THE RESISTANCE of HUMAN RED BLOOD-CORPUSCLES

IN HEALTH & DISEASE

to

HAEMOLYSIS by SAPONIN:

and a comparison of SAPONIN-HAEMOLYSIS with that of

Hypotonic Saline Solutions.

-----------------------------------------

A THESIS for the degree of M.D., UNIVERSITY of EDINBURGH

by

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The following research upon the resistance of human erythrocytes in health and disease to haemolysis, was undertaken at the instance of Sir Thomas R. Fraser. He suggested the subject to me, gave me the privileges of his laboratory, and provided me with a sample of Saponin with which the greater part of my work on this subject has been done.

This subject of the resistance of human red blood corpuscles in health and of its variations in disease is not entirely new. But comparatively little exact work has been bestowed upon it, and that has been all done by a single method, whose results are indeed definite but unfortunately throw no light on the subject they investigate - the variations in resistance of human red-cells to natural or pathological destruction in corpore. The method I refer to is that of the resistance of red-cells of man to laking by hypo-tonic salt solutions.

Maragliano (1) was one of the earliest investigators. He exposed human red blood-corpuscles to various destructive agents, - heat, drying, etc: and by the microscope observed that in different conditions of disease, the rate of cell-disintegration was greater than in health. He also studied the slow deterioration/
deterioration that took place in red-cells placed in sealed preparations, and from variations in the rapidity of that deterioration drew conclusions as to the vigour of the corpuscles in health and disease. Such methods did not lend themselves to quantitative measurement, and his results were necessarily indefinite and inexact, but they broke ground on a new subject, and stimulated further research.

Hamburger (2) in his elaborate researches on the haemolysis of mammalian erythrocytes, in saline solutions of varying strengths, gave to clinical medicine a new gauge for measuring the resistance of human red blood corpuscles, and variations in that resistance. Such a method was much better suited for accurate standardisation, and it was applied with various modifications by investigators in France, Germany, Russia and Italy. These researches and their results will be fully discussed later.

A different method is the measurement of the electrical resistance of the blood in health and disease. Several investigators have adopted this principle, of whom E. Buffa (3) may be quoted as an example. But it is impossible to draw conclusions as to variations in the resistance of red-cells in corpore.
corpora from the results obtained, and the attempt to do so has practically been given up.

Meantime, it can be said that, with the exception of Maragliano's work, and of a few observations on the electrical resistance of the blood, which have been discredited by such an authority as v. Limbeck (4), all the data on the resistance of human erythrocytes in health and disease, have been obtained by using hypotonic solutions of different salts, and observing differences in resistance to "laking" shown by blood corpuscles, in various pathological conditions.

This is surprising in view of the great mass of researches, that have been conducted in recent years, on other haemolytic agents comprising the group of haemolytic glucosides, the snake venoms, and the natural and acquired haemolysins in blood-sera. All these haemolytic agents have been, more or less, accurately standardised for the erythrocyte of different mammals, and the nature and conditions of their action on those cells carefully studied.

In all these investigations, the sensitivity of the moiety of erythrocytes to haemolysis was kept constant, by use of the blood of healthy animals, and the release of haemoglobin was used as an index.
for measuring the activity of the lytic substances and for studying the conditions of their activity. The idea of Sir Thomas Fraser, which initiated this research was to change the point of view, and in the interaction of the haemolytic substance and the red blood corpuscles, to use the haemolysin as the constant and to make it the indicator of possible changes in the sensitiveness of the cell to haemolysis. This idea, was therefore, a new one. It sought by means of the physiological activity of a substance, to investigate the pathology of human erythrocytes, in respect of their resistance to haemolysis. And, prima facie, it seemed a superior method to that which made use of a hypotonic salt solution. It has not even been suggested that haemolysis normally occurs in corpore, by the red blood corpuscle entering a hypotonic fluid medium, and it would seem dangerous to draw conclusions as to the resistance of erythrocytes from their behaviour in such unnatural surroundings. But it was now proposed to suspend the cells in an isotonic fluid, and to subject them, in it, to the action of a haemolytic substance. Such a method at least reproduced the conditions of toxicity of body fluids, and, in so far, was a closer approximation to the conditions of haemolysis in corpore.
5.

**SAPONIN.**

The powerful haemolytic property of Saponin, Cyclamin Digitalin and Solanin have been well known for some time, and especially in recent years have been carefully studied. *Ransom* (5) using the blood of dogs showed that red-cells, washed free of their serum, became much more sensitive to the action of Saponin: and that some element both in the serum and in the corpuscle, entered into a firm combination with it rendering it inactive for further haemolysis. By experiment, he concluded that this substance in serum and in the red corpuscle, which combined with Saponin, was Cholesterin. He clearly showed that the presence of Cholesterin in serum artificially induced, was highly protective of the corpuscles, against the haemolysis of Saponin.

The idea thrown out by Ransom of the protective action of Serum and plasma, was pursued further by Hedon (7). The latter endeavoured to standardise various blood-sera, from the point of view of this protective action. He found that the sera of cold blooded animals, such as frogs and eels, was many times more protective than those of the common laboratory/
In using Saponin for a clinical investigation, new and different conditions, not present in laboratory experiments, were introduced, which made it necessary to elaborate a special technique. This task proved to be a difficult one, attended by many fallacies. As, in its course, some new methods of investigation were devised which are capable of wider application, and as, by means of these, peculiar and interesting phenomena of Saponin-haemolysis were discovered, which also may have a general significance, the progress and development of this technique will be described in detail.

At the outset, it followed the general line of the methods laid down by the very numerous investigations on Haemolysis in recent years. Simply stated the main principle of these studies is to find the dose of the haemolytic substances which will exactly dissolve a given quantity of the washed red blood corpuscles of an animal, in a given time. The tech
technical manipulation employed, may be divided under two heads.

(1). The preparation of a suspension of washed blood corpuscles.

(2). The determination of the point of complete haemolysis.

(1). A suspension of washed corpuscles is obtained by bleeding the animal profusely. The large quantity of blood secured, is defibrinated. It is then washed free of serum by repeated centrifugation, removal of the supernatant fluid, and addition of saline solution. To a measured quantity of the sedimented corpuscles, a measured quantity of saline solution is added. Thus a suspension of washed corpuscles, of known percentage, is provided. Equal amounts of this suspension are placed in a series of tubes. A graduated series of doses of the haemolytic solution having been added, the tubes are placed in a constant temperature for a constant period.

(2). At the end of the selected period, the first tube whose contents show a complete transparency to the eye, indicates complete haemolysis, and gives the complete haemolytic dose.

Before describing my application of this technique, I may state, at the outset, that the sample of Saponin used was obtained from E. Merck, Darmstadt, and was marked "Saponin, Extra Pure, Very White."
All the results in the following research were obtained with this sample. The saline solution employed was of pure Sodium Chloride, recrystallised, supplied by Duncan & Flockhart, Edinburgh. Its strength was always 0.85grm per 100c.c. of sterile distilled water. This solution was used both as the solvent for the Sapponin, and for the suspensions of red blood corpuscles.

(1). PREPARATION OF SUSPENSIONS OF WASHED BLOOD CORPUSCLES.

In a clinical investigation, it is only possible to obtain small quantities of blood: and an entirely different procedure from that detailed above is called for. At first I proceeded as follows. By the usual enumeration of the cells by the Thoma-Zeiss Haemocytometer, I determined the number of erythrocytes, per cubic millimetre of entire blood, in the case under investigation. Into each of 4 or 5 tubes, 20 cubic millimetres of entire blood, obtained by puncture of the ear or finger, were placed. To each tube had already been added 1.98 cubic centimetres of the saline solution (0.85%), There was thus a bulk of 2c.c. of blood-suspension in each tube.

The/
The suspension having been thoroughly mixed by vigorous rotation, the tubes were centrifuged. 1·5 c.c. of supernatant fluid was withdrawn by pipette from each, and the bulk again restored to 2 c.c by fresh saline solution. This washing centrifugation and pipetting off were thrice repeated. After the final pipetting off, one therefore had a series of tubes, each with the number of red-cells contained in 20 c.mm. of entire blood. And these red-cells were now washed free of their serum. To the series of these tubes, different and increasing doses of a solution of Saponin were given, that amount of Saline solution having been first added which, with the amount of the Saponin fluid, would bring the bulk of the tube contents to 2 c.c. By this means, one exposed equal samples of washed blood suspensions, whose corpuscular content was known, to varying doses of Saponin solution. At the end of a constant time interval one was able, therefore, to determine the dose of Saponin, which was completely haemolytic for that number of erythrocytes in that time. But, at the very outset, a difficulty presented itself. It is evident that in conditions of oligocytæmia, the corpuscular content of 20 c.mm. of blood, would be less than in health or polycytæmia. For/
For example, a case of Pernicious Anaemia, with 1 million erythrocytes per c.mm. of blood, would give a series of tubes, each containing 20 million red cells. But 20 c.mm. of the blood of a healthy person would give approximately 100 million red cells per tube.

STANDARD NUMBER OF ERYTHROCYTES. Prima facie, it would appear safe to assume that the haemolytic dose of 100 million cells would be 5 times that for 20 million cells of the blood of the same individual. But, in addition Bashford (6) makes the following clear statement upon this point. He says, "It was determined that the relation between corpuscles and the amount of glucoside (saponin, cyclamin, digitalin, solanin) necessary to effect a solution, was simply a quantitative one: i.e. a multiple of the solvent dose of glucoside, dissolved the same multiple of the standard quantity of blood."

I confirmed this statement of Bashford in the following experiment. Two tubes of blood corpuscle suspension, each with 20 c.mm. of my own blood, were thrice washed with saline solution. After the final washing, tube 2 was brought to a bulk of 2 cc. with saline solution, and shaken to a uniform suspension/
suspension. Of this, 1 cc. was removed. The remaining 1 cc., therefore, contained the erythrocyte content of 10 c.mm. of my blood. Erythrocyte count of my blood was 5,090,000 per c.mm. Complete Haemolytic dose, for 100 million of my washed erythrocytes, was previously found to be 0.80 of Saponin solution .004 grms per 100 cc.

Incubator 37° C. Saponin Solution .004%.

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Blood Suspension</th>
<th>Sap.Soln. Interval</th>
<th>Haemolysis</th>
<th>No. of red cells in Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.4 cc. + 0.60 cc</td>
<td>2 hours</td>
<td>Complete</td>
<td>100 mlns.</td>
</tr>
<tr>
<td>2</td>
<td>1.7 cc. + 0.30 cc</td>
<td>&quot;</td>
<td>&quot;</td>
<td>50 mlns.</td>
</tr>
</tbody>
</table>

This experiment confirms Bashford's statement, quoted above. I therefore, resolved to make my standard number of corpuscles, 100 millions, in conditions of health and disease alike. In that way I would be able exactly to express the resistance of the erythrocytes of different individuals in terms of the complete haemolytic dose of Saponin for an equal number of corpuscles. Thus in the above hypothetical case of Pernicious Anaemia, where the erythrocytes numbered 1 million per c.mm of blood, the solvent dose for 20 millions erythrocytes, the content/
content of 20c.mm. of blood, would be directly ascertained. This figure multiplied by 5 would give the haemolytic dose for 100 million erythrocytes, the standard number.

