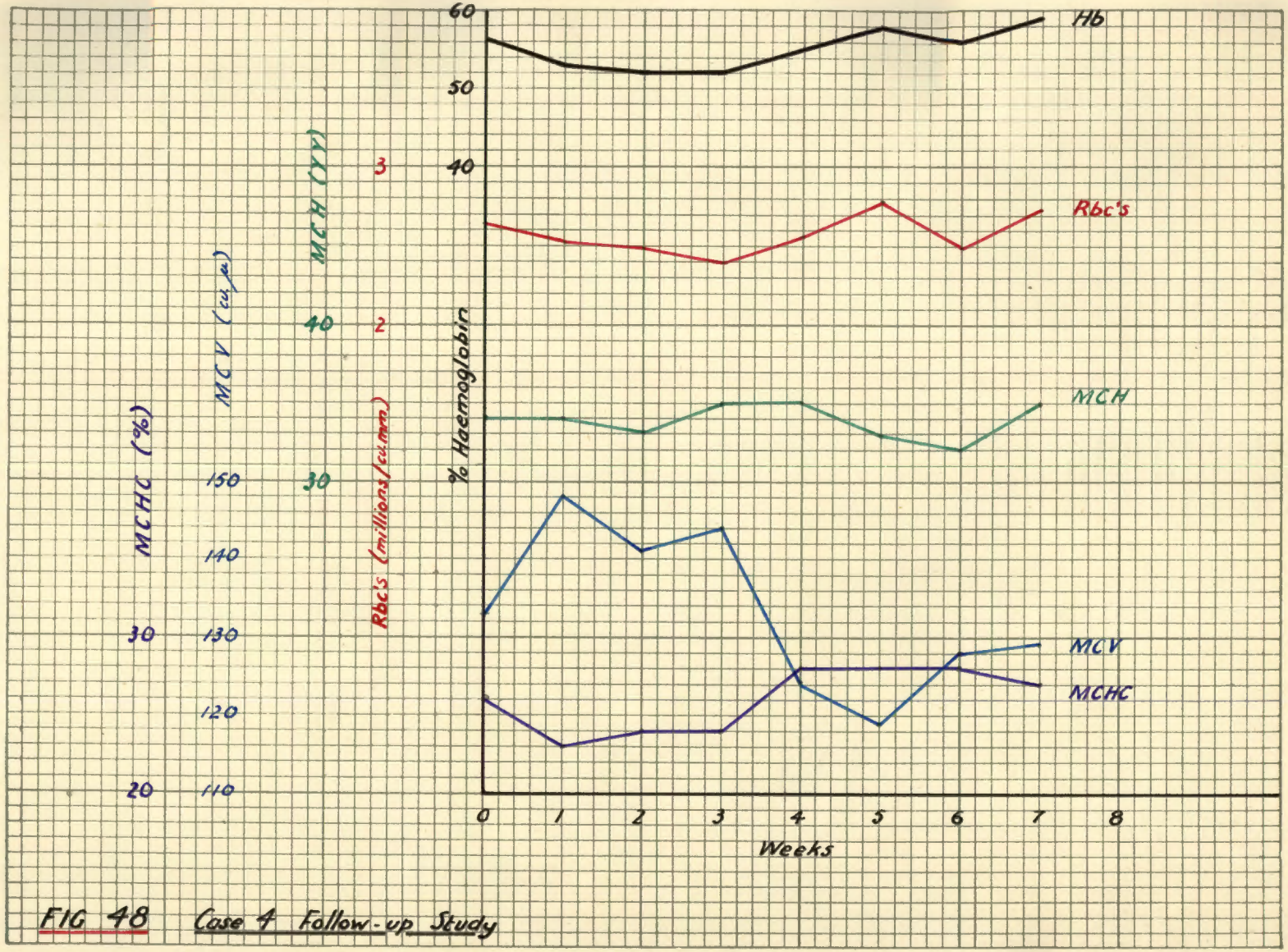


FIG 48 Case 4 Follow-up Study

k) MAIN CONCLUSIONS.

In spite of the small number of cases studied, the following conclusions concerning the anaemias of chronic infections seem warranted by the evidence presented.

1) The total red cell count shows only slight reduction in most cases. In only one quarter of the cases is the red cell count reduced by more than 1 million cells per cubic millimetre.

2) The haemoglobin shows relatively greater reduction, most readings falling between 46 and 67%.

3) The colour index and the mean corpuscular haemoglobin are raised in a few instances, i.e. a hyperchromic anaemia may be found in cases of chronic infection, due to this factor alone.

4) In about half the remaining cases the colour index and mean corpuscular haemoglobin are normal and in the rest they are below normal, i.e. hypochromic and orthochromic blood pictures are about equally common and account for most of the cases.

5) The volume of packed red cells tends to be higher than would be predicted from the haemoglobin, red cell count and colour index figures.

6)This is explained by the fact that the mean corpuscular volume shows a marked tendency

to be raised, figures above normal being seen in over a third of the cases.

7) In all cases the mean corpuscular volume is larger than would be predicted from the colour index or mean corpuscular haemoglobin.

8)The erythrocytes show a similar tendency to have an increased diameter, and in about twe-fifths of the cases the diameter is above normal.

9) In keeping with these findings, the Price Jones curves show large numbers of macrocytic cells. Microcytes are few in number.

10) The thickness of the corpuscles falls within normal limits in most of the cases.

11) Taking the above findings in relation to the findings with regard to mean cell diameter it is to be expected that the cells would tend to be flat. This is borne out by the figures for the diameter-thickness ratio.

12) The red cells show a degree of anisocytosis that is only slight.

13) The fragility of the red cells is frequently reduced, i.e. there is a tendency for the cells to show abnormal resistance to hypotonic salt solutions.

14) This tendency to resistance on the part of the red blood cells can be related in general to the tendency of the cells to be flat, although in individual instances the relationship is not always close.

15) The presence of target cells, sometimes in high percentage, forms a striking feature of the blood films in many of the cases.

16) The presence of target cells in the blood films of these cases is closely correlated with the resistance of the cells to hypotonic saline.

17) There is no evidence of any haemolysis in these cases, as shown by the low plasma bilirubin content in the presence of poor liver function, and the usual absence of reticulocytosis.

18) The reticulocytes may be raised to a very small extent on occasion, either transiently or for longer periods. Normoblasts also may appear in the peripheral blood.

19) The white cells vary according to the primary disease causing the anaemia.

20) The platelets are normal in number.

21) Bone marrow studies show very little if any depression of the erythropoietic function in these cases. The activity of the leukopoietic tissue does not affect the erythropoietic tissue. The erythropoiesis is normoblastic in type.

22) Depression of liver function is a very common finding in cases of chronic infections, as shown by defective excretion of hippuric acid in the Quick test and by the finding of excess of urobilin in the urine.

23) Clinically and histologically no abnormality of the liver is found other than fatty change.

24) The secretion of hydrochloric acid by the stomach is normal.

25) In the two cases followed up, the blood picture tended to remain stationary in spite of iron therapy in one case and treatment of the primary condition in both.

DISCUSSION.

IV. THE MECHANISM OF PRODUCTION OF THE RED CELL CHANGES IN CHRONIC INFECTIONS.

A) MISCELLANEOUS FACTORS.

No adequate analysis of the data with regard to factors such as the age, sex, and race of the patients could be carried out because the number of cases studied was too small for this purpose.

Age.

The distribution of the cases by decades is as follows:-

0 - 10 None; 11 - 20 4; 21 - 30 6; 31 - 40 6; 41 - 50 4; 51 - 60 2; 61 - 70 3.

It is unlikely that age would make a difference, except in children, where the haemopoietic system is undergoing changes and is more labile than in the adult.

Sex.

There were 15 males and 10 females in the series. This is in keeping with the fact that the hospital has a larger number of beds for males than for females.

Race.

It was considered unlikely that race would make a difference to the results, except in so far as the Cape Coloureds and Natives belong to

a much lower income group than the Europeans and thus could have changes in the blood antedating the development of the chronic infection, due to nutritional deficiencies.

It was impossible to control this aspect of the cases. It may be mentioned, however, that the cases showed no clinical evidences of vitamin deficiencies.

Duration of Illness.

This factor does not seem to have been of importance in these cases from inspection of the data. In long standing illness it might be anticipated that owing to poor appetite and extra demands on metabolism, that deficiencies may arise. However, as stated above no obvious signs of deficiency developed.

Iron deficiency, which might be expected to occur, is unlikely in view of the findings of Gross, Sandberg and Holly (1943) of normal or excessive iron stores in cases of chronic infections.

The duration of the illness varied from 2 weeks to seven years, distributed as follows:- 2 weeks to 3 months - 14 cases; 3 months to 6 months - 4 cases: 6 months to 1 year - 2 cases; 1 case each - 18 months, 2 years, 31 months, 4-5 years and 7 years.

The Cause of the Chronic Infection.

In view of the suggestions made later with regard to the actual mechanisms whereby the changes in the blood are brought about, and the demonstration by Menkin (1940) that the chemical substances responsible for the local and general phenomena of inflammation are non-specific and do not depend on the type of infection, it seems legitimate to group the rather diverse cases studied together under the common heading of anaemia due to chronic infection.

The State of the Blood before the Chronic Infection.

As has been stated several times before, no control was possible over this source of error. From the clinical histories it was not thought that any ill-health preceded the chronic infection, since all cases were perfectly fit until the onset of the disease which brought them into hospital.

Severity of the Illness.

This is in any case a difficult matter to state quantitatively and to correlate with other findings. A rough guide to this question is the termination of the illness in death. 9 of the patients died. From inspection of the data there appears to be no significant difference between the blood picture in those that

died and those that survived.

B) THE TYPE OF ANAEMIA PRESENT.

Anaemias have been classified into three main types by Witts (1932) and Vaughan (1934), according to the mechanism of production of the anaemia.

1. Posthaemorrhagic Anaemia.

Great care was exercised in the selection of patients for study to exclude all sources of bleeding, either acute or chronic. There is no evidence that chronic infection in the cases investigated gave rise to any bleeding.

Bleeding can complicate the picture of the anaemia in chronic infection, e.g. a case with pulmonary tuberculosis may show haemoptysis, which may alter the blood picture in addition to aggravating the anaemia. Also in very severe chronic infections or in certain special types one of the features may be purpuric manifestations from toxic affects on the capillary endothelium. Haemorrhage from this mechanism may be sufficient to alter the blood picture.

Haemorrhage may cause a reduction in the saline fragility of the red cells, e.g. in pregnancy anaemia (Elliott, 1941).

The discrepancies between the findings in this investigation and those of other observers may be explained in part by the fact that many

of the reported cases are such that they may easily have bled either acutely or chronically.

2. Haemolytic Anaemia.

The findings recorded here and those in the literature suggest that no haemolysis is taking place in these cases.

It is recognised however, that haemolytic anaemia may complicate the blood picture in certain cases, either due to chronic infection itself or to the therapy given for the infection.

3. Dyshaemopoietic Anaemia.

In view of the above, it must be concluded that the anaemias of chronic infections belong to the dyshaemopoietic group of anaemias. It is therefore necessary first of all to consider the various factors known to produce dyshaemopoietic anaemias.

I) Deficiency Dyshaemopoietic Anaemias.

a) Interference with iron metabolism.

It has been shown in the literature that haemoglobin metabolism is interfered with in these cases. The finding in the present investigation of low mean corpuscular haemoglobin concentration in practically all the cases supports this view. The usual way in which haemoglobin is rendered deficient in any case is by interference with metabolism of iron.

(i) Defective intake of iron.

(ii) Defective absorption of iron.

The findings of normal or excessive iron stores in various organs of the body (Gross, Sandberg and Holly, 1943) in chronic infections argues very strongly against the possibility that iron deficiency results either from deficient intake or defective absorption of iron in these cases. Defective intake of iron is easily remedied by iron administration, but this therapy exerts no or very little influence on the anaemia in chronic infection.

One factor known to be of importance in the absorption of iron is the presence of hydrochloric acid in the stomach. Where gastric hydrochloric acid is deficient, iron absorption may be defective. In the cases studied it was shown that the secretion of hydrochloric acid by the stomach is normal. Thus this factor would not play any part in the anaemia under consideration.

(iii) Excessive loss of iron.

The large iron deposits again argue against the excessive loss of iron from the body in these cases. The absence of haemorrhage has already been discussed.

(iv) Defective utilisation of iron by the bone marrow.

The large iron deposits in cases of chronic

infection indicate that in spite of the anaemia and deficiency of haemoglobin, the demand for iron is less than the supply available. This must be interpreted to mean that the bone marrow is incapable of using iron in the normal manner. This concept is analogous to the explanation advanced in the case of the haematinic factor in cases of "Achrestic anaemia" (Israels and Wilkinson, 1936).

The evidence thus points to the following explanation of the interference with haemoglobin formation:- It results from disturbance of the function of the bone marrow, as a result of which it is unable to utilise iron for the production of haemoglobin.

The bone marrow picture is in keeping with what is found in iron deficiency anaemias only in so far as erythropoiesis is normoblastic in type. Iron deficiency usually leads to hyperplasia of the red cell precursors whereas here this hyperplasia is absent and the erythroblasts are in fact present only in amounts near the lower limit of normality. This finding may be explained by depression of the erythropoietic function of the marrow in chronic infection.

A further striking difference exists between the anaemias of chronic infections and other

anaemias in which interference of iron metabolism occurs due to one of the first three mechanisms discussed above. In the latter cases the important features of the blood picture are hypochromia and microcytosis. It has already been noted that in cases of chronic infection only hypochromia is seen and microcytosis is not a feature. In fact (fig. 12) the cell size in anaemias of chronic infections is always larger than would be expected if interference with haemoglobin formation was the only factor in the production of the anaemia.

It seems therefore that in order to explain the cell size two mechanisms at least must be operative in these cases.

1) Interference with haemoglobin production, which, in addition to causing hypochromia, tends to decrease red cell size.

2) Some other factor which tends to produce an increase in the cell size.

The findings recorded in fig. 12 can be explained best by combinations of these two factors in varying amounts. Thus where the interference with haemoglobin metabolism preponderates, the cells are microcytic (2 cases). Where the second factor predominates, macrocytosis is found (9 cases). Where the two factors are more nearly

equally active, the cells are normal in size (14 cases). The influence of each factor is seen in all cases - hypochromia of the individual cell is seen in all cases and in every case the cells are too large for their haemoglobin content.