MODIFIED METHOD OF PREPARING EQUAL SAMPLES OF WASHED ERYTHROCYTES.

After some time, I adopted another method of preparing the samples of washed erythrocytes. The one which I have just described was liable to error in two directions. Firstly, in the course of blood-flow from a puncture wound, the proportions of plasma and corpuscles might vary so that that moiety of blood, which yielded the erythrocyte-enumeration by the Zeiss haemocytometer, might contain a greater or less bulk of plasma to corpuscles than those portions of 20c.mm. which I obtained for the suspension samples. Secondly, in the frequent manipulations and measurements during the course of washing, an accumulation of experimental error might produce some defect, or excess of the bulk of fluid. The following modification removes both these sources of error.

A quantity of blood, 50 to 100 c.mm. was taken from the patient, and placed in a tube containing from 4 to 5cc. of Saline solution/
solution. This blood-suspension was thrice washed free of serum, with saline solution: and the washing was now made more thorough, in that one removed by the pipette, after each centrifugation as much of the supernatant fluid as possible. Finally a suspension of washed corpuscles of about 2.5 cc. in bulk was made. From this, 4 portions, each of 0.5 cc., were measured off, and placed in 4 tubes. Of the remainder, 0.25cc. was taken, and diluted 1 in 4, or 1 in 8, with saline solution. A drop of this diluted suspension was placed in the Zeiss counting chamber, and enumeration of the red cells made. Having thus obtained the number of cells per c.mm. of the diluted suspension, one could, by a simple calculation, arrive at the erythrocyte content of 0.5cc. of the undiluted suspension. The following example will illustrate the method. It will be seen that two separate enumerations were made; that in each preparation 5 fields of 16 squares were counted; and that the subsequent calculation was based on their sum-total.

Example:— My own blood. 80c.mm. of entire blood placed in saline and washed as above described. The final suspension divided into portions of 0.5cc. From the remainder 0.25cc. brought to 2cc. with saline, dilution 1 in 8.

DILUTED/
DILUTED SUSPENSION. COUNTING CHAMBER PREPARATION.

Second Preparation.

Fields of 16 squares. Fields of 16 squares.

<p>| | |</p>
<table>
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<tbody>
<tr>
<td>50</td>
<td>47</td>
</tr>
<tr>
<td>51</td>
<td>39</td>
</tr>
<tr>
<td>40</td>
<td>52</td>
</tr>
<tr>
<td>(1)</td>
<td>(3)</td>
</tr>
<tr>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>45</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>235</td>
<td>229</td>
</tr>
</tbody>
</table>

If the two separate enumerations did not closely correspond, fresh preparations were made until two successive counts approximated as above. The calculation now proceeded.

Total for 10 fields of 16 squares (160 squares)

\[ = 235 + 229 = 464. \]

\[ \text{No. of erythrocyte per lc.mm. of original suspension} = \frac{464 \times 4000 \times 8}{160} \]

\[ = 0.5 \text{cc.} \]

\[ \text{No. of erythrocyte per 0.5 cc.} = \frac{464 \times 4000 \times 8 \times 500}{160} \]

\[ = 46,400,000. \]

In this above example, one obtained a series of...
of 0.5 cc. samples of blood-suspension, each of which contained 46,400,000 washed erythrocytes. In severe oligocythaemia 0.5 cc. samples similarly obtained, would contain much fewer erythrocytes. This modified method of determining the number of erythrocytes in the samples was superior in several points, to the original one employed. The enumeration was now made directly from the blood suspension that furnished those samples. Further, the suspension from which the count was made, was diluted at the most 1 in 8, as compared with the dilution of 1 in 200 of entire blood in the Zeiss haemocytometer; so that the error of enumeration was now, only multiplied by 8, whereas, by the former method it was multiplied by 200.

As before, the solvent dose of Saponin ascertained by experiment for the samples prepared, was converted, by easy calculation, into the solvent dose for 100 million erythrocytes. - the standard number.

THE DETERMINATION OF HAEMOLYSIS.
COMPLETE AND INCOMPLETE.

The second portion of the technique of haemolytic investigation, now falls to be considered.
INCOMPLETE HAEMOLYSIS.

In the vast majority of researches on haemolysis, Incomplete Haemolysis is indicated, in its various degrees, by such terms as "slight", "moderate", "considerable", "almost complete": or by the use of such signs as +, ++, +++, etc. These intermediate stages of haemolysis are obtained by the judgment of the observer, on the degree of opacity of the blood-suspension. Such rough and ready measurements must be inaccurate. It was very desirable to obtain, for the present investigation, a more exact, and if possible, a numerical determination.

Mioni and Henri, in their quantitative investigations upon haemolytic sera, have attempted the solution of this problem. Both have done so, by the use of colorimetric methods, each of which is based on the determination of the amount of Haemoglobin, in the supernatant fluid after centrifugation, by Fleischl’s haemoglobinometer. Mioni (8) expresses his results as grammes of Haemoglobin. Henri (9) presents his as percentages of the total Haemoglobin content.
content. Both these methods are suitable for investigations on healthy blood, where the Haemoglobin-value of a fixed amount of blood is constant. But they are cumbersome and difficult to work, and they have not been employed in the great bulk of recent researches upon the haemolysis of sera, of snake-venoms, and of the group of glucosides, of which Saponin is a member. And, certainly, neither is applicable to a clinical investigation of the blood, in various diseases, where the haemoglobin-value of the corpuscle varies widely.

COLORIMETRIC METHOD FOR A CLINICAL INVESTIGATION.

I prepared the followed colorimetric method. As already described, a number of equal samples of washed corpuscles of the blood under examination were prepared. To one of these an excessive dose of Saponin was given. The resulting haemolysis was indubitably complete, both from the perfect transparency of the fluid and from the absence of any sediment of erythrocytes, after centrifugation. The bulk of the tube-contents was 2 cc. Therefore, the depth of colour/
colour of the fluid in this tube, gave a standard of complete haemolysis for all equal samples of the same blood, made up to the same bulk, by adjustment of the saline & saponin solutions added. From this standard of complete haemolysis, a number of diluted samples were prepared. The dilutions were made by accurate adjustment of proportions of the original standard and of saline solution, by means of graduated pipettes. These diluted samples placed in small tubes, were arranged to represent a series of intermediate degrees of haemolysis, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%; and with the original standard of complete haemolysis and a tube containing saline solution only, gave a complete scale of degrees of haemolysis from 0% to 100%. When, therefore, a sample of washed cells, brought to a bulk of 2cc. with saline solution and a certain dose of Saponin solution, had been incubated for a fixed period at 37°C., it was centrifuged. From the supernatant fluid, a small quantity was drawn off by pipette, and placed in a small tube equal in calibre to those which contained the series of standards already prepared. That standard which most nearly matched its tint gave the degree of haemolysis effected by the known dose of Saponin. In this way the amount of intermediate/
intermediate Haemolysis in each of a number of tubes, containing equal samples of washed erythrocytes and varying doses of Saponin, could be expressed by a figure and one which was approximately accurate. For each case investigated, a fresh series of standards were of course prepared. And, in each case, the standard of complete haemolysis was 100%, no matter what the actual haemoglobin value of the blood, as compared with that of health, might be.

The method is neither tedious, nor difficult to work. I proceed to give an example.

Entire blood used.

Blood (my own).

Saponin solution 0.02grms. per 100cc.

Incubator 37°C. Incubation period 2 hours.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20c.mm. +</td>
<td>1.78cc.</td>
<td>+0.20cc.</td>
<td>20-30%</td>
</tr>
<tr>
<td>2</td>
<td>&quot; +</td>
<td>1.76cc.</td>
<td>+0.22cc.</td>
<td>50-60%</td>
</tr>
<tr>
<td>3</td>
<td>&quot; +</td>
<td>1.74cc.</td>
<td>+0.24cc.</td>
<td>80-90%</td>
</tr>
<tr>
<td>4</td>
<td>&quot; +</td>
<td>1.72cc.</td>
<td>+0.26cc.</td>
<td>100%</td>
</tr>
<tr>
<td>5</td>
<td>&quot; +</td>
<td>1.48cc.</td>
<td>+0.50cc.</td>
<td>100%</td>
</tr>
<tr>
<td>6</td>
<td>&quot; +</td>
<td>1.98cc.</td>
<td>+0.00cc.</td>
<td>0%</td>
</tr>
</tbody>
</table>

In the above Table, Tube 6 is the control. 
Tube 5 gave the standard of complete haemolysis, and the diluted standards.

The Table shows the moderate precision of the method, in fixing intermediate degrees of haemolysis; and, in so far, its great superiority to the very vague differentiation that is given, by the use of such terms as "slight haemolysis", "moderate haemolysis", etc. But it was more important that this method should be exact in fixing the point of complete haemolysis, for the main object in the investigation of a series of doses of Saponin was, to find the least one which would completely haemolyse the sample of erythrocytes. This, therefore, raises the second, and more important point of the end point of haemolysis.

(2). DETERMINATION OF COMPLETE HAEMOLYSIS.

The test of complete haemolysis in all recent researches on Haemolysis, is the perfect transparency to the eye of the tube-contents after being shaken up. In the Table just given, while Tube 3, before centrifugation, showed a slight opalescence, Tube 4 lined quite clear. Therefore, Tube 4 showed a Complete Haemolysis both by the Colorimetric Method
I had devised, and by the usual gauge of transparence. But, after centrifugation it was not difficult to observe in the bottom of Tube 4, a faint red powder of Sediment which the microscope proved to consist of unhaemolysed erythrocytes. So that both these methods were found to be at fault, in the determination of the end point of haemolysis.

Lamb (10) in his researches on the haemolysis of certain snake venoms, determines the point of complete haemolysis by the transparence of the fluid. But he hints at inadequacy of the test, for in the description of his technique he advises that where there is any doubt as to the perfect transparence of the fluid, the centrifuge and the microscope should be used to confirm the matter. But his subsequent tables of results do not show that this microscopic confirmation was ever employed: and it may be inferred that it was not. The same thing may be confidently said of the enormous mass of researches on the action of haemolytic sera, in connection with the subject of Immunity. In them, too, the end-point of haemolysis has been entirely determined by the inaccurate test of transparence. I will presently show that the error, so introduced, is not trifling or negligible, but serious.
serious and important. As these researches claim to be of an exact and quantitative nature, it is of the highest importance if it can be shown that the determination of the end point of haemolysis, which is the foundation on which their results are built, is obtained by a faulty test.

ENUMERATIVE METHOD OF DETERMINING COMPLETE HAEMOLYSIS.

In consideration of a more exact means of determining Complete Haemolysis, it occurred to me to count this small remnant of cells. In those cases, therefore, where centrifugation showed this faint sediment and indicated that haemolysis was almost, but not quite complete, the supernatant fluid was not removed. The tube was again shaken up, to produce an equal suspension of the remaining red-cells. A drop of this suspension was placed in the Zeiss counting chamber, and the number of red-cells per c.mm. obtained in the usual way. The total bulk of the tube contents and the original number of red-cells exposed to haemolysis being known, it was easy to get first the total number of unhaemolysed cells, then the total of haemolysed cells, and to express the latter figure as a percentage/
percentage of the original number of cells exposed to haemolysis.

The following example will illustrate.

Entire blood of myself used.
Sap.Soln. 0.02grms. per 100cc.
Incubator 37°C.
Incubation-period 2 hours.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1</td>
<td>20c.mm</td>
<td>1.72cc.</td>
<td>0.26cc.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>1.70cc.</td>
<td>0.28cc.</td>
<td>98%</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>1.68cc.</td>
<td>0.30cc.</td>
<td>99.23%</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>1.44cc.</td>
<td>0.54cc.</td>
<td>100%</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>1.98cc.</td>
<td>0.0cc.</td>
<td>0%</td>
</tr>
</tbody>
</table>

I now give the materials from which these figures were obtained.

**ZEISS COUNTING-CHAMBER PREPARATIONS.**

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>105 red-cells in 400 squares.</td>
<td>41 cells per 400 squares.</td>
<td>0 cells in 1200 squares.</td>
</tr>
<tr>
<td>Total number of red-cells in tube = 105x4000x2000</td>
<td>Total number of red-cells in tube = 41x4000x42000</td>
<td></td>
</tr>
<tr>
<td>= 2,100,000.</td>
<td>= 82,000.</td>
<td></td>
</tr>
</tbody>
</table>
The ordinary enumeration of my blood, by the Zeiss haemocytometer, gave 5,250,000 erythrocytes per c.mm. The erythrocyte content of each tube in the above table was, therefore, 105,000,000. Simple calculation gives as percentages, 2% of unhaemolysed cells in Tube 2, and 0.77% in Tube 3. The percentages of haemolysed cells in these tubes were, therefore, 98%, and 99.23% respectively, as determined by this method of enumeration.

I next proceeded to carry out the Colorimeter method in these same tubes. I give side by side the results obtained by both methods. Some of the details already given in the first table are omitted.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.26 cc.</td>
<td>90-100%</td>
<td>Distinct red spot</td>
</tr>
<tr>
<td>2</td>
<td>0.28 cc.</td>
<td>98%</td>
<td>100% Faint spot</td>
</tr>
<tr>
<td>3</td>
<td>0.30 cc.</td>
<td>99.23%</td>
<td>100% Faint powder</td>
</tr>
<tr>
<td>4</td>
<td>0.54 cc.</td>
<td>100%</td>
<td>100% None</td>
</tr>
</tbody>
</table>

The above Table shows clearly that, as haemolysis approaches completion, the Colorimetric Method
Method becomes inaccurate while the Enumerative Method is extremely exact. Further it becomes at once apparent; that the latter method might be extended to all the intermediate stages of haemolysis. For that purpose it promised an incomparably greater degree of precision than the Colorimetric Method could give.

For the determination of Complete Haemolysis, its superiority over both this and the Transparence-Method was the equally decisive one of the microscope over the unaided eye.

**VERIFICATION OF ENUMERATIVE-METHOD.**

But it was possible that fallacies might vitiate the Enumerative-Method. And, in consideration of these, an important question became raised as to the mode of Saponin-haemolysis. In a collection of erythrocytes, exposed to the action of Saponin, did haemolysis proceed by a successive total destruction of cell by cell; or by a simultaneous partial and progressive release of Haemoglobin, from all the cells? For example, when in a case, a haemolysis of 50% was determined, did this represent the total Haemoglobin content of half the erythrocytes? Or did it represent half/
half the Haemoglobin-content of the total number of erythrocytes? I am not aware that such a question, as the nature of haemolytic action, has ever been stated. And yet, it may have a wider significance, than the verification or the vitiation of the Method of Enumeration, I proposed to adopt. It may be important with regard to the division of physiologists into two parties, over the histology of the mammalian erythrocyte. A red cell containing its Haemoglobin free, within a protoplasmonic envelope, might be expected to release its Haemoglobin in a different way, from one whose Haemoglobin was enmeshed in addition in a stromatous network. In the former case, a total successive haemolysis of cell by cell would, prima facie, be the more probable. In the latter, a simultaneous progressive oozing of Haemoglobin, from all the cells might occur.

I was able to bring the matter to proof, by a combination and comparison of the results obtained by Colorimetric & Enumerative Methods, which I had devised. Thus, if, by enumeration, I found a disappearance of 50% of the original number of cells, the measurement of the Haemoglobin in the supernatant fluid of the tube after centrifugation, would determine the point/
point at issue. If the Haemoglobin-content of this fluid were greater than 50%, the remaining red cells, though visible to enumeration, must have suffered a partial loss of their colour-matter. But, if the Haemoglobin proved to be just 50%, that Haemoglobin would be fully accounted for by the escape from the destroyed cells, and, the red-cells visible under the microscope, would be proved to have retained their complement of Haemoglobin.

The latter alternative was shown to occur, in a quite decisive way. The following example will serve to illustrate.

**EXAMPLE.**

Blood of myself, washed three times, as usual.

Final blood suspension contained 46,400,000 red cells per 0.5 cc.

Saponin Solution 0.004 grms. per 100cc.

Incubator 37° C. Incubation period.

2 hours.

Each tube contains 0.5cc. of washed blood-suspension brought to a bulk of 2cc. with saline and Saponin solutions.
Tubes | Dose of Sap. Soln. | Haemolysis by Enumeration | Haemolysis by Colorimeter
--- | --- | --- | ---
1 | 0.06cc. | 20.26% | 20%
2 | 0.08cc. | 44.96% | 40-50%
3 | 0.10cc. | 83.19% | 80%
4 | 0.14cc. | 99.75% | 100%
5 | 0.25cc. | 100% | 100%

Tube 5 was taken as the standard of complete haemolysis. On centrifugation, no sediment of erythrocytes was obtained, even to microscopic examination. From it the various standards of intermediate haemolysis were obtained by dilution as described. The enumeration was first made in each tube. The tube was then immediately centrifuged, and the colorimetric determination of the supernatant fluid made. Each of the two methods was, therefore, tested on the same tube.

Such comparison of the results of these two methods was made several times; and decisively confirmed.

This confirmation of the Method of Enumeration is very satisfactory. It enables one to use with/
with confidence, a very exact and delicate method, which is superior to the Colorimetric Method in fixing intermediate degrees of haemolysis, and is incomparably superior to it and to the test of transparence in determining the end point of haemolysis. It, perhaps, does not afford direct evidence, in favour of the view that the erythrocyte contains its Haemoglobin free within an envelope, without an internal stromatous framework; but it might fairly be considered corroborative, and, as such, might be added to the body of evidence already existing in support of that view.

RETARDATION OF HAEMOLYSIS JUST BEFORE COMPLETION.

On the application of this enumerative method the inaccuracy of apparent transparence, as a test of Complete Haemolysis, was soon completely established. When a tube whose contents seemed perfectly transparent to the naked eye, was examined by this new method, a few cells remaining showed that haemolysis was almost but not quite complete. But it was further shown that the error introduced was not small, but great. For example when a certain dose of Sapconin produced in 2 hours a haemolysis of 99%, the additional Sapconin/
Saponin required to complete haemolyses was not small, but very considerable. In fact the increment formed a large proportion of the total haemolytic dose. The observation is a new one, and has such important consequences, that I give below a series of results to illustrate it. In all these, the Saponin Solution was .004 grms. per 100 c.c. The blood-suspensions were washed, and counted as described. The Incubator temperature was 37°0, and the incubation-period was 2 hours.

RETARDATION AT COMPLETION OF HAEMOLYSIS.

1. Blood of myself. Health. 0.5cc. of blood-suspension contains 79,400,000 erythrocytes.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5cc. + 1.34cc. + 0.16cc. 98.62%</td>
</tr>
<tr>
<td>2</td>
<td>&quot;  + 1.26cc. + 0.24cc. 99.95%</td>
</tr>
<tr>
<td>3</td>
<td>&quot;  + 1.18cc. + 0.32cc. 100%</td>
</tr>
</tbody>
</table>

2. Blood of A.T.M. Health. 0.5cc. of blood-suspension contains 57,800,000 erythrocytes.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5cc. + 1.34cc. + 0.16cc. 99.62%</td>
</tr>
<tr>
<td>2</td>
<td>&quot;  + 1.30cc. + 0.20cc. 99.96%</td>
</tr>
<tr>
<td>3</td>
<td>&quot;  + 1.26cc. + 0.24cc. 100%</td>
</tr>
</tbody>
</table>
3. Blood of J.P.M. 

Health.

0.5cc of Blood-suspension, contains

74,000,000 erythrocytes.

<table>
<thead>
<tr>
<th>TUBES</th>
<th>BLOOD SUSPENS.</th>
<th>SAL. SOLn.</th>
<th>SAP. SOLn.</th>
<th>HAEMOLYSIS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5cc + 1.32cc + 0.18cc</td>
<td>93.52%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&quot; + 1.28cc + 0.22cc</td>
<td>98.03%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&quot; + 1.24cc + 0.26cc</td>
<td>99.97%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&quot; + 1.20cc + 0.30cc</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. W.P. aet.54 

Pernicious Anaemia.

0.5cc of Blood-suspension contains

34,700,000 erythrocytes.

<table>
<thead>
<tr>
<th>TUBES</th>
<th>BLOOD SUSPENS.</th>
<th>SAL. SOLn.</th>
<th>SAP. SOLn.</th>
<th>HAEMOLYSIS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5cc + 1.38cc + 0.12cc</td>
<td>93.46%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&quot; + 1.34cc + 0.16cc</td>
<td>99.86%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&quot; + 1.30cc + 0.20cc</td>
<td>99.96%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&quot; + 1.22cc + 0.28cc</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Mrs H.L. aet.59 

Pernicious Anaemia.

0.5cc of Blood-suspension contains

29,200,000 erythrocytes.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5cc.</td>
<td>1.44cc.</td>
<td>0.06cc.</td>
<td>30.8%</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>1.40cc.</td>
<td>0.10cc.</td>
<td>90.9%</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>1.36cc.</td>
<td>0.14cc.</td>
<td>98.98%</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>1.32cc.</td>
<td>0.18cc.</td>
<td>99.92%</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>1.28cc.</td>
<td>0.28cc.</td>
<td>100%</td>
</tr>
</tbody>
</table>


0.5cc. of Blood-Suspension contains 40, 600,000 erythrocytes.