The concept advanced here is supported by the findings in cases of certain tropical nutritional anaemias to which the name "Dimorphic Anaemia" was given by Trowell (1943). Here the blood picture is macrocytic and hypochromic and it has been shown that both iron and liver deficiencies are present.

The theory is put forward therefore that the anaemia of chronic infection is also a dimorphic anaemia.

b) Interference with the liver factor.

In looking for a cause for macrocytosis in any case, attention is immediately focussed upon the liver. Calder, Steen and Baker (1939) suggested that liver disturbance was responsible for the changes in the red cells in the cases of brucellosis that they investigated. They made this suggestion purely from the red cell picture and had no direct evidence of abnormal liver function. Munger and Huddleson (1937) record the presence of the "liver failure" leucocytes of Isaacs in the blood in cases of brucellosis. In the present investigation it has been established beyond doubt that the liver function is defective in cases of chronic infection.

It seems reasonable therefore to suggest that this depression of liver function is the factor that is responsible for the macrocytic tendency seen in the cases of anaemia due to chronic infection. In addition, the data in table XXI and fig. 49 are to some extent suggestive of an inverse relationship between the cell size and the hippuric acid excretion test results, i.e. the lower the hippuric acid the higher the mean cell volume.

It is not clear however, how liver disease gives rise to anaemia at any time, especially

TABLE XXI.

The Effect of Deficient Liver Function on the Size of the Erythrocytes.

Case No.	Hippuric Acid Test gms. benzoic acid excreted	M.C.V. cu. μ.
8	3.9	93
2	3.3	83
14	3.3	86
15	3.3	70
12	2.8	92
22	2.8	111
9	2.7	114
16	2.7	80
5	2.5	78
1	2.3	121
11	1.9	83
3	1.9	90
13	1.6	90
4	1.5	133
6	1.2	125

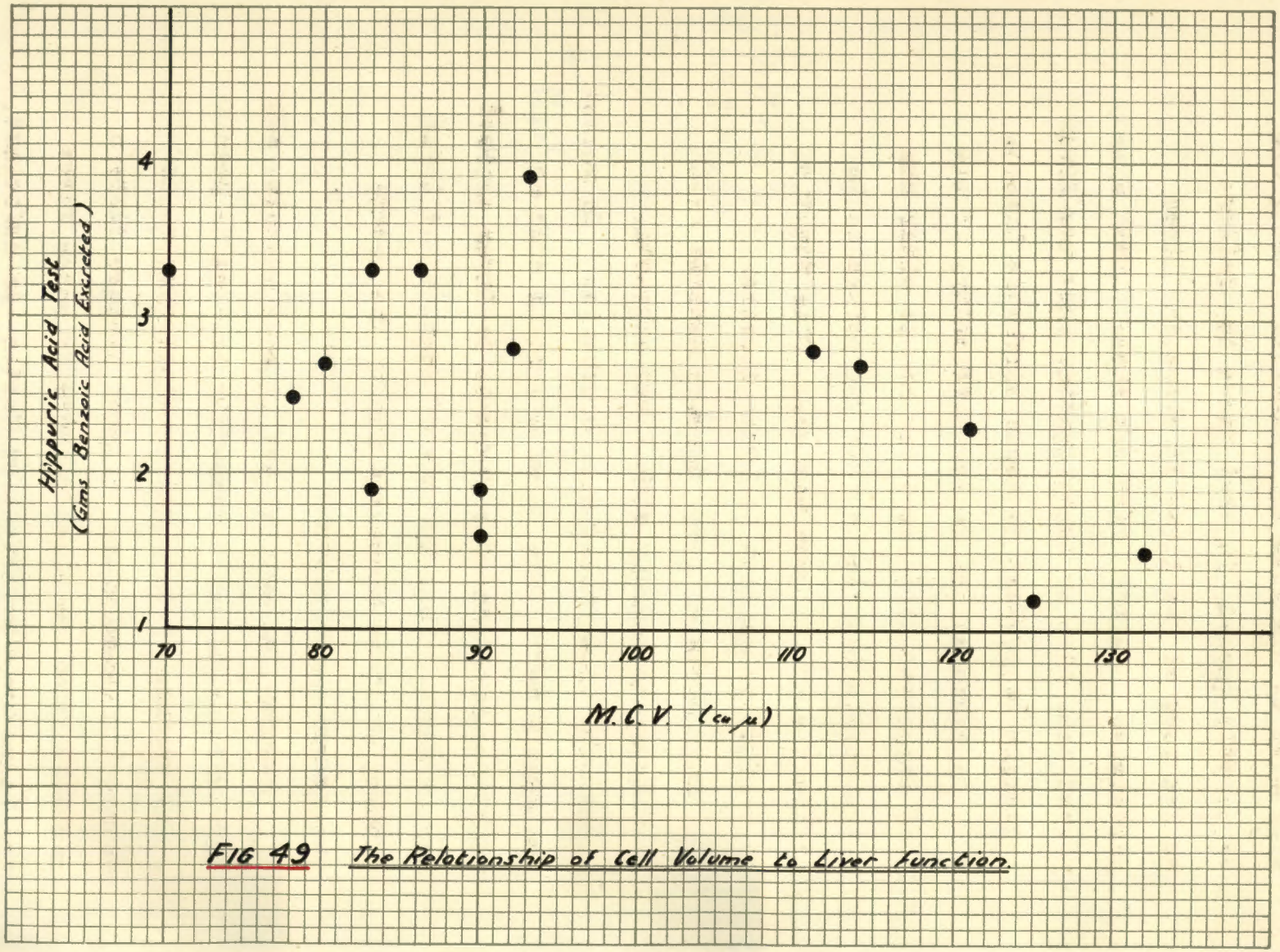


FIG 49 The Relationship of Cell Volume to Liver Function.

of the macrocytic variety. One explanation, suggested by Wintrobe and Shumacker (1933), and repeatedly quoted, is that there is failure on the part of the liver to store the liver principle. Schiff, Rich and Simon (1938) disproved this idea by demonstrating that the liver principle is present in human cirrhotic livers.

To overcome the latter difficulty, it has been suggested that the liver has to alter the haematinic principle in some way before it is utilisable by the bone marrow. This function is thought to be lost in liver disease.

Many cases of liver disease anaemia fail to respond to therapy with liver extracts, especially those cases with cirrhosis. In milder and easily reversible liver disorders such as infective hepatitis the response to liver extract may be good. This failure of response to liver therapy could be accounted for by the second theory mentioned above.

However, as pointed out by many authors, all macrocytosis is not necessarily related to pernicious anaemia. In fact the blood picture in macrocytic anaemia due to liver disease differs markedly from the blood picture in cases where the haematinic principle of Castle is deficient, in that there is uniformity of cell size which contrasts markedly with the gross anisocytosis

seen in pernicious anaemia. Liver disease anaemias also lack the poikilocytosis characteristic of pernicious anaemia.

This lack of identity between the anaemia of liver disease and that of cases in which Castle's factor is deficient has been stressed recently by Dameshek (1943b).

Wintrobe (1942) in his textbook also draws this important distinction, and classifies the anaemias of liver disease under the heading of simple chronic anaemias.

The association of a pernicious anaemia blood picture and bone marrow with cirrhosis of the liver is rare, and was found in only 2 cases out of a series of 386 by Ratnoff and Patek (1942).

A reviewer of Mogensen's book (1938) on the red cell diameters mentions that the author suggests that the changes in the blood in liver disease arise in the cells as they circulate.

There is very little doubt therefore that liver disease is capable of giving rise to a macrocytic anaemia, but the exact mechanism of how this arises is completely unknown. Liver disease may give rise to macrocytosis even in the absence of anaemia (Wintrobe and Shumacker, 1933).

c) Other deficiencies.

Other factors, deficiency of which are known to result in anaemia are thyroxin, the vitamins, and certain liver fractions which are not Castle's haematinic principle, which are extracted during the preparation of purified liver extracts. The only one that need be considered in detail is vitamin C. This has been shown to be defective in many cases of chronic infections (Parsons, 1938) and in some instances the disease (e.g. rheumatic fever) has actually been attributed to vitamin C deficiency. It does not appear possible to consider this factor any further as no data are available, but this aspect merits further study.

II) Toxic Dyshaemopoietic Anaemia.

Toxic factors produce anaemia by depressing or interfering with bone marrow function. The known causes of toxic dyshaemopoietic anaemia such as benzol derivatives have been excluded in the selection of the cases for study.

The study of the bone marrow permits the following conclusions:-

1) There is probably some degree of depression of the erythropoietic function of the bone marrow.

2) The reticulocyte counts suggest that there is a diminished release of red cells into the circulation. This is also suggested by the fact that the red cell count tends to become fixed or to drop lower in cases of chronic infection in the absence of haemolysis of the circulating cells. This reduction in the number of cells released into the circulation may be greater than is apparent - this is suggested on the fact that the plasma bilirubin is extremely low in the presence of defective liver function, this feature indicating possibly that the rate of red cell destruction is actually lower than normal, even allowing for the reduced total red cell mass.

The fact that the red cells are resistant may also indicate that they are able to survive longer in the peripheral blood, although a

close correlation between in vitro tests and what happens in vivo is not always seen e.g. sickle cell anaemia and Mediterranean disease cases show cells resistant to saline solutions and yet the cases show excessive haemolysis clinically.

3) In the discussion of the fragility test results, it was pointed out that the alterations in red cell shape did not accurately explain the changes in resistance to hypotonic saline. This suggested that some abnormality of the cell envelope exists. This abnormality of the cell envelope may arise during the formation of the red cells in the marrow.

From the preceding discussions the conclusion can be drawn that in infections the bone marrow is interfered with by the toxic process present. This results in depression of erythropoiesis and in inability of the bone marrow to utilise iron.

When iron deficiency exists the peripheral blood shows hypochromia. In such cases there may be reduction in the number of erythrocytes in the blood or the total red cell count may remain normal. It is not known what factors determine in a particular case whether less cells than normal are released from the bone marrow or whether cells are liberated in normal amounts.

Where less cells than normal reach the peripheral blood from the marrow, iron therapy not only corrects the hypochromia, but restores the red cell count to normal as well, so that the reduced liberation of red cells does appear to be connected in some way with iron deficiency.

In chronic infections there is diminished release of red cells into the peripheral blood. This may be due to the interference with iron metabolism, however this factor produces such an effect, or to the depression of erythropoiesis already discussed.

The interference with liver function produced by the infection also influences the red cell picture, possibly through some action on the bone marrow.

Certain of the features of the blood picture are not easily explained by the mechanisms discussed above. These are the macrocytosis, the resistance of the cells to hypotonic saline, the increased numbers of target cells in the peripheral blood, and the flatness of the erythrocytes. Unfortunately these features are not sufficiently well developed in the cases of chronic infection to be easily investigated by the methods that could be devised.

Cases of jaundice show many features identical with those seen in chronic infection (tables XXII, XXIII, and XXVI. It will be seen that macrocytosis is found with normoblastic marrow, liver and bile duct disease is present, there is marked resistance of the red cells to hypotonic saline and target cells are seen in great numbers. The problem of explaining the cell changes in jaundice is therefore very similar to the problem of explaining the cell changes in cases of chronic infection. Since jaundice cases show the changes to a more marked degree, it was therefore decided to investigate the problem in cases of jaundice. This study is recorded in the next section.

c) SUMMARY.

The following factors play a part in the production of the anaemia of chronic infections:-

1) Interference with haemoglobin metabolism. This results from inability on the part of the bone marrow to utilise iron. This factor accounts for the hypochromia present.

2) Some factor present tends to cause a macrocytosis. This factor may be the defective liver function demonstrated, although it is not clear how liver function deficiency causes macrocytosis.

3) Interaction of the above two factors accounts for some of the findings present in the red cells.

4) There is toxic interference with the bone marrow function, resulting in depression of erythropoiesis in deficient release of red cells into the circulation. Abnormalities in the erythrocyte envelope may also result from this factor.

5) The anaemia of chronic infection is therefore a toxic dimorphic dyshaemopoietic anaemia.

6) Five features of the anaemia are not fully explained by the data available at this point:-

a. Macrocytosis.

b. The anomaly of macrocytosis and normoblastic erythropoiesis.

c. Increased resistance of the red cells to hypotonic saline.

d. Target cells.

e. Flatness of the erythrocytes.

D) THE RED CELL CHANGES IN JAUNDICE.

1) Introduction.

It is well known in obstructive jaundice the red cells become larger than normal in diameter. The cell thickness does not increase correspondingly and may even become smaller, so that large diameter, flat cells result. Together with this, target cells appear in abnormal numbers in the peripheral blood and the red cells show abnormal resistance to hypotonic saline. The volume of the cells may or may not be increased.

It will be seen that these features resemble those of the anaemias of chronic infection that were not adequately explained in the previous section.

From tables XXII and XXIII below it will be seen also that the cause of the obstructive jaundice is not important in this connection. These changes occur in jaundice due to extrahepatic biliary tract obstruction, e.g. as occurs in carcinoma of the head of the pancreas, and also in toxic infective jaundice with an obstructive element as shown by the direct prompt positive v.d. Bergh reaction, and the clinical features of pale stools and dark urine.

TABLE XXII.

The Blood in Non-Haemolytic Jaundice.

Case	Duration of Jaundice	Hb. % Dare	R.B.Cs. m/cu.mm.	CI	VPC %	MCV cu.mm.	MCH γγ	MCHC %
Obstructive Jaundice								
96	2 weeks	63	3.63	0.95	30	83	28	34
97	3½ weeks	80	4.29	1.03	38	89	30	34
98	3 weeks	63	3.16	1.09	33	104	32	31
102	3 weeks	78	4.66	0.92	42.5	91	27	30
104	3 weeks	67	3.63	1.02	35.5	98	30	30
105	7 weeks	80	4.56	0.97	40	88	28	32
113	5 days	80	4.89	0.90	44	90	26	29
114	6 weeks	68	3.32	1.13	34	102	33	32
120	10 days	56	2.97	1.04	28.5	96	31	32
122	4 months	86	5.00	0.95	42	84	28	33
Toxic-Infective Jaundice								
51	2 weeks	89	4.34	1.13	46.5	107	33	30
65	12 days	56	2.56	1.21	28.5	111	35	32
95	3 weeks	40	---	---	20.5	---	31	--
99	3½ weeks	81	4.58	0.97	46	100	28	28
100	2 months	47	2.15	1.21	24.5	114	35	31
101	4 weeks	39	2.07	1.04	22	106	33	31
106	3 weeks	78	4.54	0.93	38.5	85	27	32
116	4 weeks	73	3.62	1.10	38.5	106	32	30
118	6 days	72	3.82	1.03	30	103	30	29
119	9 weeks	69	4.50	0.84	34	76	24	32
121	2 weeks	53	2.85	1.02	26	91	30	33
Obstructive Biliary Cirrhosis								
103	22 months	59	3.70	0.88	32.5	88	25	29
Stone in Common Duct with Cholangitis								
112	2 weeks	92	5.67	0.90	49	86	26	30

TABLE XXIII.