<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5cc.</td>
<td>1.34cc.</td>
<td>0.16cc.</td>
<td>99.81%</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>1.30cc.</td>
<td>0.20cc.</td>
<td>99.97%</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>1.26cc.</td>
<td>0.24cc.</td>
<td>100%</td>
</tr>
</tbody>
</table>

Many more similar examples could be given, but these are enough to prove, beyond doubt, that, as the haemolysis of any given sample of washed erythrocytes by Saponin approaches completion, the increment of dose required, to complete haemolysis, is out of all proportion to the number of cells remaining unhaemolysed.

Thus in Table 5 while 0.06cc. of Saponin Solution produces 30% haemolysis, and 0.10cc. produces 90%.
90%. it requires a dose of 0.22cc. to complete haemo-
molysis i.e. double the amount that produces 90% haem-
molysis.

These tables further show, that this in-
crement of dose to complete haemolysis is proportion-
ally greatest between 99%, and 100% or Complete Haem-
molysis. Thus in Table 4 the amount of Saponin has
to be increased from 0.16cc. to 0.28cc. to carry hae-
molysis from 99.66% to 100% — an increment of 1/3 of
the total haemolytic dose. This was in a case of
disease — Pernicious Anaemia. But the same fact is
as strikingly demonstrated in healthy blood in
Table 2, where the increment required to bring 99.62%
to Complete Haemolysis, is also 1/3 of the total haem-
olytic dose.

INADEQUACY OF TEST OF TRANSPARENCY.

But, it is impossible for the unaided eye to
discern a defect of transparence in a tube, the enumer-
ation of whose contents shows a haemolysis of 99.5%,
from complete transparence, in a tube where enumeration
shows haemolysis to be complete. The human eye is
quite unable to discriminate such fine shades of
difference/
difference, and yet very great alterations, in the amount of the active solution, are required to effect these inappreciable changes in transparence.

I have already pointed out, that the vast majority of recent and current researches on haemolysis of different substances and especially of the natural and acquired haemolysin's in sera, determine Complete Haemolysis by the test of transparence; and that this determination is the great key by which their results are obtained. I have also shown, in the case of Saponin, that this test is inadequate, and that its use introduces error which is not small, but very gross. It is still possible that a haemolytic serum may act differently, and in the closing stages of haemolysis, the employment of the test of transparence may not be liable to this serious fallacy. But, it is much more likely that this remarkable feature of Saponin-haemolysis also marks the action of other haemolytic substances, which attack the envelope of the erythrocyte.

I will show later, that in the case of Saponin it is based, not on the nature of the haemolytic substance, but on the peculiar structure of the collective tissue on which that substance act - and therefore, that it probably accompanies the action of other/
other haemolysins. In the meantime, it may be said, that the demonstration of this fact in Saponin haemolysis, places the accuracy of much recent work on Immunity, which is based on the quantitative results of haemolytic researches under grave suspicion.

RETARDATION BY HAEMOLYSIS IN POINT OF TIME.

The fact can be further confirmed in another way. In the example given below, I selected a dose of Saponin which, for the sample of washed cells under investigation, would effect on almost complete haemolysis in 2 hours. During the period of incubation, I determined the degree of haemolysis by enumeration at half-hour intervals.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5cc. + 1.38cc. + 0.12cc.</td>
<td>94.88%</td>
<td>99.28%</td>
<td>99.88%</td>
</tr>
</tbody>
</table>

The above result demonstrates, in another way/
way, the same fact, the great retardation that takes place in haemolysis as it very nearly approaches completion. The former tables, showed the great increment of dose required to convert a haemolysis of 99% into 100%. The latter shows how a dose that produces in two hours an almost complete haemolysis, produces in half an hour a 94% haemolysis; in one hour 99%, and yet in another hour is unable to carry haemolysis to completion.

In view of this established fact I determined to adopt as the end-point, not Complete Haemolysis but Haemolysis almost Complete, i.e. between 99·8% and 99·97%. For Complete Haemolysis might not be an exact point: it might easily be overreached. But the end-point I proposed to adopt expressed a definite stage which could easily be used for comparative purposes.

CLINICAL RESULTS. FIRST SERIES.

With this specialised technique, in the preparation of equal samples of washed erythrocytes, in the method of determining haemolysis, and in the end-point of haemolysis selected, I began to collect results/
results in condition of health and disease.

The procedure in each case investigated was as follows. The number of erythrocytes in the equal samples of washed blood suspensions was carefully determined. By experiment and using a series of doses of Saponin Solution, that dose was obtained which effected an almost complete Haemolysis in 2 hrs. at 37°C. By calculation the amount of Saponin solution which would produce an equal haemolysis in 100 million erythrocytes was obtained.

Thus in Table 1 p. 30, with my own blood, it was shown that 0.24cc. of Saponin Solution produced 99.95% haemolysis in 2 hours at 37°C. And here the sample of blood-suspension contained 79,400,000 erythrocytes. Therefore the haemolytic dose for 100 million erythrocytes would be

\[
\frac{0.24 \times 100,000,000}{79,400,000} = 0.30\text{cc.}
\]

In the three cases of health, as shown in Tables 1, 2, & 3, the conversion into the haemolytic dose for the standard number of erythrocytes was as follows:

<table>
<thead>
<tr>
<th>Name</th>
<th>No. of erythrocytes in sample</th>
<th>Sap.Sol. Haemolysis of Erythrocytes</th>
<th>Haemolytic Dose for standard No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.Me.N</td>
<td>79,400,000</td>
<td>+0.24cc. 99.95%</td>
<td>0.30cc.</td>
</tr>
<tr>
<td>A.T.M.</td>
<td>57,800,000</td>
<td>+0.20cc. 99.96%</td>
<td>0.34cc.</td>
</tr>
<tr>
<td>J.P.M.</td>
<td>74,000,000</td>
<td>+0.26cc. 99.97%</td>
<td>0.35cc.</td>
</tr>
</tbody>
</table>
Tables 4, 5, & 6 p. show the results obtained in three cases of Pernicious Anaemia. Their conversion was as follows:

<table>
<thead>
<tr>
<th>Name</th>
<th>No. of Erythrocytes</th>
<th>Haemolytic Dose</th>
<th>No.of Erythrocytes</th>
<th>Sap.Sol.</th>
<th>Haemolysis of Erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.P.</td>
<td>34,700,000</td>
<td>0.20cc</td>
<td>99.96%</td>
<td>0.57cc.</td>
<td></td>
</tr>
<tr>
<td>Mrs. H.L.</td>
<td>29,200,000</td>
<td>0.18cc</td>
<td>99.92%</td>
<td>0.61cc.</td>
<td></td>
</tr>
<tr>
<td>Mrs. H.M.</td>
<td>40,600,000</td>
<td>0.20cc</td>
<td>99.97%</td>
<td>0.49cc.</td>
<td></td>
</tr>
</tbody>
</table>

These cases of Pernicious Anaemia, therefore, show a considerable increase in the resistance of their erythrocytes to destruction by Saponin, as compared with that of the red corpuscles of health.

ERROR OF TECHNIQUE.

During six months, many results in different pathological conditions were collected and compared in this way. It is sufficient, however, to have given the few examples above; for at the end of this period, I discovered a fallacy in the technique employed. This error was so serious, as render all these results worthless. It proceeded from the fact that, according to the corpuscular richness of the blood, the number/
number of red cells in 0.5cc. samples of washed blood-suspension varied widely, from 10 millions, in very severe oligocythaemia, to between 80 & 90 millions in the blood of health. The correctness of my results depended on Bashford's statement already quoted p. that "a multiple of the solvent dose of glucoside dissolved the same multiple of the standard quantity of blood", and on the confirmatory experiment I made p.

**INFLUENCE OF BULK OF FLUID UPON HAEMOLYSIS.**

When I now repeated that original experiment, I obtained an entirely different result. I give below a clear example. The Saponin solution 0.004grms. per 100cc. Incubator temperature 37°C. Incubation period 2 hours. These three factors continue constant hereafter, unless where specially mentioned.


0.5cc. washed blood-suspension contains 44,400,000 erythrocytes.

Tubes.
In this example Tube 2 contains half the number of red cells in Tube 1. Therefore half the dose of Saponin given in Tube 1 should according to Bashford's statement, produce an equal haemolysis in Tube 2, in the same time. But in the above example, it is very far indeed from doing so. The haemolysis effected in Tube 2 is approximately only half that in Tube 1. But on consideration of the conditions of the above experiment it will be seen, that in Tube 2, while the number of erythrocytes and the doses of Saponin have been halved, the total bulk of the tube contents has been kept identical with that of Tube 1. That is to say, the factor of bulk of fluid has not been adjusted, correspondingly to the alteration of the other factors. The enormous discrepancy in the results, therefore, points to an important influence of this factor of bulk of fluid upon haemolysis.

In the following experiments, therefore, this factor of bulk was alone altered. The other conditions were kept unchanged.
Blood of myself.

0.5 cc. of Blood suspension contains
54,300,000 erythrocytes.

<table>
<thead>
<tr>
<th>TUBE</th>
<th>BULK OF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5 cc.</td>
</tr>
<tr>
<td>2</td>
<td>0.5 cc.</td>
</tr>
<tr>
<td>3</td>
<td>0.5 cc.</td>
</tr>
<tr>
<td>4</td>
<td>0.5 cc.</td>
</tr>
</tbody>
</table>

At first sight it would appear that the approximation of haemolysis in Tubes 3 & 4, viz., 99.93% & 95.74% is much greater than in Tubes 1 & 2 85.5% & 44.07%. But the great retardation which has been shown to occur in haemolysis as it approaches completion, removes entirely this apparent inconsistency.

This last Table confirms what the other had suggested, the importance of the bulk of fluid on which the cells are suspended, upon Saponin haemolysis. I, therefore, prepared a series of tubes in some of which the three factors, amount of Saponin, number of erythrocytes, and bulk of fluid, were equally and simultaneously altered: while in others the first two factors were equally altered, but the factor of bulk was left unchanged.
Blood of myself.

0.5cc. Blood suspension contains 50 millions of erythrocytes.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1(a) 0.5cc+ 1.32cc</td>
<td>+0.12cc</td>
<td>2cc</td>
<td>99.8%</td>
<td></td>
</tr>
<tr>
<td>2(a) 0.25cc+ 1.69cc</td>
<td>+0.06cc</td>
<td>2cc</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>3(a) 0.25cc+ 0.69cc</td>
<td>+0.06cc</td>
<td>1cc</td>
<td>99.24%</td>
<td></td>
</tr>
<tr>
<td>1(b) 0.5cc+ 1.26cc</td>
<td>+0.24cc</td>
<td>2cc</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>2(b) 0.25cc+ 1.63cc</td>
<td>+0.12cc</td>
<td>2cc</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>3(b) 0.25cc+ 0.63cc</td>
<td>+0.12cc</td>
<td>1cc</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

Let us consider, first, the results obtained from the tubes of Series (a). Tube 2a demonstrates, that when the number of erythrocytes and the amount of Saponin are equally reduced, but the original bulk of content retained, an equivalent haemolysis is not obtained. Tube 3a demonstrates, that when the factor of bulk is adjusted equally to the variation of the other two factors - the number of erythrocytes and the amount of Saponin, the haemolysis is now exactly equivalent, so far as the range of experimental accuracy will permit.