The Blood in Non-Haemolytic Jaundice.

Case	Target Cells %.	Reticulocytes %.	MCF	Direct v.d.Bergh	Plasma Bilirubin
Obstructive Jaundice					
96	22.6	0.7	0.274	p +	7.6
97	0.6	0.9	0.300	p +++	26.7
98	8.7	1.0	0.240	p +++	26.2
102	11.5	0.1	0.248	p +	14.2
104	9.2	0.4	0.272	p +++	15.8
105	0.0	---	0.340	p +++	17.5
113	0.0	0.2	0.384	f.p.	2.9
114	1.6	0.1	0.290	p +++	23.7
120	---	---	0.265	f.p.	1.5
122	0.0	0.2	0.348	f.p.	2.0
Toxic-Infective Jaundice					
51	9.8	---	F.T.R. 1	p	25.0
65	0.0	---	0.380	p ++	17.9
95	3.5	---	0.346	p	4.1
99	9.0	0.4	0.300	p	12.5
100	---	1.8	0.310	p +	5.6
101	---	---	0.324	p +	5.8
106	3.4		0.325	f.p	7.3
116	6.9	1.3	0.300	p +++++	35.0
118	5.8	---	0.270	p ++	12.3
119	---	---	0.400	p	2.0
121	0.0	---	0.300	p +++	26.7
Obstructive Biliary Cirrhosis					
103	5.4	1.0	0.330	-	---
Stone in Common Duct with Cholangitis					
112	0.0	0.8	0.367	p +	2.6

There are two chief ways in which these changes in the red cells could arise.

1) The cells delivered from the bone marrow may be abnormal in the features described above.

2) It is possible that normal cells are delivered from the bone marrow and that these become altered during their life in the peripheral circulation.

The purpose of this portion of the investigation was to determine which of these two possibilities is the correct one, and to attempt to reach a more detailed explanation of the alterations observed in the red cells.

2) The Site at which the Changes occur.

Information derived from four types of study lead to the conclusion that the changes in the red cells in obstructive jaundice are the result of alterations occurring in the red cells as they circulate in the peripheral blood.

a) The rate at which the changes occur in the cells.

Twenty three cases of non-haemolytic jaundice were studied. In these cases the following investigations were carried out:- haemoglobin estimations, total red cell counts, haematocrit determinations, hypotonic saline fragility tests, v.d. Bergh reactions and plasma bilirubin estimations. Price-Jones curves, reticulocyte counts and target cell counts were performed in some of the cases. In addition a careful clinical history was recorded to determine the duration of the jaundice. The results are recorded in tables XXII, XXIII, together with the diagnosis in each case. The Price-Jones curves of three cases are shown in figs. 50, 51 and 52.

The most important features shown by these cases as far as the present problem is concerned is that the changes in the red cells are present in very marked degree as early as 10 days (case 120) and 2 weeks (cases 51 and 96) after the onset

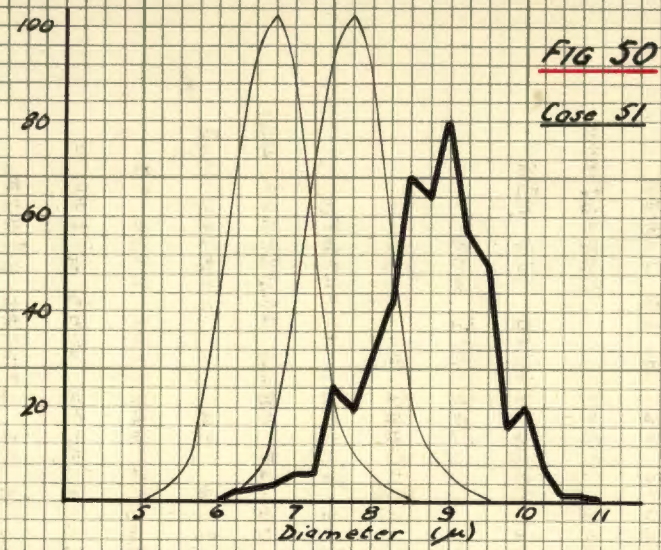


FIG 50

Case 51

Case	51	99	98
M.C.D (μ)	8·703	8·509	8·966
σ (μ)	0·759	0·616	0·690
v (%)	8·7	7·2	7·7
M.C.A.T. (μ)	1·800	1·758	1·647
D : T Ratio	4·8	4·8	5·4
Macrocytosis (%)	64·4	59·2	73·2

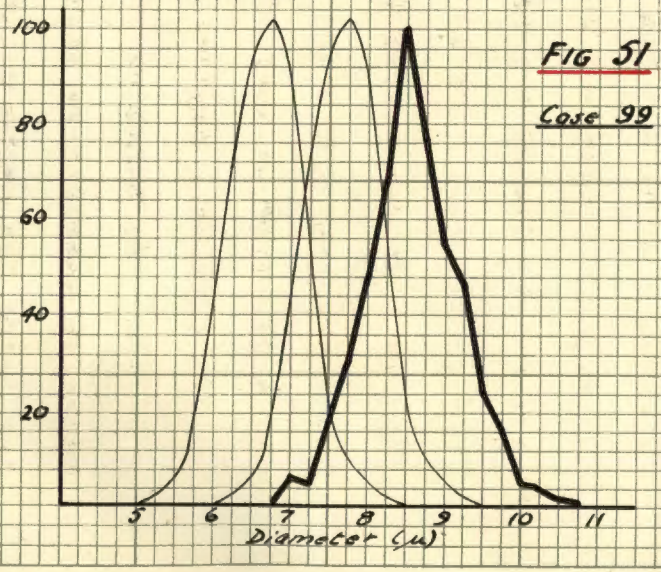


FIG 51

Case 99

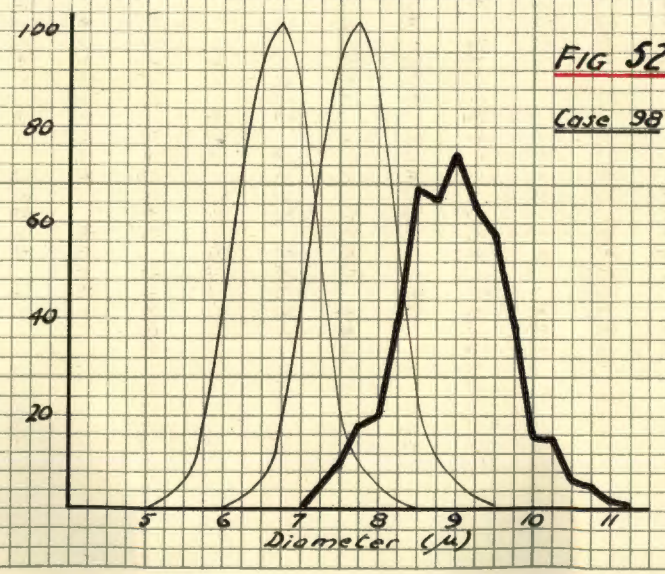


FIG 52

Case 98

of the jaundice. Furthermore, a study of the Price-Jones curves show that there is only a slight degree of anisocytosis. Sigma is only slightly raised and the coefficient of variation (v) is only slightly above normal. The height and shape of the curves approximates that of the normal curves closely. It appears therefore that the whole curve is shifted bodily to the right from the normal position. 59.2, 64.4 & 73.2% of the cells are macrocytic in the three cases respectively.

It seems impossible that the entire cell population could be replaced in 10-14 days by new large diameter flat cells. The normal survival time of the red blood cells is about 100 days (Mollison and Young, 1942). Therefore in order to change the cell population the old generation would have to be killed off and regeneration would have to proceed at 7-10 times the normal rate. The blood, however, shows evidence neither of rapid cell destruction, nor of rapid cell regeneration (reticulocytes 0.1 - 1.8%). In addition pigment studies done in cases of obstructive jaundice show that after a few weeks the serum bilirubin no longer rises. Ottenberg (1943). Part of the explanation of this may be diminished production of bilirubin due to decreased breakdown of red blood cells.

The resistance of the corpuscles to hypertonic saline & lysolecithin (Singer, 1940) haemolysis may be evidence supporting this suggestion.

b) The reversibility of the changes.

Case 99 was studied at intervals during the course of her illness. The results are shown in table XXIV and figs. 51, 53 and 54.

It will be seen that the blood returned towards normal during the 10 days in which cell diameters were measured. This change took place gradually, the whole Price-Jones curve retaining its shape and moving bodily to the left. The fragility test curve moved steadily to the right.

The same arguments as those brought forward in connection with the rapid development of the blood changes apply here too.

TABLE XXIV.

Case 99.

Date	Hb.	R.B.Cs.	CI	VPC	MCV	MCH	MCHC	Retics.	MCF	v.d. Bergh	Plasma Bilirubin.
21.11.43	81	4.58	0.97	46	100	28	28	0.4	0.300	⊕p	12.5
23.11.43	83	4.56	1.00	45.5	100	29	29	0.2	0.313	p ++	5.4
24.11.43	84	4.92	0.93	45	93	27	30	0.0	0.335	p +	8.0
26.11.43	78	4.04	1.06	41	101	31	30	0.6	0.334	p	7.3
30.11.43	79	4.24	1.03	39.5	93	30	32	0.6	0.344	⊖f.p.	4.9
7.12.43	77	4.15	1.02	39.5	95	30	31	1.3	0.345	f.p.	1.9

⊕p = positive.
⊖f.p. = feeble positive.