Series (b) apparently contradicts Series (a), and/
and reasserts Bashford's statement, and my original confirmation of that statement. In reality it does neither of these things. For in Tube (b) the dose of Saponin produces 100% Haemolysis. That appears to be an exact stage, but most probably it is not exact and is obtained by an amount of Saponin, which exceeds the complete haemolytic dose. That excess of Saponin is able to counteract the influence of the factor of bulk in Tube 2 (b). That factor in Tube 2 (b) is not called into extreme play, as in the previous Table p. where the difference in bulk is between 1cc and 4cc. But though its operation in Tube 2(b) is not indicated in the determination at the end of 2 hours, it is most probable that evidence of it would have been obtained, had a determination of haemolysis been made in Tubes 1(b) and 2(b) at the end of one hour.

THE FACTOR OF TIME.

That suggestion introduces the question of a fourth factor, the factor of time. And the following result shows that it, too, plays an important part in haemolysis.

Blood/
Blood of myself.
0.5cc. of Blood-Suspension contains 50,200,000 erythrocytes.

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Blood Suspension</th>
<th>Sal. Sap. Soln.</th>
<th>Soln. Bulk</th>
<th>Haemolysis after 1/2 hour</th>
<th>1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5cc.</td>
<td>1.28cc+0.22</td>
<td>2cc.</td>
<td>63%</td>
<td>97.88%</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>0.26cc+0.22</td>
<td>1cc.</td>
<td></td>
<td>98.22%</td>
</tr>
</tbody>
</table>

In Tubes 1 & 2 the relation of the four factors is as follows. The number of erythrocytes, and the dose of Saponin are equal in both, but the factor of bulk is halved in Tube 2. The variation of this factor, brings into operation a variation in the rapidity of haemolysis in the two tubes, so that at the end of half an hour, there is 63% haemolysis in Tube 1, while there is 98.22% in Tube 2 at the same point of time. But in twice this period, i.e. one hour, Tube 1, with a bulk twice that of Tube 2, reaches 97.88 haemolysis, which is, approximately, equal to that attained by Tube 2 in half an hour.

THE FOUR FACTORS IN A HAEMOLYTIC EXPERIMENT: THEIR MUTUAL RELATION.
The above series of experiments on the interplay of these four factors, show Bashford's statement, that "a multiple of the solvent dose of glucoside dissolved the same multiple of the standard quantity of blood to be inadequate in two respects (1) with regard to the bulk of fluid in which red cells and Saponin interact, and (2) with regard, to the factor of time".

As it stands, the statement is accurate, for it mentions only the two factors of the number of erythrocytes and the amount of Saponin: and variation of these two factors by multiplication or division is followed automatically by equal variation in the third factor of bulk. But these three factors having been properly adjusted, the fourth factor of time remains undisturbed. But it is inadequate, in that it takes no account of the enormous influence, which the bulk of fluid exerts upon the haemolysis of these glucosides. And, it is also, seriously defective in making no mention of the factor of time. In fact both Hedon & Bashford entirely neglect this important factor. Hedon(7) in comparing the action of Saponin with that of Cyclamin and Solanin, describes the action of the former as slower than the latter, haemolysis taking place with Saponin, "avec un retard de quelques minutes/
minutes." Bashford (6) seems to have tacitly adopted this standard of practically instantaneous haemolysis. At any rate, in his tables he does not mention any period at which readings of complete haemolysis were made, and it can only be assumed that they were made shortly after the doses of Saponin were added.

Throughout my investigations, the determinations of haemolysis were made at the end of 2 hrs. And it was absolutely plain in these investigations that the action of Saponin was not exhausted at once, or in "a few minutes", but proceeded throughout this period of 2 hrs. It is evident, therefore, that the dose of Saponin which will instantaneously effect complete haemolysis, will be much greater than that one which will take 2 hours to produce an equal result. The term "Complete Haemolysis" as employed in the researches just mentioned is tacitly assumed to be an absolute one and independent of the factor of time. But, in effect, it is Complete Haemolysis determined at an indefinite period, shortly after the preparation of the experiment. As such it sets up an entirely different standard from that obtained by a reading of results at the end of 2 hours.

The/
The selection of such a period was arbitrary. But the fixation of a constant time period, was necessary to allow a just comparison of results, and the time chosen seemed to be suitable, in allowing a considerable time for the active substance to spend itself upon the reactive material. Indeed, at the outset, I believed that the action of any dose of Saponin, upon washed erythrocytes, would be completed in 2 hours at blood-temperature. But the following table shows that this assumption was very far from being true. The tubes were incubated for 6 hours, and determinations made at the periods indicated. After this, they were allowed to stand at room-temperature 15°C, and a final reading taken at the end of 22 hours from the preparation of the tubes. This table also illustrates the action of bulk of fluid upon the rapidity of haemolysis, and upon the degree of haemolysis at any given time, in fact, the interaction of the four factors in a haemolytic experiment.

Blood of myself.

0.5cc of washed blood-suspension contains 50,200,000 erythrocytes.
The selection of each a body were primarily for the period of a couple of hours, necessary to allow a short spinning of the material. The time elapsed seems to be essential in adjusting the crockery to the section of the reaction material, regardless of the character of the material. I followed that the section of any one of the several many reaction products, many to complete the following table shows that the reaction was very fast from the point given. The time was important for a complete and get information about the reactions involved.

At the time, they were allowed to stand for 48 hours at 120° C. and a slight residue was the case of the figure. The use from the determination of the figure. The figure shows the section of the body. The diagram shows the section of the body.
<table>
<thead>
<tr>
<th>PBS. BLOOD SUSP.</th>
<th>BULK</th>
<th>HAEMOLYSIS after 1 hr. 2 hrs. 3 hrs. 4 hrs. 6 hrs. 22 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 cc + 1.28 cc + 0.22 cc</td>
<td>2 cc</td>
<td>97.88% 99.88% 99.88% 99.88% 99.92% ----</td>
</tr>
<tr>
<td>0.5 cc + 3.28 cc + 0.22 cc</td>
<td>4 cc</td>
<td>57.44% 89.12% 94.56% 96.72% 98.24% 98.24%</td>
</tr>
<tr>
<td>0.25 cc + 1.39 cc + 0.11 cc</td>
<td>2 cc</td>
<td>53.92% 84.64% 91.68% 92.16% 95.20% 98.16%</td>
</tr>
<tr>
<td>0.25 cc + 3.39 cc + 0.11 cc</td>
<td>4 cc</td>
<td>0.00% 0.00% 0.00% 0.00% 20.00% 60.32%</td>
</tr>
<tr>
<td>0.5 cc + 1.38 cc + 0.12 cc</td>
<td>2 cc</td>
<td>44.00% 78.00% 89.28% 93.22% 95.24% 98.40%</td>
</tr>
<tr>
<td>0.25 cc + 1.44 cc + 0.06 cc</td>
<td>4 cc</td>
<td>6.00% 12.00% 40.00% 46.00% 65.44% 68.32%</td>
</tr>
</tbody>
</table>

Such a table may be graphically illustrated (two graphic charts). The above table may be divided into two groups, Tubes 1 to 4 showing the effect of variation of bulk with a large dose of Saponin, and Tubes 5 and showing the same effect with a smaller dose. This division has been made in the graphic charts.
It establishes some new results, and confirms and extends others already shown.

1. It shows that the haemolytic action of Saponin in those doses, far from being completed in two hours, proceeds for at least six hours at blood-temperature, and even continues for many hours after at 15° C. This result does not invalidate the selection of a time-period of two hours, so long as that period is kept constant in all cases.

2. It strongly suggests, if it does not quite prove, that if haemolysis is not complete at an early period, it never becomes so.

3. It shows that where a dose of Saponin produces in two hours a haemolysis that is nearly complete, the subsequent haemolysis is very slow and small: while where a smaller dose of Saponin has produced in two hours a minor degree of haemolysis, the subsequent haemolysis is more rapid and of greater amount, but yet at the end of the period of observation does not reach the degree attained by the larger dose. This is illustrated in Tube/
Tube 1 and Tube 5. At the end of two hours the haemolysis in these tubes is respectively 99.88% and 78.00%. At the end of six hours it is respectively 99.92% and 95.24%. At the end of twenty-two hours Tube 5 shows 98.40% haemolysis, which is still short of the stage reached by Tube 1 in six hours, and even of that of Tube 1 in two hours. That is to say, the greater dose of Saponin has produced the maximum of its action in a shorter time. The smaller dose has taken a longer time to effect its maximum action, and, even at the end of the period of observation, that maximum is still short of the degree of haemolysis attained by the greater dose at a much earlier stage. This last point is more than a confirmation of the great retardation that has been shown to occur in the final stages of haemolysis. It is an amplification of it, for it shows that with smaller doses of Saponin this retardation takes place at an earlier stage of haemolysis.

4. It illustrates again, but in an even more striking way than before the enormous influence of the factor of bulk of fluid in which the action takes place upon haemolysis, and that influence is shown to/
to affect, not only the rapidity of haemolysis, but also the final degree of haemolysis. The series of Tubes 1 to 4 demonstrates this. In all these tubes, the relation of amount of Saponin to number of erythrocytes is equal, but the bulk of fluid in which haemolysis takes place is increased in a regular sequence. The result is seen in an extreme degree in comparison of Tube 4 and Tube 1. In each the dose of Saponin per quantity of corpuscles is the same, but in Tube 1 there is 99.88% haemolysis at the end of 4 hours, while in Tube 4, at the same time, there is 0%; and the result in Tube 4 at the end of twenty-two hours shows that there is not merely a retardation of haemolysis, but, in addition, an actual and large deficiency of haemolysis. To this last statement it may be objected that the time-limit of twenty-two hours is arbitrary, and that possibly, had a determination been made of Tube 4 after a still more prolonged period, the degree of haemolysis in that tube would have passed 60%, and have reached that recorded of Tube 1 in six hours. That is a natural criticism, but in discussing the explanation of these novel results I will immediately adduce reasons to show it/
it is not correct.

5. It is also shown that the operation of the factor of bulk of fluid cannot be expressed by any simple formula. The early stages of haemolysis in Tubes 1, 2, and 3 would suggest that this might be so: but the later stages of these tubes, and the whole course of haemolysis in Tube 4, show that the adjustment of the factors of bulk and time in haemolysis by Saponin is a very complex one. But that is not surprising in view of the comparison already frequently drawn between the small doses that will effect a great part of haemolysis, and the relatively very large doses that are required to complete the haemolysis. It is probable that all these facts are related, but before discussing them, it will be well to sum up the conclusions to be drawn from the variations of these factors in haemolysis.