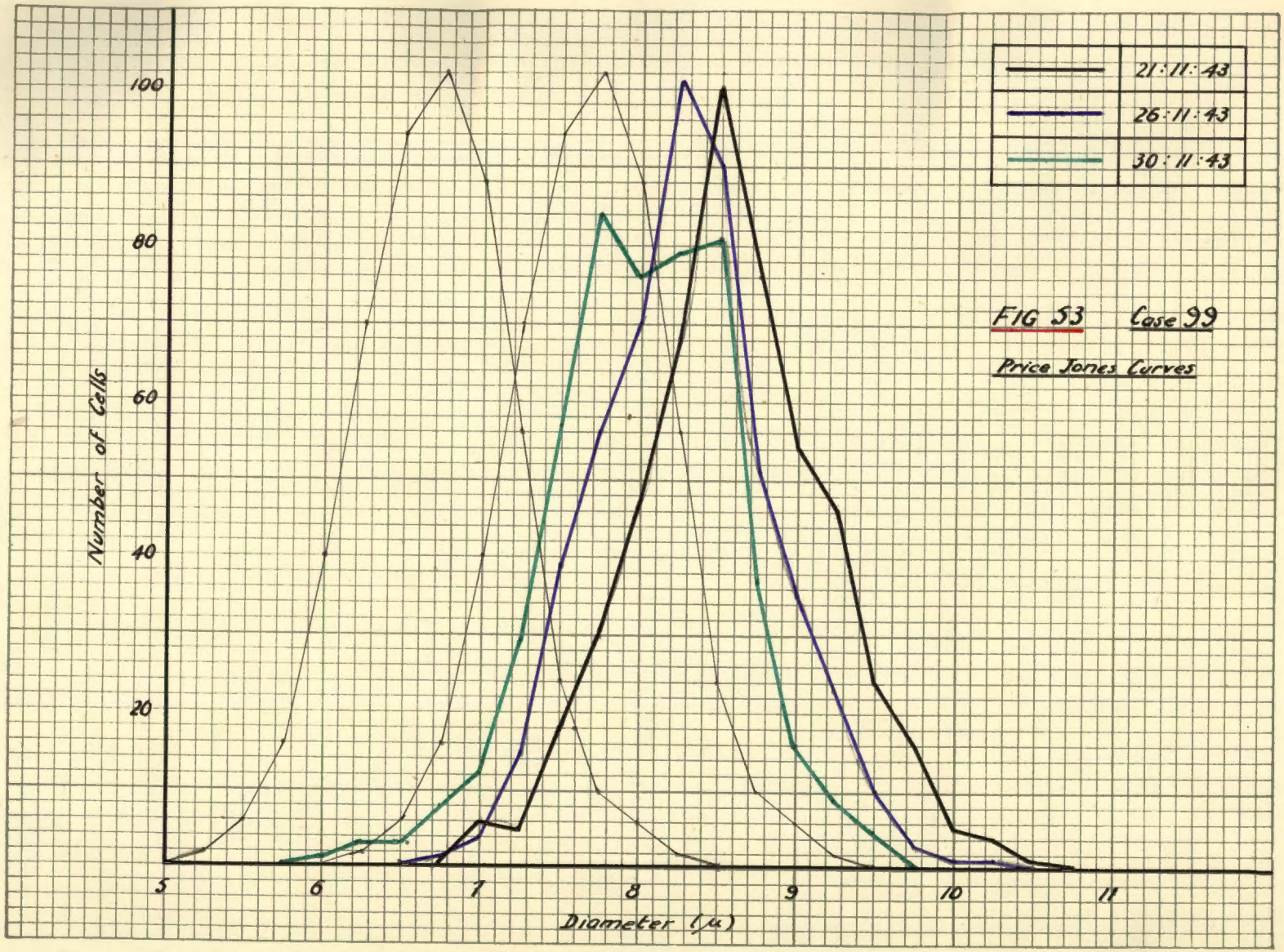


FIG 53 Case 99

Price Jones Curves

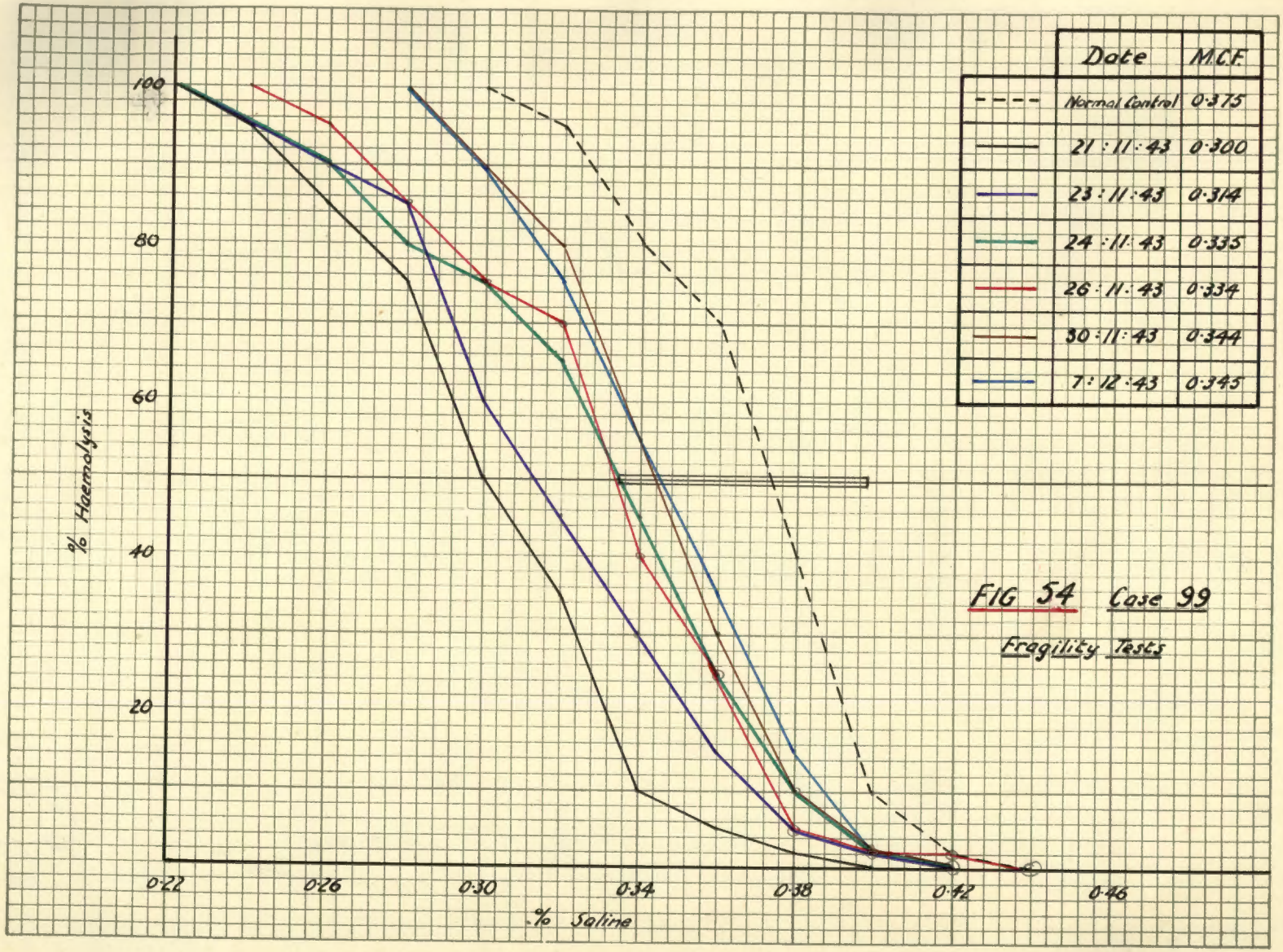
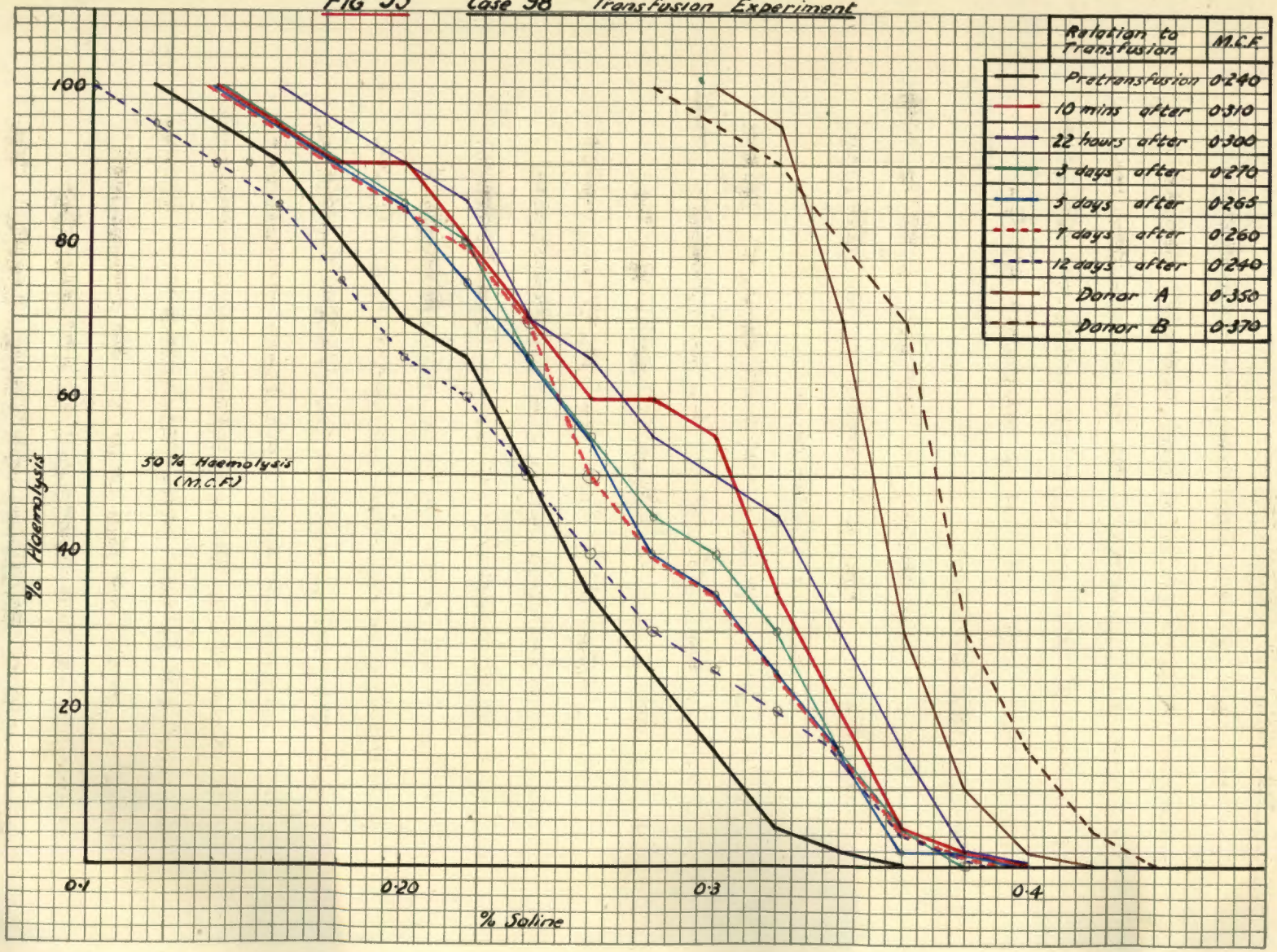


FIG 54 Case 99

Fragility Tests

FIG 55 Case 98 Transfusion Experiment



g) Transfusion of normal red cells into a case of obstructive jaundice.

Patient 98 was selected for this study, because her red cells showed the most marked resistance to hypotonic saline in the series. The cause of the jaundice was shown at operation and later at autopsy to be carcinoma of the head of the pancreas.

After a preliminary investigation of the red cells, she was given a transfusion of 1200 cc. of blood from 2 normal donors.

The investigations were repeated at intervals for 14 days following the transfusion. The results are shown in table XXV and fig. 55.

The graphs show that the transfused cells produce a considerable hump on the fragility curve done immediately after the transfusion. After this the curve rapidly returns towards its pretransfusion shape and position. These changes are reflected also in the MCF readings. It will be noticed also that these changes run their cycle while the transfused cells remain in full numbers in the recipient's blood. It was intended to follow the fate of the transfused corpuscles by the technique of differential agglutination (Mollison and Young, 1940). Unfortunately the patient belonged to Group IV and no M and N or Rh sera were available, so that

TABLE XXV.

Case 98.

Date	Hb. % Dare	R.B.Cs. m/cu.mm.	CI	VPC %	MCV cu.mu.	MCH	MCHC %	MCF % Saline	v.d.Bergh Direct	Plasma Bilirubin
22.11.43	63	3.16	1.09	33	104	32	31	0.240	^p +++	26.2
		Transfusion given 25.11.43. 1,200 ccs. in 5 hours.								
*25.11.43	73	4.02	1.00	38	95	29	31	0.300		
26.11.43	78	4.09	1.05	42.5	104	31	29	0.300	p +++	19.6
28.11.43	79	4.00	1.09	42	105	31	30	0.270		
30.11.43	78	3.88	1.11	41	106	32	30	0.265	p +++	23.8
2.12.43	78	4.18	1.03	42	100	30	30	0.260	p +++	26.0
7.12.43	75	3.50	1.17	40	114	34	30	0.240		
14.12.43	70	3.35	1.15	38	113	33	30	0.250	p +++	27.4
Donor A	83	4.33	1.04	41.5	96	31	32	0.350		
Donor B	84	4.79	0.96	41	86	28	33	0.370		

*Specimen taken 10 minutes after conclusion of transfusion.
^p = positive.

this part of the investigation had to be dispensed with. However, from the haemoglobin estimations, the red cell counts and the haematocrit readings it appears that the transfused cells survived with very little loss for at least a week, during which time the fragility curve more or less returned to the pre-transfusion position.

This study shows that normal cells transfused into a jaundiced patient become so changed that after a little more than a week their presence as normal cells in an abnormal blood is not recognisable.

d) The Bone Marrow in Jaundice.

The bone marrow was studied by sternal puncture in 5 cases. The counts are shown in table XXVI.

The results indicate that the marrow is not hyperplastic, as would be expected if the cell population was being replaced rapidly. Furthermore the marrow shows normoblastic erythropoiesis. An attempt made to measure the diameters of the primitive cells failed, but by inspection of the slides it did not appear that the erythroblasts were larger than normal.

These studies also suggest that normal cells are delivered by the bone marrow to the peripheral blood.

The association of normoblastic marrow with macrocytic red cells is again worthy of notice.

Conclusion.

From the evidence brought forward there seems to be little doubt that the changes found in the red blood cells in jaundice result from alterations produced in the erythrocytes as they circulate in the peripheral blood.

TABLE XXVI.

The Bone Marrow in Jaundice.

Case	Erythroblasts % of total nucleated cells	Type I % of erythroblasts	Type II	Type III	Type IV	MCV cu. mu.	MCF % Saline
98	20.0	2	7	36	55	104	0.240
99	18.2	1	8	30	61	100	0.300
100	10.7	0	2	15	83	114	0.310
101	6.0	1	7	26	66	106	0.324
121	11.9	6	15	51	37	91	0.300

The four investigations described above indicate that it is impossible that the abnormal red cells in jaundice are the result of the delivery of abnormal red cells from the bone marrow. The cells already present in the peripheral blood at the onset of the jaundice are changed to large diameter flat cells, which show increased resistance to hypotonic saline, and many of which are target cells. Any new cells delivered from the bone marrow may or may not be abnormal. Studies of the bone marrow indicate that the cells delivered are probably normal. Furthermore, the results of the transfusion experiment indicate that any cells put into the blood stream of a jaundiced patient develop the same changes as do the red cells present in the blood of the patient at the time the jaundice starts. This latter argument is a very powerful one in favour of the theory put forward here.

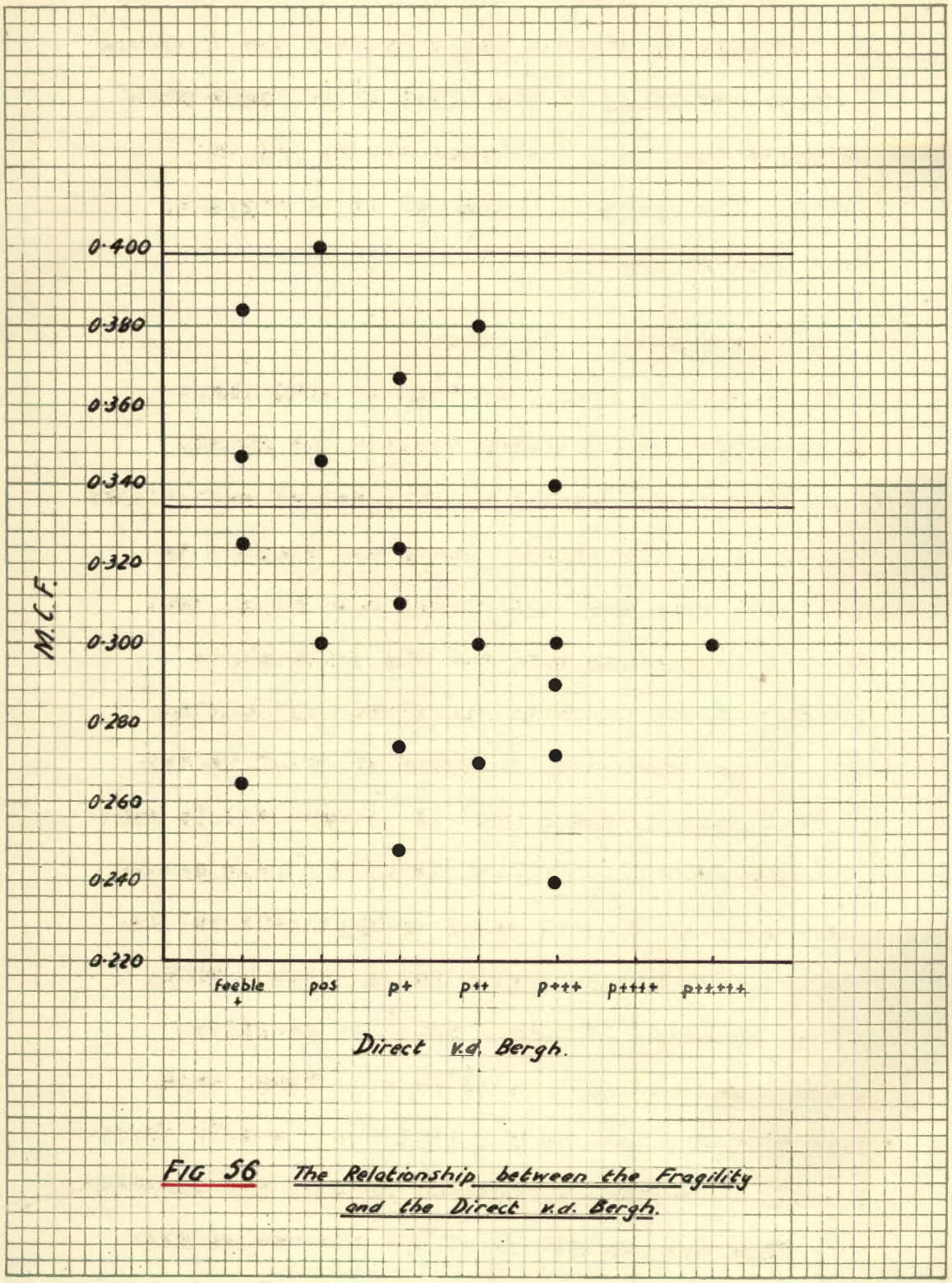


FIG 56 The Relationship between the Fragility and the Direct v.d. Bergh.