That cannot be better done than by extending Bashford's incomplete statement. Corrected, it would run thus:—"A multiple of the solvent dose of glucoside will dissolve the same multiple of the standard quantity of blood in the same time, provided that the bulk of fluid in which interaction takes place is proportionally adjusted."

And/
And that statement has now been shown to carry the following important corollaries:

1. A relative increase of the bulk of fluid in which haemolytic interaction takes place, the other factors being the same, will increase the time-period of complete haemolysis.

2. Where the increase is extreme it will increase the time-period to infinity. i.e. it will prevent complete haemolysis from occurring.

3. A relative decrease of the bulk of fluid in which haemolytic interaction takes place, the other factors being the same, will decrease the time-period of complete haemolysis.

4. The relation between variation in bulk and the resulting variation in time of haemolysis is only one of simple proportion, when two conditions are both present, - when the haemolytic dose of active substance is complete, and when the variation in bulk is small. If either of these conditions is absent, the relation between the two factors becomes complex, and its exact formula unknown.

DISCUSSION/
DISCUSSION OF THESE SPECIAL PHENOMENA.

It is now opportune to discuss, and if possible to explain, these two striking features of Saponin haemolysis - (1) the disproportionate increment of Saponin required to carry haemolysis from 99% to complete Haemolysis; (2) the enormous influence of the bulk of fluid in which interaction takes place upon the rapidity and the degree of Haemolysis.

I shall attempt to show that these new facts as to the nature of haemolytic action, which the use of the Enumerative Method of determining haemolysis has discovered, depend on the peculiar structure of the tissue which is acted upon, and on the peculiar index of activity which is used. If I succeed in doing so, these remarkable features of Saponin haemolysis must also be transferred to other groups of haemolytic substances, which act by a solution of the red-coll envelope. These facts, interesting in themselves, will thus obtain a much wider significance.
The Retardation of Haemolysis at its Close: its Explanation. In the elucidation of this question I take as my starting-point the observation of Bashford (6.) that complete Haemolysis does not terminate the interaction of Saponin and erythrocytes. His method of proving this was to give more than a complete haemolytic dose to a quantity of washed erythrocytes, and some time after complete Haemolysis had occurred, to add a fresh quantity of washed erythrocytes. These latter were found to sink to the bottom of the tube unchanged. It was shown that the Haemoglobin did not absorb or fix Saponin, and that the interaction of the latter was entirely with some element in the cell-envelope (or stroma), and continued after complete Haemolysis.

The methods which I used allowed me to give this fact quantitative exactness.

Blood of M.P., aet. 30. Exophthalmic Goitre. 0.5 cc of washed blood-suspension contains 50 million red-cells.
The dose of Saponin in Tube 3 was excessive and produced complete Haemolysis in a few minutes. After this tube had remained one hour in the incubator (37° C.), it was thoroughly shaken up, and one half of its clear Haemoglobin-tinted fluid, i.e. 1 cc, was added to a fresh sample of washed red cells of the same individual, as follows:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Blood Suspens.</th>
<th>S.A.L.</th>
<th>S.A.P. Soln.</th>
<th>HAEMOLYSIS IN TWO HOURS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.5cc + 0.5cc + 0 cc</td>
<td></td>
<td></td>
<td>0 %</td>
</tr>
</tbody>
</table>

The conclusion is that the whole of this dose of 0.8 cc of Saponin solution has been fixed by some element in the red cells. But this amount of Saponin far exceeds the complete Haemolytic dose, for 99.6% Haemolysis was produced by a dose of 0.12 cc (vide Tube 2 of this series).

A/
A similar procedure was followed in another case: and here the limit of absorption of Saponin was reached.


0.5cc. of washed blood suspension contains 50 million red-cells.

The details of the procedure were exactly as given above, and need not be repeated. But here 1.5cc. of the Saponin Solution was given to the 0.5 sample of washed red-cells; and after 2 hours' incubation at 37°. 1 cc. of the fluid of this tube was applied as before, to a fresh sample of red-cells of C.H. with the result:-

<table>
<thead>
<tr>
<th>TUBE</th>
<th>BLOOD</th>
<th>SAL.</th>
<th>FLUID OF HAEMOLYSIS IN SUSPENS. SOLN.</th>
<th>TUBE 1.</th>
<th>TWO HOURS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.5cc.</td>
<td>0.5cc. +</td>
<td>1 cc.</td>
<td>61.25%</td>
<td></td>
</tr>
</tbody>
</table>

Now, by actual experiment 0.12cc. of Saponin Solution produced 90.24% Haemolysis of 0.5cc. of blood-suspension of C.H. It is, therefore, a fair approximation to say that 1 cc. of the fluid of Tube 2 after 2 hours' incubation at 37°C contained 0.10 cc. of active/
active Saponin solution. That is to say, the whole tube contained about 0.2 cc of active Saponin solution while 1.3 cc of the haemolytic agent had been absorbed, and neutralised, qua haemolysis, by the sample of red-cells. Now the Complete Haemolytic dose for this sample of red-cells in 2 hours, was 0.24 cc of Saponin solution. The conclusion is, that 1.3 cc of Saponin solution was fixed by 50 million red-cells of C.H. in 2 hours, although 0.24 cc of the same solution was sufficient to cause Complete Haemolysis of the same number of cells of C.H. in the same time. This series of experiments, therefore, not only bears out Bashford's observation that, after the haemolysis of a number of red-cells is complete, the remains of the erythrocytes continue to absorb Saponin; it also amplifies it, by showing that they do so in amounts which far exceed that required to effect their Complete Haemolysis in the same time. But this fact can be stated in a much more illuminating way. As expressed above, it shows that after Complete Haemolysis of a collection of red-cells, the remnants of these cells absorb a far greater amount of Saponin than was sufficient to destroy them. But the fact must be equally true of one red-cell, as of millions. It may, therefore,
therefore, be stated of a single erythrocyte that after its haemolysis, it will continue to absorb Saponin and that in far greater amount than is sufficient to entirely release its Haemoglobin. That statement gives the key to the problem being discussed.

It has already been shown, p. 25 that when Saponin attacks a number of cells, it released their haemoglobin totally and successively, cell by cell, and not simultaneously and partially from all the cells. It follows, therefore, that when a dose of Saponin is given, so small as to occupy 2 hours in effecting an almost complete haemolysis, those erythrocytes which are destroyed early in this period will still continue to absorb Saponin and thus divert it from acting upon the envelopes of still intact erythrocytes. But we have just seen that the absorption of Saponin by cells, or cell-remnants, subsequent to their Haemolysis, is in far greater amount than that which is sufficient to release their Haemoglobin. The consequence is, that in the course of a Haemolysis extending over 2 hours, this diversion of Saponin from the envelopes of un-haemolysed cells, will not be small, but considerable. And, further, as haemolysis approaches completion, this diversion will exert its influence more and more, and will/
will require a disproportionate increment of Saponin to carry Haemolysis to a termination. This argument can be best illustrated by following the progress of haemolysis during 2 hours, where the dose of Saponin effects almost Complete, or Complete Haemolysis. Such an example has already been given, but may now be repeated to give clearness to the argument.

T.B. act.58 Jaundice.

<table>
<thead>
<tr>
<th>BLOOD</th>
<th>SAL.</th>
<th>SAP.</th>
<th>HAEMOLYSIS AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUSPENS.</td>
<td>SOLn.</td>
<td>Soln.</td>
<td>½hr.</td>
</tr>
<tr>
<td>0·5cc + 1·38cc + 0·12cc</td>
<td>94·88%</td>
<td>99·28%</td>
<td>99·88%</td>
</tr>
</tbody>
</table>

Here 94% Haemolysis occurs in half an hour, and yet there is not Complete Haemolysis at the end of 2 hours. The disproportionate increment here, of course, is in the factor of time, but the same fact of retardation at the close of haemolysis is illustrated. In this example a large Haemolysis has occurred early, giving the most favourable opportunity for a great diversion of Saponin from the few remaining, and more resistant cells, to the much greater quantity of cell-debris still far from saturated with Saponin.

The/
The great retardation that marks the closing stages of haemolysis thus receives a satisfactory explanation. It is due primarily, to the fact that an erythrocyte entirely releases its Haemoglobin long before it has satisfied its affinity for the haemolytic substance. It is immediately due to the results of that fact in an aggregation of erythrocytes exposed to haemolysis, so that as a greater and greater proportion of the total number of cells are destroyed, there is a greater and greater deviation of Saponin from the haemolysis of intact cells to the remnants of destroyed cells. It is the great faculty for absorption possessed by these cell remnants that makes the increment of dose between that of almost complete, and complete haemolysis, not a simple progression, but a large multiplication. This factor, of course, operates right through the progress of haemolysis, but its influence becomes more and more extreme as haemolysis approaches completion.

It should be stated that the term "cell remnant" is provisional, and does not express any definite idea as to physical condition. These "cell-remnants" do not certainly exist as solid particles, which can be sedimented by centrifugation. The term is only/
only used to express the fact, that after total haemolysis of a number of erythrocytes by Saponin, material still remains which can absorb far more Saponin than is sufficient to entirely release their Haemoglobin.

It may even be possible that Haemoglobin may play some part in interaction with Saponin. But even if it did, the argument given above would not be weakened in the least. The fundamental fact, based on experiment, remains, that after the haemolysis of an erythrocyte - i.e. the setting free of it's Haemoglobin, Saponin continues to be absorbed and fixed, and in far greater amount than was sufficient to cause that haemolysis.

In laying such stress on this deviation of Saponin as the explanation of the Retardation at the close of haemolysis, I do not mean to assert that it is the only factor. In any sample of red-cells exposed to haemolysis, there must be variations in resistance of their envelopes to Saponin. Otherwise they would be haemolysed simultaneously. The fact that they are not, is a proof of such a variation. But it is impossible that this variation could, by itself explain the extraordinary increment of Saponin required to carry haemolysis from 99% to completion. Such/
Such variation is, therefore, a factor in producing the results, and cooperates with the influence of deviation of Saponin, but when we consider how small is the total resistance of an erythrocyte to haemolysis by Saponin compared with its total affinity for Saponin before and after haemolysis, it is evident, that variations in the haemolysis-resistance of different cells will be a still smaller proportion of the total affinity. The factor of deviation of Saponin immensely outweighs the factors of variation in resistance of individual erythrocytes. But of course the two cooperate.

EXPLANATION OF THE INFLUENCE OF BULK OF FLUID UPON THE RAPIDITY & AMOUNT OF HAEMOLYSIS.