3) The Role of the Chemical Changes.

The first suggestion that arises to explain the changes in the red cells is that the chemical changes occurring in the blood of patients with jaundice produce the alterations in the red cells. The evidence presented below suggests that this is so.

a) The Direct v. d. Bergh.

It will be seen from fig. 56 that there is no direct relationship between the direct v.d. Bergh test results and the median corpuscular fragility of the cells. Resistant corpuscles are found even when the direct v.d. Bergh test is only feebly positive and the obstructive element of the jaundice therefore negligible.

It is true that the cases of jaundice due to obstruction, where the v.d. Bergh test is more strongly positive, do tend to show a more marked resistance of the red cells to hypotonic saline, the MCF readings generally being below 0.300%, while the cases of toxic infective jaundice with a less strongly positive v.d. Bergh show less markedly reduced MCF readings, usually above 0.300%. However, exceptions to this are numerous.

Thus the resistance of the corpuscles does not bear an accurate relationship to the extent of the obstructive element of the jaundice and

therefore cannot be dependent on this factor.

b) The Individual Chemical Substances.

Since the results in the previous section by no means rule out the possibility that chemical changes in the blood are responsible for the changes in the red cells, it was decided to consider each abnormal chemical constituent of the jaundiced blood separately to see if any of these could be responsible for the erythrocyte changes.

The chemical substances that require consideration are 3 in number - bile salts, cholesterol, and bilirubin.

(i) Bile Salts.

It has been shown (Harrison, 1943; Ottenberg, 1943) that bile salts remain elevated in the blood in obstructive jaundice for relatively short periods of time, returning to normal levels in about 2-3 weeks. By contrast the changes in the red cells continue for many weeks, months and even for almost 2 years (22 months in case 103). It is not likely therefore that the bile salts could be responsible for the changes in the red cells. Furthermore, because of the well-known property of the bile salts of reducing surface tension, it might be expected that the bile salts would cause abnormality of the erythrocytes in the direction of fragility rather than resistance.

Bile salts have in fact been used as haemolytic agents in experiments by Ponder (1943) and Mer, Birnbaum and Kligler (1943).

(ii) Cholesterol.

Studies were made of 5 cases in which the blood cholesterol was raised. Table XXVII shows the results.

TABLE XXVII.

Fragility of the Red Cells in Hypercholesterolaemia.

Case	Diagnosis	Plasma Cholesterol %	M.C.F. % Saline	Span of Haemolysis % Saline
53	Myxoedema	270	0.388	.44-.34
54	Xanthomatosis	700	0.357	.42-.28
75	Myxoedema	360	0.433	.48-.38
81	Xanthomatosis	355	0.357	.42-.30
117	Xanthomatosis	680	0.364	.40-.30

It will be seen that raised cholesterol in the blood is not associated with abnormal resistance of the red blood cells. The MCF is within normal limits in 4 of the cases studied and is raised in the fifth case. Cases 54 and 117 are particularly important in that there is an isolated abnormality, the raised cholesterol, in the blood; the cholesterol is at a very high level and yet the MCF is normal.

It has been shown that cholesterol is an anti-haemolytic agent (Lee and Tsai, 1943) and it has been suggested that the abnormally low blood cholesterol found in acholuric family jaundice is the factor responsible for the excessive haemolysis (Muller, 1930). However, most cases of anaemia of all varieties show abnormally low cholesterol levels (Muller, 1930; Nayar, 1942; Member, Bruger and Chassim, 1940). Most of these anaemias are not of haemolytic type and are associated with increased resistance of the red cells to hypotonic saline (Cassells, 1938).

The antihaemolytic action of cholesterol demonstrated in the work quoted above was in connection with haemolytic systems of the antigen-antibody type and were not associated with changes in cell shape or resistance in hypotonic saline.

Furthermore, it has been shown experimentally that increased fat ingestion actually leads to increased red cell destruction (Loewy, Freeman, Marchelle and Johnson, 1943).

The few cases with resistant blood cells studied in the present investigation also showed normal or abnormally low blood cholesterol levels. (Table XXVIII).

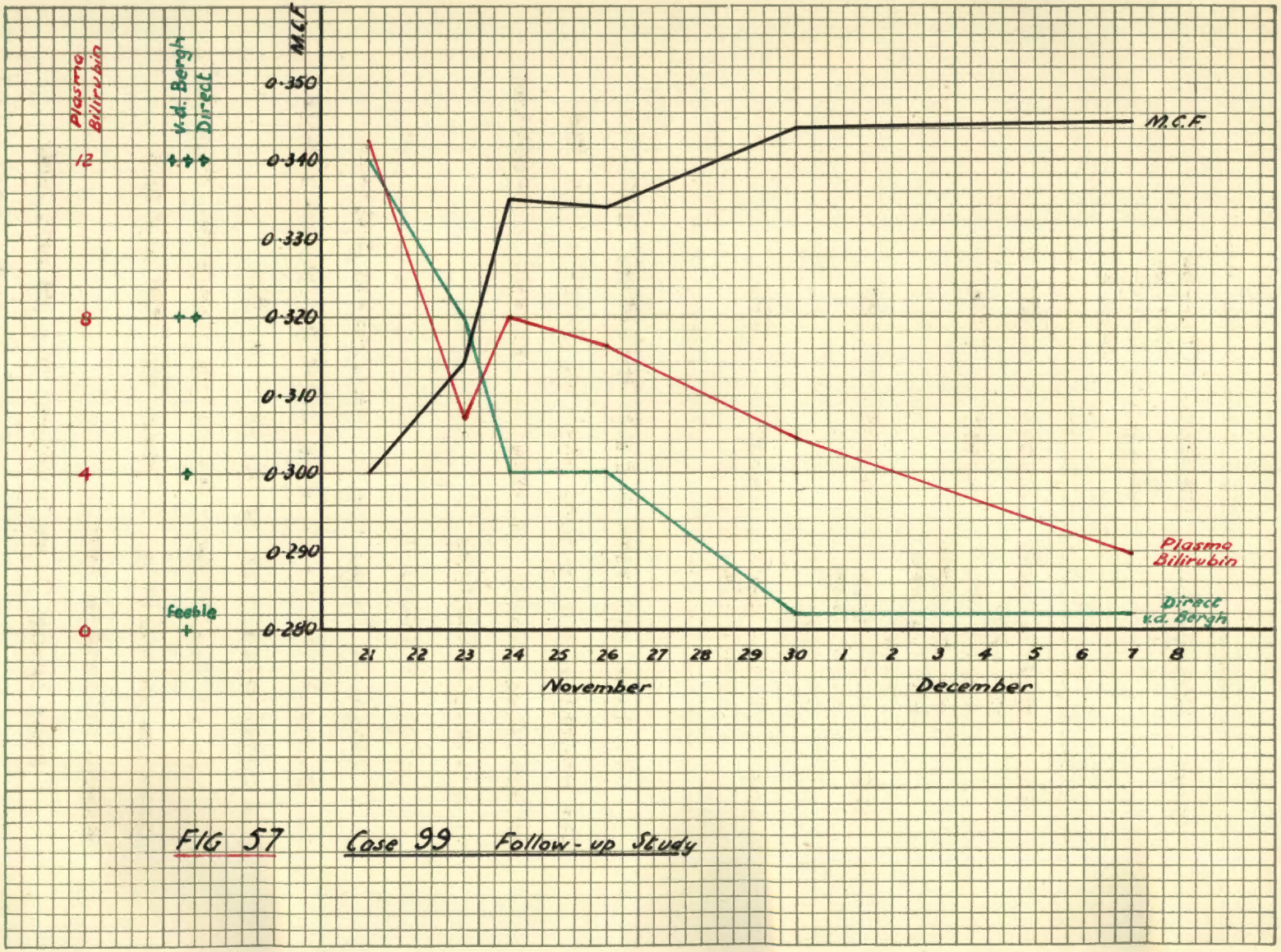


FIG 57 Case 99 Follow-up Study

TABLE XXVIII.

Plasma Cholesterol in Resistent Bloods.

Case	Diagnosis	M.C.F. % Saline	Plasma Cholesterol mg. %
55	Sickle cell anaemia	0.287	94
98	Carcinoma of the head of the pancreas	0.240	202
116	Alcoholic cirrhosis of the liver	0.300	150
120	Acute pancreatitis	0.265	104

It does not seem likely therefore that the raised blood cholesterol of jaundice is the determining factor in causing red cell resistance to hypotonic saline.

(iii) Bilirubin.

There is no accurate correlation between the MCF and the level of bilirubin in the blood. The relationship is shown in fig. 57.

In an individual case also (Case 99, Fig. 58), there is no parallel between the MCF and the plasma bilirubin level.

Some cases (e.g. numbers 51, 99 and 106) show practically only indirect reacting bilirubin

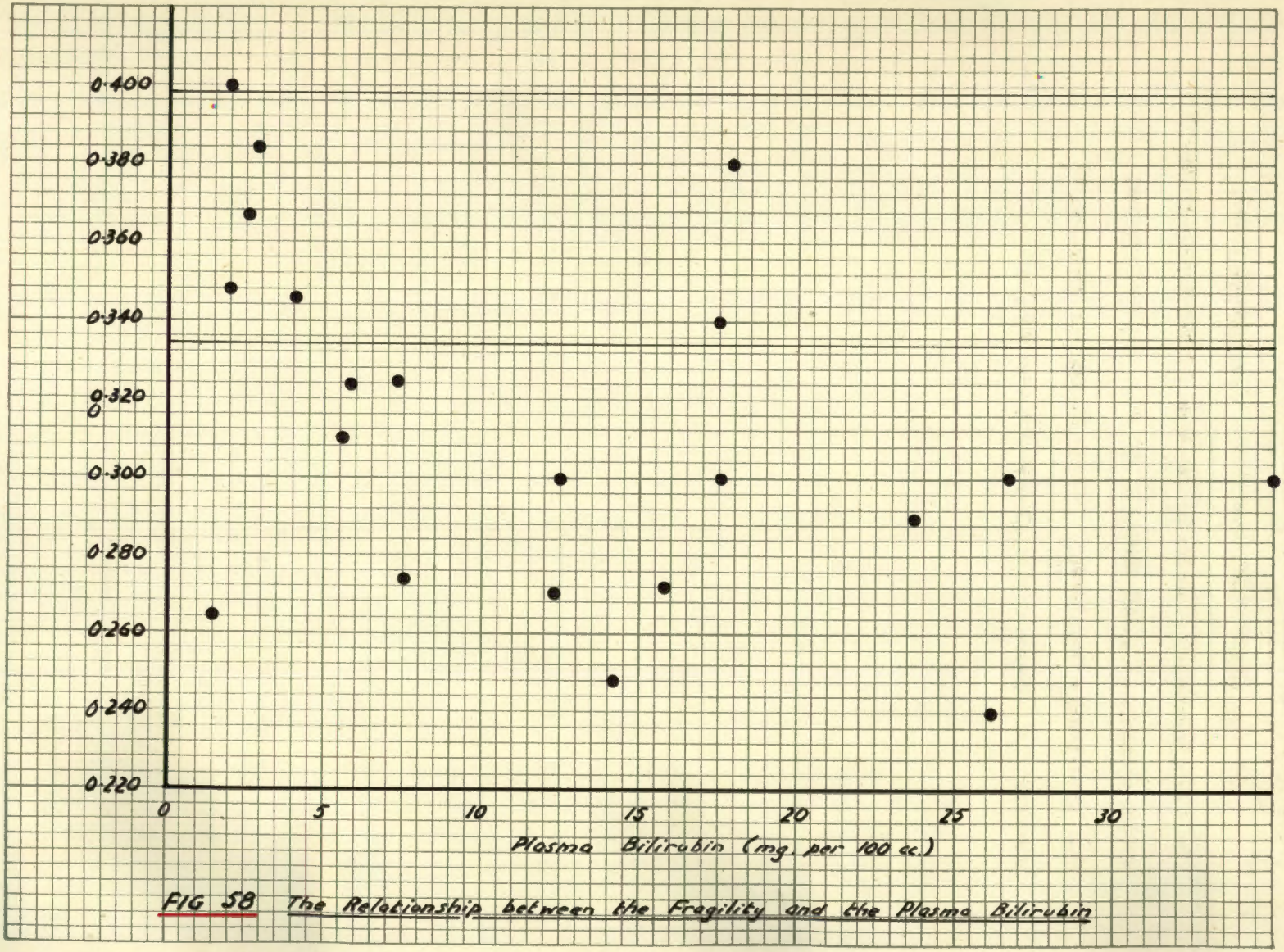


FIG 58 The Relationship between the Fragility and the Plasma Bilirubin

and are therefore chemically like haemolytic anaemias, and yet show abnormally low MCF readings.

It is also obvious from the graph that raised plasma bilirubin is not always associated with abnormal resistance of the erythrocytes to hypotonic saline. This finding occurs of course in haemolytic jaundice as well.

c) In Vitro Experiments.

In order to test the effect of the chemical changes in the blood in jaundice on the red cell fragility directly the following experiment was carried out.

Blood was drawn from a case of jaundice and also from a normal person of the same blood group. The blood was centrifuged lightly and the plasma collected from each specimen separately. The red cells were washed with normal saline until they were free from plasma. The following mixtures of red cells and plasma were then made. The proportions of cells to plasma was equal in each instance, approximately equal volumes of each being used.

i) Patient's cells + patient's plasma.
ii) Normal cells + normal plasma.
iii) Patient's cells + normal plasma
iv) Normal cells + patient's plasma.

These specimens were thoroughly mixed by inversion and then were placed in an incubator at 37°C for 1 hour and then fragility tests were performed on them.

Two cases were studied in this way, cases 98 and 99, and the results are shown graphically in figs. 59 and 60.

It can be seen that the results obtained depend entirely upon the properties of the red

FIG 59 Case 99

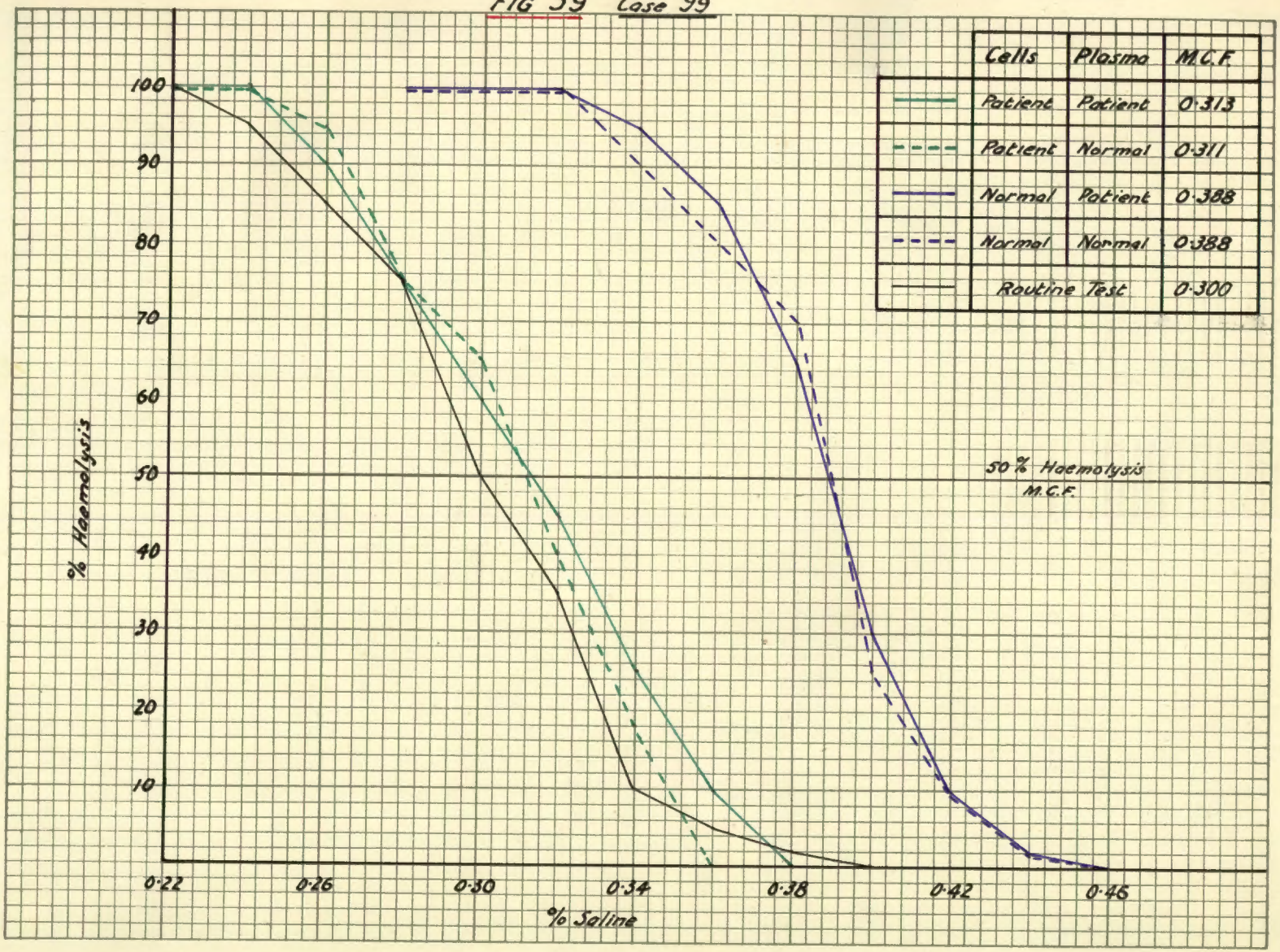
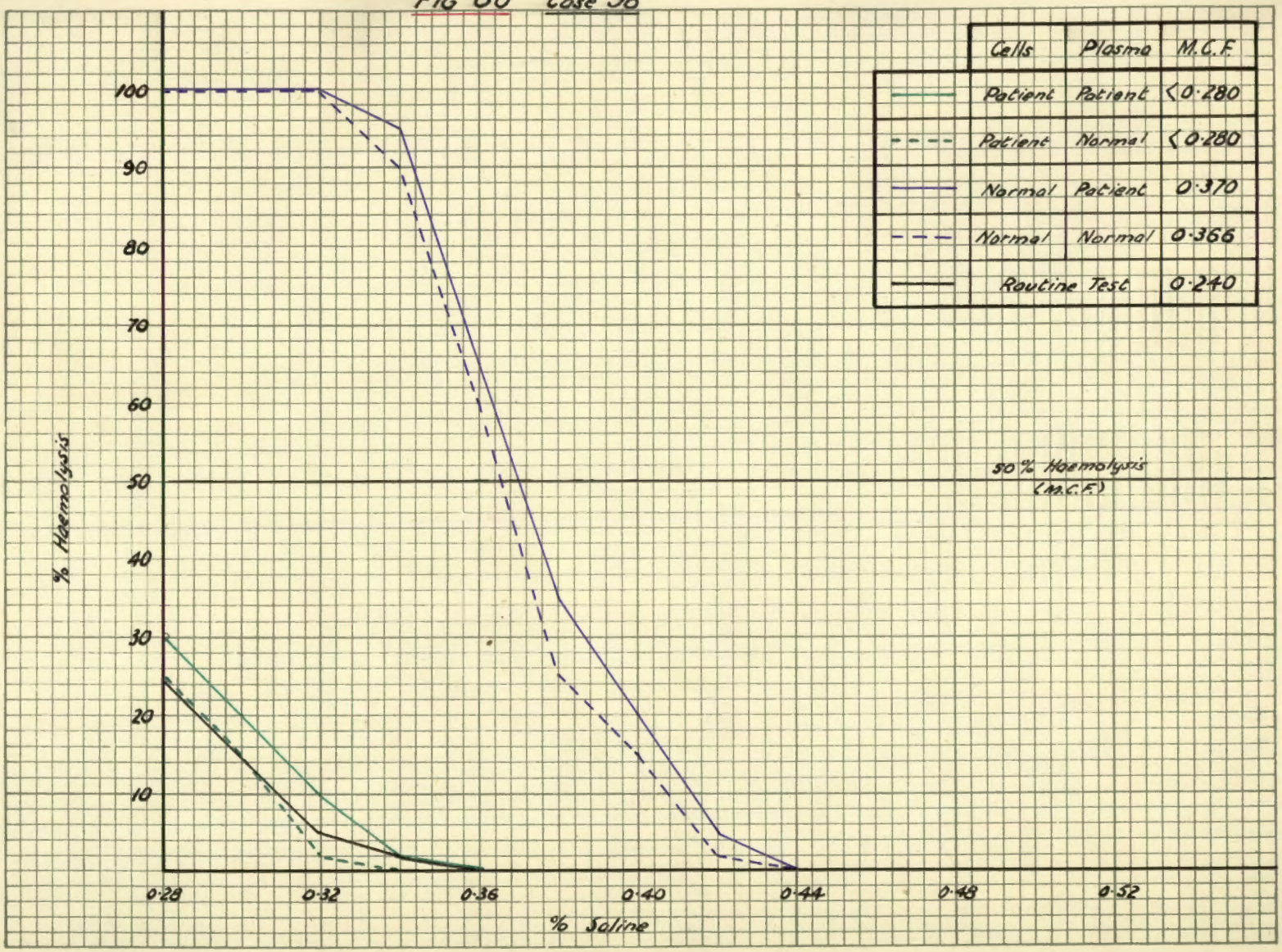


FIG 60 Case 98



cells, since the curve of haemolysis is identical for the same cells irrespective of the plasma in which they are suspended.

There are two interpretations of these results. It is possible that chemical changes in jaundiced plasma do not alter the properties of the red blood cells and the abnormalities that occur in jaundiced blood are not reversed by putting the cells into normal plasma. This interpretation would support the conclusions reached above. The second possibility is that the duration of contact between cells and plasma may not have been long enough to allow changes in the cells to occur. This time unfortunately could not be prolonged, since in order to obtain accurate results in fragility tests, it is necessary to do the test within 4 hours of drawing the blood (Dacie and Vaughan, 1938). The time consumed by the preparation of the specimens did not allow of longer contact between cells and plasma before performing the fragility tests.

An attempt was made to overcome this difficulty by returning the cell-plasma mixtures to the incubator at 37°C overnight. The following morning the degree of haemolysis was observed. There was haemolysis in all specimens, but there was very much more haemolysis in the specimens containing the normal cells.

The specimens containing the patient's cells showed only slight haemolysis. There was very little difference between the two specimens containing normal cells. Likewise the two specimens containing patient's cells showed comparable degrees of haemolysis. Thus here also the results depended entirely on the properties of the cells and were independent of which plasma the cells were suspended in.

These experiments, while not entirely satisfactory, do tend to support the conclusion that the changes in the red cells are not due to the chemical changes that occur in the blood in jaundice.

The results of the transfusion experiement also tend to support the conclusion arrived at above. It will be seen that 5-7 days elapsed before the transfused cells took on the properties of the patient's own cells. It seems reasonable to have expected that if the changes were due to altered blood chemistry, the change in the transfused cells would have occurred more rapidly, as occur in the case of haemolytic agents added to the blood stream (Dameshek and Schwartz, 1940) and in in vivo tests (Dameshek and Miller, 1943), and in the experiments on disc-sphere transformations in the red cells due to alterations in pH and albumin (Furchgott, 1940;

Furchgott and Ponder, 1940).

Conclusion.

The changes in the red blood cells in jaundice are not brought about by the abnormal chemical substances in the blood.

4) The Role of the Spleen.

In considering mechanisms that cause the phenomena under discussion, namely thin corpuscles, with abnormal resistance to hypotonic saline and the appearance of target cells in blood smears, attention is immediately focussed on the spleen.

Singer, Miller and Dameshek (1941) have summarised the changes in the blood following splenectomy. The changes in the red cells may be described under three headings:-

i) Howell-Jolly bodies and normoblasts appear in the peripheral blood.

ii) Target cells are seen in smears, the red cells show abnormal resistance to hypotonic saline, and there is diminished haemolysis.

iii) In addition less lysolecithin is demonstrable in the blood and incubation of the blood fails to increase the lysolecithin content.

i) The Howell-Jolly Bodies.

Certain of the effects of splenectomy on the erythrocytes are attributable to a "hormonal" action of the spleen, whereby it exerts an influence on the maturation of the red cells in the bone marrow. Loss of the hormonal function results in abnormal nuclear extrusion in the eryhhroblasts with the result that Howell-Jolly bodies appear in the blood and may persist for as long as 28 years

(Pepper and Austin, 1943). This idea of the hormonal action of the spleen on the bone marrow is a reasonable one, since the effects are produced at a distance from the spleen and must therefore be mediated by some chemical substance.

The concept of a hormonal action by the spleen on the bone marrow is further supported by the leucocytosis and thrombocytosis that occur after splenectomy.

That the spleen is capable of producing chemical substances that are carried in the blood stream to exert their effects on distant parts seem certain from the features of thrombocytopoenic purpura stressed by MacFarlane (1941). He pointed out that mere tying of the splenic vein at operation for thrombocytopoenic purpura results in immediate dramatic cessation of oozing of blood from the operation wound. Some success has also attended the extraction of the chemical substance responsible for the effect on the capillaries and platelets, although other attempts have failed (Rose and Boyer, 1941).

ii) Target Cells, Resistance to Saline Solutions and Diminished Haemolysis.

The appearance of target cells and the development of abnormal resistance of the red cells to

hypotonic saline could be due to abnormality in the development of the erythrocytes following splenectomy from loss of the hormonal function. From the data on the blood of jaundice cases quoted above, it seems that this is an unlikely suggestion.

A more probable explanation is that these changes occur in circulating cells as a direct result of the absence of the spleen. Normally the spleen alters the characters of the red cells circulating through it, so that the cells in the splenic venous blood are more spherocytic and show greater fragility in hypotonic saline than do cells in the splenic arterial blood (Lauda, 1933; Bergenhem and Fahreus, 1936; Heilmeyer, 1936b). When the spleen has been removed, it is reasonable to conclude that this action of the spleen is lost, and that the cells fail to become more spherocytic and fragile.

There are two schools of thought concerning the circulation within the spleen. The older theory, an account of which is given by Best and Taylor (1943), is that the splenic circulation may take one of two forms. Under conditions of stress, when the spleen is contracted, the circulation is a "closed" one, and blood passes directly from the arterial capillaries to the venous sinuses and thence to

the venous capillaries and so to the splenic vein. When the spleen is relaxed, the circulation is an "open" one. The red cells escape from the capillaries into the pulp, where they are held for some time, and it is here that the changes described above occur.

The second, more recent view was advanced by Knisely (1936a and b) and is more generally accepted (Fahreus, 1939). Studying the splenic circulation in living animals, he found that the splenic circulation is always a "closed" one. The arterial capillaries end in the venous sinuses and these in turn continue into the venous capillaries. He found that there are sphincters at either end of the venous sinus. There is a cyclic alteration in the state of the splenic circulation through this closed system. The cycle commences with closing of the sphincter at the venous side of the sinus. This results in damming up of the red cells, while the blood fluid passes out of the sinus into the pulp. This process continues until the sinus is distended with red cells and then the sphincter at the arterial end closes. The sinus may remain filled in this way for anything from a few minutes up to 10 hours.