The explanation of this fact is partly that which has been just given, and partly the established law that chemical interaction is delayed by dilution. With regard to the latter, it must be remembered that the interaction is not between two fluid substances, but between a fluid, and a solid or semi-solid substance. It is probably a solvent action resulting in a chemical union and a fluid product. That is shown/
shown by the fact that total haemolysis produces a perfectly clear fluid which yields no sediment on prolonged centrifugation, and which has lost any further haemolytic action on fresh erythrocytes. But the chemical law of dilution does not explain entirely either the great delay in the interaction, or the actual defect of haemolysis after a prolonged period. The fact that the erythrocyte, after haemolysis, is still far from satisfied in its affinity for the lytic substance, bears the most important share in the result. For the retarded haemolysis gives more prolonged play to this latter influence, and so allows a much greater deviation of Saponin than takes place in greater concentration. We, therefore, obtain an explanation of the fact already noted, that, in dilution, the retardation of haemolysis takes place at a much earlier stage, and finally stops considerably short of that attained by an equal, but more concentrated dose. To put the matter briefly, in diluted preparations of erythrocytes and haemolytic fluids, haemolysis is in the first place retarded by the effect of that dilution upon chemical interaction. But the great deficiency of haemolysis at the end of a long period in such cases is caused by the excessive deviation of Saponin to some/
some element in the haemolysed erythrocytes. That
deviation occurs in every collection of erythrocytes
exposed to Saponin, apart from the concentration or
dilution of the preparation. But it has specially
favourable conditions for doing so, in the retarded
haemolytic action of diluted preparations. If the
Table on p. is referred to, it will be seen that
this explanation is illustrated there in every particu-
lar.

THESE SPECIAL PHENOMENA OF SAPONIN HAEMOLYSIS
PROBABLY CHARACTERISTIC OF OTHER
HAEMOLYTIC SUBSTANCES.

If the above explanation is correct, and
these remarkable features of Saponin Haemolysis are
proved to depend, not on any peculiar action of the
lysin, but on the special nature of the complex tissue
acted upon – special both in the structure of the unit,
and in the aggregation of units, then there are strong
grounds for believing that they will, also, mark the
action of other groups of haemolytic substances which
also effect their action by a solution of the envelope
of the erythrocyte. It would be specially important
if these phenomena accompanied the haemolytic actions
of the natural and acquired lysins in animal sera.
In/
In that event, at least two important conclusions could immediately be drawn.

(1). In the first place, the determination of Complete Haemolysis by the test of Transparency would be shown to introduce very gross error, and to be quite unsuitable for accurate quantitative work.

(2). Secondly, many phenomena of serum-haemolysis which hitherto have been attributed to the peculiar action of these haemolysins and upon which a vast structure of theory has been reared in connection with the subject of Immunity, would very probably be found to depend upon the fundamental fact, & its consequences - namely that an erythrocyte releases its Haemoglobin, long before it has satisfied its affinity for the lytic substance. It is of course beyond the scope of this investigation to test these possibilities. But it is fair and proper to point out that these phenomena of Saponin-haemolysis may be relevant to the action of other haemolytic substances.
The reasons for this probability are:—

(1). Their occurrence in Saponin-haemolysis is shown to depend not on the nature of the haemolytic substance, but on the special structure of the red-cells.

(2). Their occurrence with Saponin was only detected by so delicate a means as the Enumerative Method: and was not detected by the test of transparence, which is universally employed in researches on snake venoms and haemolytic sera.

REMEDY OF ERROR OF TECHNIQUE.

The discovery of the error of technique introduced by the use of different numbers of erythrocytes in the same bulk of fluid, led a good deal further than was expected, and brought to light interesting and important facts of haemolytic action which, in the meantime, are only shown to occur with Saponin, but are probably of a much more general nature. Having completed the study of the different factors cooperating in a haemolytic experiment, and of the influence of their variation upon haemolysis, it was now/
now necessary to return and devise some remedy for this fallacy.

The tables given, have shown how gigantic this error might be. And as, in my clinical investigations, the corpuscular content of the samples of blood-suspension varied from 10 millions to 80 millions it was apparent that in many cases the error was very great. Two possible remedies suggested themselves.

(1). The first was to draw up a table of complete haemolytic doses of Saponin for a graduated series of blood-corpuscle suspensions, all in 2cc bulk, but with a corpuscular content ranging from 10 to 80 millions. Haemolysis, as before would be determined in 2 hours. This would be a cumbrous and not very accurate procedure; but at least, it would promise to redeem the many results I had already collected.

(2). The alternative was to adjust the blood-corpuscle-suspension so that in every case, and independant of the original erythrocyte content in the entire blood, a blood-suspension containing the same number of corpuscles in an equal sample, say 0.5 cc. would/
would be obtained. This second result would be much the more satisfactory of the two, but at first sight it seemed far more difficult to attain. But the problem proved not to be difficult, and was solved as follows:

**CLINICAL METHOD OF PREPARING EQUAL SUSPENSIONS OF WASHED ERYTHROCYTES.**

The standard blood-suspension, which I aimed at obtaining, in every case, was 50 million erythrocytes per 0.5cc sample.

To obtain 5 such samples, and some in addition for the purpose of enumeration and control, I would require roughly 300 millions red-cells from each patient. 60 cmm of entire blood, in a healthy man, would give me that quantity. But in blood, where the erythrocytes were 1 million per Cmm, 300cmm of blood would be required. For intermediate degrees of oligocytæmia, the intermediate quantities of blood necessary were similarly calculated. A little defect or excess of entire blood drawn was of course immaterial.

The quantity of blood necessary was collect-
collected in a tube containing about 5cc. of 0.85 saline. Washing (shaking up, centrifugation and pipetting off) was carried out three times. The final sediment was made into a blood-suspension of about 3cc. This blood suspension was always so adjusted as to contain more than 50 million erythrocytes per 0.5cc. Of this suspension, 0.25cc was measured off, and brought to a dilution of 1 in 8, by addition of 1.75cc. of Saline solution. A drop of this diluted suspension was placed in the Zeiss counting-chamber, and enumeration of 5 sets of 16 squares made. This enumeration was repeated in a second preparation. An example will now best illustrate the procedure.

M.R. Blood-Corpuscle-Suspension.

Dilution 1 in 8.

<table>
<thead>
<tr>
<th>FIELDS OF 16 SQUARES</th>
<th>FIELDS OF 16 SQUARES</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>66</td>
</tr>
<tr>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td>1st. Prep. 60</td>
<td>2nd. Prep. 50</td>
</tr>
<tr>
<td>58</td>
<td>68</td>
</tr>
<tr>
<td>49</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>291</td>
<td>387</td>
</tr>
</tbody>
</table>

The/
The total for 10 fields is 578 erythrocytes.
The average of 1 field is 57.8 or approx. 58.

\[
\text{in } 0.5\text{cc of the undiluted suspension hours will be:}
\frac{58 \times 4000 \times 8 \times 500}{16} \quad \text{or 58mln. erythrocytes.}
\]

There are thus 58 million cells in each 0.5cc sample, instead of 50 millions - an excess of 8 million over the standard aimed at. Therefore in 3cc of the undiluted blood-suspension, there will be an excess of 24 millions. But calculation will give the amount of saline to be added, which will make a suspension of this excess in the proportion of 50mln. cells per 0.5cc.

This for 24 millions will be
\[
\frac{0.5\text{cc} \times 24}{50} \quad \text{i.e. } 0.24\text{cc}.
\]

Having then measured off 3 cc of the undiluted blood-suspension, and having added to that amount 0.24cc of saline solution, one has now obtained a corpuscular suspension 3.24cc in bulk, and containing 50 million erythrocytes per 0.5cc.

MODIFIED/
MODIFIED METHOD OF PREPARING EQUAL SUSPENSIONS.

Before long, I modified the above method. Up to the point of enumeration of the erythrocytes in the diluted suspension, the procedure was exactly the same. But now, instead of expressing the result as the number of erythrocytes per 0.5cc of the blood suspension, I stated the number as per cmm. Thus in the example given above, the average number of red-cells in a field of 16 squares was 58.

.. the undiluted suspension contains

\[
\frac{58 \times 4000 \times 8 \text{ per cmm.}}{16} = 116,000 \text{ per cmm.}
\]

But from this figure can be calculated the number of c.mm. of the suspension that will contain 50 million erythrocytes.

This number will be \( \frac{1 \times 50,000,000 \text{ c.mm.}}{116,000} \)

or 43 cmm. or 43cc.

The amount of blood-suspension whose corpuscular content is 50 millions will vary in different suspensions. But as in every case, by adjustment of Saponin and Saline solutions, the total bulk of fluid is/
is the same - 2cc, this variation is immaterial. I give below an example to illustrate this adjustment to a total equal bulk.

<table>
<thead>
<tr>
<th>TUBE</th>
<th>BLOOD SUSPENSION</th>
<th>SAL. SOLN.</th>
<th>SAP. SOLN.</th>
<th>BULK</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0·43cc.</td>
<td>1·45cc.</td>
<td>+0·12cc.</td>
<td>2cc.</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>1·39cc.</td>
<td>+0·18cc.</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>1·33cc.</td>
<td>+0·24cc.</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Thus in all cases the factor of dilution was kept the same, & so operated equally. One could, therefore by this method expose to haemolysis the same number of red-cells - 50 millions, in the same bulk - 2cc.

This modified method of obtaining equal blood suspensions, has several advantages over the other. It is simpler, and more rapid, But especially the number of manipulators and measurements is greatly reduced in it, and greater accuracy in result is, therefore, attainable.

MODIFICATION/
MODIFICATION IN THE END-POINT OF HAEMOLYSIS.

Having by the methods described obtained equal Blood-suspensions, it only remained to ascertain the dose of Saponin that haemolysed the standard number of red-cells. The standard end-point was not Complete Haemolysis, as in most haemolytic researches where the test of transparence is employed: but almost Complete Haemolysis, i.e. 99.9% or thereby. But it has been thoroughly established by numerous tables that a very large increment of Saponin is required, to carry haemolysis from a stage of 99% to one of 99.9%. It seemed unreasonable that the haemolysis of such a trifling fraction of the total number of cells, should be allowed to increase so disproportionately the haemolytic dose of Saponin. I, therefore, determined to bring back still further the standard end-point of haemolysis and to fix it at stages ranging between 99.2% and 99.8%. It will be noted that this fresh alteration was a still further departure from the usual end-point in haemolytic investigations - the so-called Complete Haemolysis.

It was now the object of investigation to ascertain the dose of Saponin that produced a degree of haemolysis somewhere within this range.

RESULTS/
RESULTS OBTAINED BY THE TECHNIQUE IN CONDITIONS OF HEALTH AND DISEASE.

2 nd. Series.

I now proceed to give a table of results. In all cases the Saponin Solution was .004 gms. per 100cc. Incubator 37°C. Incubation-period 2 hours. Standard number of Erythrocytes:— 50 millions. The Standard Colour-Index is expressed as I.