The sinus wall does not allow red cells to pass out into the pulp. Knisely (1936b) showed that the appearance of cells in the pulp is an agonal phenomenon, occurring only immediately prior to death and never in the living healthy spleen.

Fahreus (1939) has suggested that it is while the erythrocytes are held up in the spleen in this way that they undergo the change described above, and speaks of the separator-reservoir function of the spleen.

Fahreus (1939) has shown that when blood is incubated in vitro the cells become spherocytic, and also show decreased rouleaux formation and increased suspension stability. This change is due in part to changes occurring in the cells primarily, and in part to changes in the plasma which affect the red cells secondarily. The change in the plasma consists of the formation of lysolecithin from lipoids by enzymatic action. This process is normally inhibited in circulating blood because the red cells and plasma are in free contact with each other. The red cells prevent the changes from occurring in the plasma and the plasma inhibits the alterations in the red cells.

When the red cells and plasma are separated, they undergo the changes described. When they

come together again haemolysis may occur. The conditions in the spleen are normally correct for this train of events, since the cells are separated off in the sinuses, while the plasma is probably kept separate in the pulp. When the two come together again in the splenic veins, some degree of haemolysis ensues, and the cells can be shown to be spherocytic and fragile.

After splenectomy therefore, the cells fail to undergo the changes described and haemolysis is diminished.

It is necessary to consider now why the cells actually become flattened after splenectomy. It is not clear why absence of the separator function of the spleen should not result merely in cells that have the characters of the cells entering the spleen normally in the splenic artery.

Several facts suggest that some mechanism exists in the body normally for flattening the red blood cells. These are as follows:-

a) One striking evidence is the evidence mentioned above that splenectomy results in flat cells, and not normal cells.

b) This suggests that the spleen constantly has to act on the red cells and change them in the

direction of a more spherocytic form in order to maintain the cells in their "normal" shape.

c) Conversely, the fact that the spleen is constantly changing the erythrocytes in this direction suggests that some mechanism must be tending constantly to change them in the opposite direction, since if this were not so the cell population would gradually become more and more spherocytic and no state of equilibrium such as is seen in the peripheral blood could be established.

d) The abnormally spherocytic cells from the splenic vein are not recognisable in the peripheral blood.

One possibility that arises is that the cells released from the bone marrow are in a flattened form and assume the "normal" shape after being acted upon by the spleen. This is unlikely in view of the evidence put forward above that "normal" cells change to flat cells in the peripheral blood. Such a mechanism would also make it very difficult to explain the normal constant size of the red cells as discussed above.

The possibility that the spleen acts selectively on flat red cells is unlikely if Knisely's concept of the splenic circulation is correct, and also in view of the fact that the

cells in the splenic vein are not "normal" but spherocytic.

There is no evidence at present to indicate what this flattening mechanism might be. One factor that may be of importance is the demonstration by Furchgott and Ponder (1940) that the crystalbumin of the plasma prevents the sphering of the red cells that occurs when blood is placed between two glass surfaces. Thus antisphering substances are present in the blood plasma.

It is suggested therefore that some mechanism for flattening the red cells exists. The spleen tends to make the cells round. The "normal" state of the red blood cells is the result of the equilibrium established between the actions of these two opposing mechanisms, and the red cells are in constant state of flux as regards shape. This state of flux is complicated further by the fact that the red cells become more spherocytic and fragile in venous blood and then return to their "normal" state in arterial or oxygenated blood.

After splenectomy the action of the spleen is lost and the cells become flat. This fact remains true whatever the mechanism of the flattening.

iii) Disturbed Lysolecithin Metabolism.

It is possible that the reduced lysolecithin content of the blood after splenectomy is due to loss of the separator action of the spleen whereby the plasma is separated off from the red cells and traverses the pulp. Here there is normally a formation of lysolecithin. After splenectomy less lysolecithin is formed. It is difficult to understand, however, why no increase in lysolecithin is seen after incubation of the plasma in splenectomised patients. Singer, Miller and Dameshek (1941), only speak of disturbed lysolecithin metabolism and do not consider its mechanism any further.

The conclusion relative to the present study that may be drawn from the above discussion is that the flattening of the cells after splenectomy is due to loss of the separator function of the spleen. It seems conceivable that this separator function of the spleen may be lost, without actual removal of the spleen, from disturbance of the normal vascular cycle. This possibility is supported by the experimental study of Miller, Singer and Dameshek (1942) in which they produced target cells in the blood of animals simply by interfering with the splenic circulation.

It is suggested therefore that in jaundice there is loss of the separator function of the spleen, from disturbance of its vascular mechanism, as a result of which the red cells are not held up in the spleen and so fail to undergo the changes normally produced by the action of the spleen. The possibility that haemolysis is reduced also has been discussed already and would support this suggestion of impaired splenic function.

The fact already referred to that transfused cells in jaundice require 5-7 days before showing the properties of flatness, resistance to hypotonic saline and target cells also appears to be more in keeping with this concept than with the idea that they are caused by some abnormal chemical constitution of the plasma.

There is no indication of how this disturbance of the splenic circulation might be brought about. The only factor that appears to be common to all the cases of jaundice is disturbance of the liver function.

The only feature not accounted for satisfactorily is the macrocytosis, although Cassells (1938), counting cells above 7.25 mu. in diameter as macrocytes, found an average macrocytosis of 77% in 5 cases in which the spleen had been removed for reasons other than haemolytic anaemia.

Since the presence of target cells in a blood film indicate that the red cells are flat and are resistant to hypotonic saline, it may also be suggested that the presence of target cells indicate that the splenic function is disturbed.

5) Summary of the red cell changes in jaundice.

1) The red cells in jaundice show certain changes - flattening, increased resistance to hypotonic saline haemolysis and target cells.

2) These abnormal features result from changes brought about in normal red cells during their circulation in the peripheral blood. The cells released from the bone marrow are probably normal.

3) The chemical changes in the blood in jaundice do not appear to be responsible for this alteration in the red blood cells.

4) The changes in the erythrocytes probably arise because there is disturbance of the normal function of the spleen. This may arise because of alteration in the circulation through the spleen, whereby the cells are not held up in the spleen and thus fail to undergo the changes found normally in erythrocytes in splenic venous blood.

E) POSSIBLE ROLE OF HYPOSPLENISM IN THE ANAEMIAS OF CHRONIC INFECTIONS.

It has already been pointed out that certain features of the red blood cells in chronic infections are not easily explained on the basis of alteration in erythropoiesis whereby abnormal cells are delivered to the blood stream.

It was also noted that these very changes that occur in chronic infections also occur in jaundice. The latter problem has been investigated and the conclusion has been reached that the changes result from interference with the functions of the spleen, and that this interference with splenic function may occur secondarily to abnormalities in the liver and biliary apparatus.

It is obvious too, as in the case of the red cell changes in jaundice, that if the red cell changes in chronic infections cannot be explained fully by abnormal erythropoiesis, then some of these changes must occur in the peripheral blood. From analogy with the mechanism suggested for the changes in the erythrocytes in jaundice, it is suggested that in cases of chronic infections there is also an upset of the function of the spleen.

The possibility discussed above that

there is less cell destruction than normal in cases of chronic infection would fit in well with this concept of underfunction of the spleen.

There are several ways in which this upset in the splenic function could be brought about in cases of chronic infections.

1) The agent (see below) responsible for the other changes in chronic infections may act on the spleen directly, producing alterations in its normal vascular physiology.

2) The changes in splenic function may be secondary to the disturbed liver function that has been demonstrated in these cases, just as in the case of jaundice the abnormality of splenic function is likely to be secondary to the disturbance of the liver.

There is no available evidence to indicate which of these two mechanisms is at work.

It is possible also that interference with splenic hormonal function is responsible for the occasional normoblastosis that occurs in chronic infections. This is suggested by the fact that normoblastosis is frequently seen after splenectomy. The idea is supported by the case reported by van Buchem and Borman (1940) of reticuloendotheliosis in which normoblastosis was a prominent feature and in which autopsy

revealed an atrophic spleen weighing no more than 6 grammes.

It is interesting to speculate as to how far a splenic mechanism as suggested above might account for other cases with red blood cells resistant to hypotonic saline and with increased numbers of target cells. Singer, Miller and Dameshek (1941) have already advanced this concept of "hyposplenism" and its clinical recognition. It seems at least possible that this concept will account for most examples.

1) After haemorrhage and other blood loss target cells are frequently seen (Bohrod, 1941). It is well known that after haemorrhage the spleen is contracted and thus the circulation through it must be altered.

2) In any non-haemolytic anaemia it is likely that red cells are not retained within the spleen, but that all available erythrocytes are kept in the circulating blood stream.

3) In liver disease with or without jaundice, target cells are often seen. It is well known that disturbances of the liver and of the spleen often occur together and it is possible that the spleen is affected in these cases of liver disease where target cells are present.

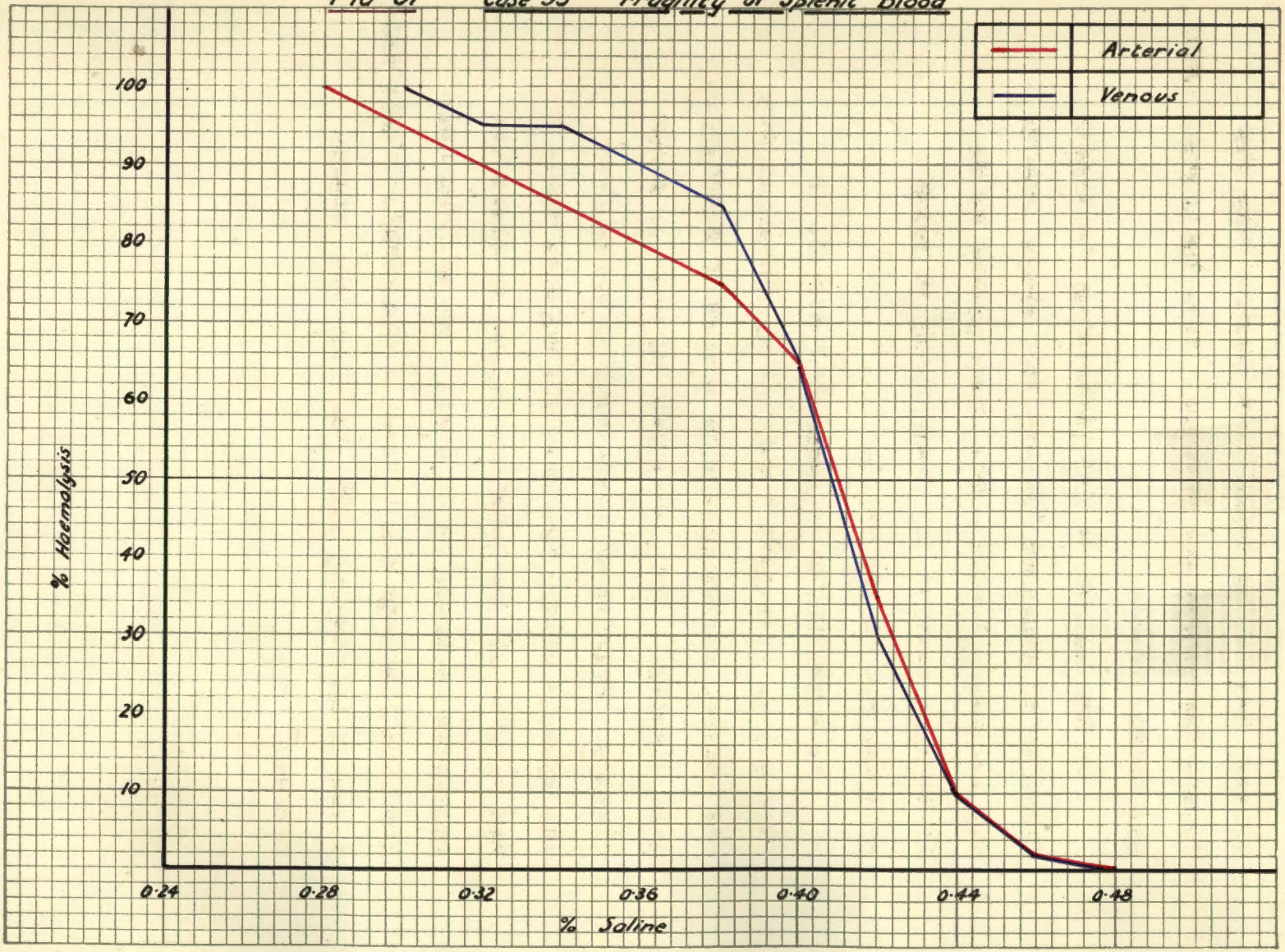
4) In the cases of Mediterranean disease it is possible that there is some inherited defect of the spleen and not of haemopoiesis which explains some of the phenomena observed.

5) In sickle cell anaemia the spleen is often atrophic and shows pathological changes long before this. Some defect of the spleen may be involved here too.

In the latter two instances it might be difficult at first sight to reconcile this concept with the fact that there is excessive haemolysis in both these conditions. However, it is known that splenectomy fails to relieve the haemolytic anaemias of both these diseases, so that the spleen appears to play very little role in the haemolytic process here. Also in sickle cell anaemia the haemolysis proceeds unchecked even after the spleen has atrophied to almost nothing, and the characteristic target cells and resistance to hypotonic saline may be seen while the spleen is still palpable clinically (Berk and Bull, 1943).

A method of investigating this hypothesis that suggests itself is to test the fragility of the red blood cells in the splenic artery and in the splenic veins of cases showing the abnormalities under discussion. Normally there

FIG 61 Case 93 Fragility of Splenic Blood



is a distinct difference between the fragilities of the blood entering and leaving the spleen, the latter being more fragile than the former (Bergenhem and Fahreus, 1936; Heilmeyer, 1936 and Lauda, 1933).

If the spleen is not performing its normal function of changing the red cell shape towards the spherocytic side, then there should be no difference between the fragilities of splenic arterial and venous blood.

An opportunity to carry out such an investigation presented itself at an operation for the removal of the spleen in a case of Mediterranean disease. This case was reported by Brock (1944). Samples of blood were obtained from the splenic artery and from the splenic vein. The results of the fragility tests on these two specimens is shown in fig. 61. It will be seen that the readings are virtually identical. This evidence is in keeping with the views put forward above.

F) THE HUMORAL AGENT INVOLVED.

It has been shown that infections bring about certain of their bodily effects through the agency of chemical substances produced at the site of the inflammation and absorbed from there into the blood stream (Menkin, 1940). The substance most fully studied to date is the leucocytosis promoting factor.

Willisen (1939) working on rabbits in which a constant level of anaemia was maintained by bleeding, demonstrated that the toxins of various bacteria (tetanus, diphtheria, streptococci, staphylococci) depressed bone marrow activity. This was shown by reduced reticulocytosis and the fact that less bleeding was required to keep the count constant while the toxins were being administered.

In the experiment of Robscheit-Robbins and Whipple (1936) referred to earlier it was shown that sterile pleurisy induced by turpentine injections also inhibited the formation of new haemoglobin. Since the effects occurred at a distance from the site of infection, they must have been mediated by either a nervous or a humoral agency. In view of the work of Menkin the humoral theory seems the most likely. In both Menkin's and Whipple's experiments it

has been shown that the presence of neither bacteria nor their toxins are necessary for the production of the humoral agent. The latter is a non-specific product resulting from the tissue breakdown that occurs with the inflammatory reaction.

It seems likely therefore that some abnormal breakdown product or products produced at the site of the inflammatory reaction and absorbed into and carried by the blood stream are the actual means whereby chronic infection brings about the changes in the red blood cells.

A curious feature of the anaemia in chronic infection is the fixation of the level of the anaemia over long periods of time and the difficulty in altering this level by transfusions or haematinic therapy. This has been noted by Vaughan and Saifi (1939) and is demonstrated in the two cases that were followed up. It is easily confirmed by ordinary clinical experience and by the statements of many authors that the grade of anaemia is usually mild.

Gordon (1939) has also noted that the bone marrow probably assumes a static level of activity.

This fixation of the degree of anaemia suggests

that the activity of the bone marrow is set at a new, abnormally low level of production in much the same way as the temperature regulatory mechanism is set to operate at a higher level than normal in infection. This fixation is probably brought about by the humoral mechanism discussed above.

The concept of the mechanism of the blood changes in chronic infections which has been arrived at in this thesis may thus be summarised as follows:-

As a result of the inflammatory reaction present in cases of chronic infections, non-specific abnormal products of tissue breakdown are absorbed into the blood stream. These produce many effects which have a bearing on the blood changes observed.

1) There is interference with the activity of the bone marrow as a result of which haemoglobin metabolism is disturbed by inability of the marrow to utilise iron and erythropoiesis is depressed. As a result of this interference with marrow function fewer cells than normal are released into the circulation and these are insufficiently haemoglobinised.

2) Liver function is interfered with and this may be the cause of the absolute or relative macrocytosis found.

3) There is interference with the functions of the spleen. This arises either directly or secondarily from the disturbance of liver function.

4) There is probably some abnormality of the red cell envelope, but it is not clear where it arises.

These combine to give the red cell changes in cases of chronic infection, which may be summarised as follows:-

a. Anaemia.

b. Hypochromia.

c. Macrocytosis, absolute or relative.

d. Target cells.

e. Flatness of the erythrocytes.

f. Abnormal resistance to hypotonic saline.

The combination of these features in a blood picture is suggestive of the presence of a chronic infection.

GENERAL SUMMARY.

V. GENERAL SUMMARY.

1) The literature on the anaemias of chronic infections has been reviewed. There is considerable difference of opinion as to the morphological features of the anaemia and with regard to the pathogenesis of the changes in the blood.

2) The cases studied in this investigation were described and the methods of study outlined in detail.

3) A new modification of the hypotonic saline fragility test has been introduced and shown to give accurate and valuable information.

4) A series of 20 normal cases were studied in order to test the accuracy of the technical methods employed and to serve as normal standards of reference.

5) The importance of standardising the oxygen tension of blood samples in doing haematocrit determinations has been emphasised.

6) A study of target cells is reported. The main conclusion reached about their significance is that their presence in a blood film indicates that the red cells are resistant to hypotonic saline.

7) The plan of investigation was designed to eliminate as far as possible extraneous

factors which influence the blood picture.

8) The following were found to be the main features of the red cells in chronic infections:-

a. The blood picture may be macrocytic hyperchromic, macrocytic normochromic, normocytic normochromic, normocytic hypochromic or microcytic hypochromic.

b. On analysis of the individual characters of the red cells it was found that hypochromia of the individual cells was an almost constant finding.

c. Large mean cell volume readings are common, and if the cells volume is considered in relation to the colour index, then a tendency to macrocytosis in all cases becomes evident.

d. The cells tend to be flat.

e. There is a marked tendency for the red cells to show abnormal resistance to hypotonic saline.

f. There is evidence that the erythrocyte envelope may be abnormal.

g. Target cells are present in abnormal numbers in the blood films of cases of anaemia due to chronic infection.

h. There is no evidence of haemolysis in these cases, although a small degree of reticulocytosis may be seen. Normoblasts occasionally appear in the peripheral blood.

j. The changes in the white cells are dependent on the primary cause of the anaemia.

j. The platelets are normal in number.

k. The bone marrow shows erythropoiesis of normoblastic type.

l. Liver function is very commonly depressed in cases of chronic infections, sometimes to a very marked degree.

m. The secretion of hydrochloric acid by the stomach is normal in these cases.

n. The level of haemoglobin and red cell count tends to remain fixed in cases of anaemia due to chronic infection and are not influenced by any form of therapy.

9. The following suggestions are made with regard to the pathogenesis of the changes in the red cells:-

i) The blood picture is dimorphic i.e. there are two factors at work producing the changes in cells size and haemoglobinisation.

ii) There is interference with the normal function of the bone marrow, as a result of which it is unable to utilise iron for the production of new haemoglobin and erythropoiesis is depressed.

iii) The factor causing the macrocytic trend may be the disturbance of hepatic function.

iv) Depression of liver function can result in macrocytosis, but the mechanism of this is in doubt.

v) The flatness of the cells with increased resistance to hypotonic saline and target cells in the blood, and the macrocytosis in association with normoblastic erythropoiesis are not explicable on the data available at this point.

10. Most of these characters of the red cells in chronic infections which are difficult to explain have also been described in cases of non-haemolytic jaundice. These findings have been confirmed. They are present to a much more marked degree in jaundice than in chronic infection.

11. Investigation of these characters in jaundice resulted in the following conclusions being reached.

(a) The changes in the red cells in jaundice result from alterations in the red cells as they circulate in the peripheral blood and not from the production of abnormal erythrocytes by the bone marrow.

(b) The changes in the red cells cannot be accounted for by the effect of the retained chemical substances in jaundice, viz. the bile salts, cholesterol or bilirubin.

(c) It is suggested that the changes result because of defective splenic function.

(d) The splenic function is defective probably because of disturbance of the circulation of the blood through the spleen.

12. It is suggested by analogy with cases of jaundice that chronic infection leads to defective splenic function, either directly or because of disturbed liver function.

13. The changes in the red cells in chronic infection are brought about by the absorption of non-specific breakdown products of the inflammatory reaction.

14. These abnormal breakdown products are absorbed into the blood stream, in which they reach the bone marrow, liver and spleen, and so produce the effects discussed, namely a toxic dimorphic dyshaemopoietic anaemia with hypo-splenism.

SUGGESTIONS FOR FURTHER STUDY.

VI. SUGGESTIONS FOR FURTHER STUDY.

Arising out of the work reported in this thesis, numerous lines of investigation suggest themselves.

Most important of these is the question of defective splenic function. The first subject to tackle for confirmation of this theory would be to study the splenic arterial and venous blood, as has already been done in one case of Mediterranean anaemia. It appears that it would be necessary to investigate the problem experimentally in dogs. Lysolecithin metabolism should be investigated in chronic infections.

The second striking suggestion is that many problems of haematology lend themselves to study by the technique of transfusion and investigation of the cell changes. The importance of changes in the red cells as they circulate in the peripheral blood are brought to attention in conditions other than haemolytic anaemias in which they have been extensively studied in recent years. It is suggested that it is important not only to follow the cells by Mollison's differential agglutination technique, which only counts the cells, but also by fragility tests and by Price-Jones curves. An

example of the problem that may be solved in this way is the microcytosis of pernicious anaemia.

Another important haematological problem that requires investigation by these techniques is that of the red cell changes in cases of liver disease unaccompanied by jaundice.

The anaemias of chronic infections themselves should be subjected to this method of analysis.

Another possible line of investigation into the splenic function is suggested by the work of Watson and Paine (1943). Adrenaline injection may release cells from the spleen which can be recognised and studied in the peripheral blood by the techniques employed in this investigation.

The survival time of cells transfused into anaemic cases and of cells from anaemic cases transfused into normal subjects would also give further insight into erythrocyte metabolism, as has already been done in the case of acholuric jaundice by Dacie and Mollison (1943).

The study of the anaemias in chronic infections should be extended to the closely allied anaemias occurring in malignant disease and in nephritis.

Investigations should be directed towards isolating and identifying the humoral agent responsible for the changes occurring in the blood in chronic infections. This might profitably be approached along the lines suggested by Menkin's investigations of the chemical factors concerned in inflammation.

It also seems desirable to investigate the role of possible vitamin C deficiency in the anaemia of chronic infection.

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VII. ACKNOWLEDGEMENTS.

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APPENDIX.

THE AGE, SEX and RACE OF THE CASES OF CHRONIC INFECTION.

Case	Age	Race	Sex
1	42	Native	Male
2	28	Native	Male
3	11	Coloured	Male
4	63	Coloured	Male
5	56	European	Female
6	65	Coloured	Male
7	30	Native	Female
8	47	European	Male
9	54	European	Male
10	16	Coloured	Female
11	32	Coloured	Female
12	49	European	Male
13	17	Coloured	Male
14	33	Coloured	Male
15	23	European	Female
16	23	Coloured	Female
17	33	Coloured	Female
18	35	European	Male
19	28	European	Female
20	34	Native	Female
21	26	Native	Female
22	18	Coloured	Male
23	22	European	Male
24	65	European	Male
25	49	European	Male

IX. THE CASES STUDIED.

1. Tuberculous pleural effusion.
2. Tuberculous glands in the neck.
3. Tuberculous glands in the neck.
4. Tuberculous pleurisy.
5. Rheumatoid arthritis.
6. Lung abscess.
7. Tuberculosis of the spine, lungs and larynx.
8. Empyema following pneumonia
9. Subphrenic abscess.
10. Tuberculous pleurisy.
11. Rheumatoid arthritis.
12. Broncho-pleural fistula following pneumonia.
13. Tuberculous spine with lumbar abscess.
14. Tuberculous pleural effusion.
15. Tuberculous empyema and lungs.
16. Tuberculosis of the lungs.
17. Subacute bacterial endocarditis.
18. Subacute bacterial endocarditis.
19. Pneumonia.
20. Tuberculous pleurisy.
21. Chronic osteitis.
22. Generalised tuberculosis (Case 5 reported by Berk, 1943,a).
23. Subacute Bacterial endocarditis.
24. Tuberculous glands and pleurisy.
25. Chronic eczema.
26. Nil abnormal found.

27. Healed gastric ulcer.

28. Normal (nurse).

29. Healed duodenal ulcer.

30. Hypertension.

31. Normal (doctor).

32. Normal (doctor).

33. Normal.

34. Normal (doctor).

35. Normal (doctor).

36. Normal (doctor).

37. Normal (nurse).

38. Normal (nurse).

39. Normal (nurse).

40. Normal (nurse).

41. Normal (doctor).

42. Normal (doctor).

43. Normal (doctor).

44. Normal (nurse).

45. Normal.

46. Hypertension.

47. Polycythaemia.

48. Sciatica.

49. Cirrhosis of the liver.

50. Cirrhosis of the liver.

51. Syphilitic hepatitis.

52. Pernicious anaemia.

53. Myxoedema.

54. Xanthomatosis.

55. Sickle cell anaemia.

56. Polycythaemia.

57. Hodgkin's disease.

58. Acute monocytic leukaemia.

59. Chronic myeloid leukaemia.

60. Chronic lymphatic leukaemia.

61. Chromic myeloid leukaemia.

62. Normal (nurse).

63. Cirrhosis of the liver.

64. Hepatitis.

65. Nodular hyperplasia of the liver with subacute necrosis.

66. Cirrhosis of the liver.

67. Scleroderma with liver disease.

68. Cirrhosis of the liver.

69. Carcinoma of the bronchus.

70. Nutritional iron deficiency anaemia.

71. Chronic ulcerative colitis.

72. Carcinoma of the stomach.

73. Haemophilia.

74. Pernicious anaemia.

75. Myxoedema.

76. Reticulosis.

77. Constrictive Pericarditis.

78. Acholuric jaundice.

79. Cirrhosis of the liver.

80. Secondary carcinomatosis.

81. Xanthomatosis.

82. Scurvy.

83. Pernicious anaemia.

84. Duodenal ulcer.

85. Refractory anaemia.

86. Acholuric jaundice.

87. Polycythaemia.

88. Hodgkin's disease.

89. Pernicious anaemia.

90. Cirrhosis of the liver.

91. Pernicious anaemia.

92. Acute lymphatic leukaemia.

93. Mediterranean anaemia.

94. Pernicious anaemia.

95. Hepatitis accompanying lobar pneumonia with empyema.

96. Stone in the common bile duct.

97. Carcinoma of the head of the pancreas.

98. Carcinoma of the head of the pancreas.

99. Alcoholic hepatitis.

100. Cirrhosis of the liver.

101. Hepatitis.

102. Carcinoma of the head of the pancreas.

103. Congenital obliteration of the bile ducts with obstructive biliary cirrhosis.

104. Primary carcinoma of the liver with obstruction at the porta hepatis.

105. Carcinoma of the head of the pancreas.

106. Syphilitic hepatitis.

107. Reticulosis.

108. Chronic lymphatic leukaemia.

109. Acute myeloid leukaemia.

110. Chronic lymphatic leukaemia.

111. Acholuric jaundice.

112. Hepatitis.

113. Stone in the common bile duct.

114. Carcinoma of the head of the pancreas.

115. Chronic haemolytic anaemia.

116. Cirrhosis of the liver.

117. Xanthomatosis.

118. Hepatitis.

119. Hepatitis.

120. Acute pancreatitis.

121. Cirrhosis of the liver.

Summaries of the clinical features of cases 1 - 25 have been lodged with the Registrar, University of Cape Town, from whom they may be obtained.