I. HEALTH.

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>AGE</th>
<th>NO. OF RED CELLS PER cm³</th>
<th>COLOUR INDEX</th>
<th>SAP. HAEMOLYSIS</th>
<th>COL. SAP.</th>
<th>SOL.</th>
<th>IN 2 HRS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>W.M.S.</td>
<td>25</td>
<td>5,080,000</td>
<td>1.03</td>
<td>0.14cc</td>
<td>99.63%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>J.P.M.</td>
<td>28</td>
<td>5,300,000</td>
<td>0.97</td>
<td>0.14cc</td>
<td>99.39%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>A.T.M.</td>
<td>26</td>
<td>4,830,000</td>
<td>0.98</td>
<td>0.16cc</td>
<td>99.64%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>J.N.</td>
<td>25</td>
<td>5,100,000</td>
<td>1.06</td>
<td>0.16cc</td>
<td>99.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>J.A.G.</td>
<td>26</td>
<td>5,400,000</td>
<td>1.01</td>
<td>0.16cc</td>
<td>99.84%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>M.S.</td>
<td>22</td>
<td>4,890,000</td>
<td>1.04</td>
<td>0.16cc</td>
<td>99.52%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>C.M.</td>
<td>26</td>
<td>5,200,000</td>
<td>1.0</td>
<td>0.16cc</td>
<td>99.8%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All the above cases are males.

These results were so remarkably uniform that I considered their number in the meantime sufficient.
sufficient, and next examined the blood in various conditions of disease by the same technique and with the same standards. The results are set forth in the next table.

II. DISEASE.

<table>
<thead>
<tr>
<th>NO.</th>
<th>NAME</th>
<th>AGE</th>
<th>CELLS PER CMM</th>
<th>INDEX</th>
<th>DISEASE</th>
<th>SAP. HAEMOLYSIS</th>
<th>SOL. IN 2 HRS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M.R. (f)</td>
<td>25</td>
<td>1,170,000</td>
<td>1.2</td>
<td>Pernicious Anaemia</td>
<td>0.16cc</td>
<td>99.44%</td>
</tr>
<tr>
<td>2</td>
<td>G.J. (m)</td>
<td>25</td>
<td>1,170,000</td>
<td>1.3</td>
<td>&quot;</td>
<td>0.14cc</td>
<td>99.90%</td>
</tr>
<tr>
<td>3</td>
<td>P.C. (m)</td>
<td>37</td>
<td>1,970,000</td>
<td>1.01</td>
<td>Splenic Anaemia</td>
<td>0.16cc</td>
<td>99.64%</td>
</tr>
<tr>
<td>4</td>
<td>W.S. (f)</td>
<td>14</td>
<td>2,790,000</td>
<td>0.6</td>
<td>Simple Anaemia</td>
<td>0.18cc</td>
<td>99.8%</td>
</tr>
<tr>
<td>5</td>
<td>E.M'C (m)</td>
<td>20</td>
<td>2,330,000</td>
<td>0.7</td>
<td>&quot;</td>
<td>0.16cc</td>
<td>99.88%</td>
</tr>
<tr>
<td>6</td>
<td>M.D. (f)</td>
<td>20</td>
<td>4,130,000</td>
<td>0.58</td>
<td>&quot;</td>
<td>0.16cc</td>
<td>99.2%</td>
</tr>
<tr>
<td>7</td>
<td>MRS. A. S. (f)</td>
<td>37</td>
<td>3,290,000</td>
<td>0.6</td>
<td>Secondary Anaemia</td>
<td>0.16cc</td>
<td>99.84%</td>
</tr>
<tr>
<td>8</td>
<td>W.H. (m)</td>
<td>37</td>
<td>3,520,000</td>
<td>0.8</td>
<td>Malaria</td>
<td>0.12cc</td>
<td>99.36%</td>
</tr>
<tr>
<td>9</td>
<td>MRS. G. (f)</td>
<td>44</td>
<td>3,500,000</td>
<td>1.0</td>
<td>Exophthalmic Goitre</td>
<td>0.16cc</td>
<td>99.76%</td>
</tr>
</tbody>
</table>

CONCLUSIONS.

In view of the marked differentiation of haemolytic doses of saponin in health and disease previously obtained there/
these results were very surprising. For they showed that in conditions of health and disease alike the dose of Saponin, required to produce a haemolysis of about 99.5%, was 0.16 cc; or varied so little from it, and so indifferently on either side of it that no conclusions could be drawn from the divergence. Of course the number of diseased conditions in which the blood was examined was not numerous; but they included a representative group of the Anaemic diseases in which above all others a divergence from the standard of health might have been expected, and these entirely failed to show any appreciable variation. These results, therefore, though small in number, were so concordant that no promise was held out of appreciable variations being discovered if their number was increased.

Three conclusions might be drawn from that result.

(1) That in health, and in varied conditions of disease the resistance of the red cells to haemolysis does not vary within appreciable limits, or

(2) That variations in resistance on the part of the red cells may exist, but Saponin haemolysis/
haemolysis does not test them,

(3) But there is a third and quite different possibility. The gauge of an equal resistance in these tables lay in the fact that an approximately complete haemolysis was obtained at the end of 2 hours. By such a gauge, no substantial differentiation was obtained. But it still remained possible that the progress of haemolysis had not been the same in all cases. In some, haemolysis may have proceeded more rapidly at first, and later more slowly; in others, it may have begun more slowly, and in its later stages have been more rapid: so that finally the same degree of haemolysis had been reached simultaneously by all.

Such a suggestion is not merely a speculation. It is a hypothesis for which preceding pages show a sound experimental basis. It has already been shown that as haemolysis approaches its close, the destruction of the remaining cells proceeds much more slowly. It was also shown that this retardation, though at its height at the close of haemolysis, operates also, though in less degree, throughout/
throughout its course. The explanation of this was founded on the fact that the total affinity of the erythrocyte for Saponin is very great, and that only a small proportion of this affinity is satisfied when the cell is haemolysed.

It therefore was a legitimate hypothesis that in a sample of cells of feeble resistance, haemolysis in the early stages of the incubation period may have proceeded rapidly and to a considerable degree; but that subsequent haemolysis may have progressed much more slowly, because of the partial absorption of the unfixed and available Saponin by the "cell-remants" of the corpuscles already destroyed. And the hypothesis was equally fair that in a sample of cells of high resistance, haemolysis for a considerable period might be of small amount. During this stage a smaller fraction of the total cells would be destroyed, and there would thus be less deviation of Saponin to "cell-remants" than in the former case, and a greater fixation of Saponin by the envelopes of intact cells. The operation of the latter result would in the later stages effect a rapid haemolysis which might obliterate the early retardation.

This/
This hypothesis was at once tested by experiment. The only way to do so was to follow the haemolysis in a tube from the beginning to the end of the incubation-period. Happily the method of Enumeration which I had devised, allowed this easily to be done. At regular selected intervals, a tube was removed from the Incubator. Its contents having been shaken to obtain a uniform suspension, and a drop of this having been removed by a small pipette and placed in the Zeiss chamber, the tube was immediately replaced in the Incubator. The degree of haemolysis at any intermediate stage of the Incubation period could thus be obtained.

(1) The aim was to find a dose of Saponin which would haemolyse the great majority of 50 million erythrocytes in 2 hours. A haemolysis of 99% was not now aimed at. In fact it was more desirable that the stage of haemolysis in 2 hours should be less than that. A haemolysis of 90% would be much more suitable, for in this case there would be a much slower haemolysis at the outset of the incubation period, and therefore, much less deviation of Saponin from the/
the envelopes of intact red cells. To follow the progress of haemolysis with such a dose of Saponin, would give an interesting grouping of the cells of the sample according to their resistance.

(2) But in addition to a dose of Saponin that would haemolysse the great bulk of the cells, I proposed to give a much smaller dose, one which, in a sample of healthy erythrocytes, was only able to destroy at the end of the 2 hours a small minority of the total, this fraction consisting of the feeblest cells. With such a dose, the deviation of Saponin would occur in a minimal degree: for by the time haemolysis had commenced, the great bulk of the dose of Saponin would already be distributed among and fixed to the cell-envelopes of the rest of the sample.

That minimal haemolytic dose would compare with and confirm the early stage of haemolysis in the larger dose: Indeed, as a test of the feeblest cells in the sample it would be superior to that given by the larger dose. In the more rapid and powerful/
powerful action of the latter, a greater deviation of Saponin to haemolysed cells would take place than in the much delayed haemolysis that occurs with the smaller dose. The group of feebly resistant cells separated by the prolonged action of the smaller dose would thus be a more accurate one than that revealed by the swifter action of the larger dose.

I shall now illustrate with full details the investigation of a case by the method outlined above.


Ordinary Blood Determination.

Erythrocytes: 1,090,000 per c.mm.
Haemoglobin: 30%
Colour Index: 1.4
Leucocytes: 4,600.

This blood determination was, of course, made on the same day and at the same time as the rest of the investigation.

300 c.mm. of entire blood obtained at 11-20 a.m. by puncture of the lobe of the ear, and placed in a tube containing 6 cc. of saline solution. This blood suspension transferred to a sterile centrifuge/
centrifuge-tube, shaken up, centrifuged, the supernatant fluid removed by pipette, and fresh saline solution added. This washing (shaking up with saline solution, centrifugation, and removal of fluid) carried out three times. To the final sediment, saline solution added in amount that would roughly approximate to a blood-suspension containing 50 million red-cells in 0.5 cc.

Of this blood-suspension, 0.25 cc. measured off; 1.75 cc of saline solution added to it, giving a dilution 1 in 8 of the blood-suspension.

Enumeration of this diluted suspension made and confirmed in the Thoma–Zeiss counting chamber, as follows:–

<table>
<thead>
<tr>
<th>FIELDS OF 16 SQUARES</th>
<th>FIELDS OF 16 SQUARES</th>
</tr>
</thead>
<tbody>
<tr>
<td>56 red-cells</td>
<td>56, red-cells</td>
</tr>
<tr>
<td>51 &quot;</td>
<td>51 &quot;</td>
</tr>
<tr>
<td>1st Prep. 53 &quot;</td>
<td>2nd Prep. 53 &quot;</td>
</tr>
<tr>
<td>54 &quot;</td>
<td>60 &quot;</td>
</tr>
<tr>
<td>214 &quot;</td>
<td>220 &quot;</td>
</tr>
</tbody>
</table>

These confirmatory counts make a total of 434/
434 red-cells in 128 squares.

\[ \frac{434 \times 4000 \times 8}{128} \quad \text{or} \quad 108,000 \text{ red-cells are contained in 1 cmm. of the original blood-suspension.} \]

50 million red-cells are contained in

\[ \frac{1 \times 50,000,000}{108,000} \text{ c.mm. i.e. in 460 cmm.} \]

or 0.46cc.

Samples of the blood-suspension, 0.46cc were measured off, and haemolytic preparations were prepared as under.

<table>
<thead>
<tr>
<th>TUBES</th>
<th>BLOOD SUSPENS.</th>
<th>SAL.</th>
<th>SAP.</th>
<th>IN-HAEMOLYSIS AFTER TUBES</th>
<th>½hr</th>
<th>1hr</th>
<th>1½hrs</th>
<th>2hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.46cc</td>
<td>1.48cc</td>
<td>0.06cc</td>
<td>2.15pm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>63.8%</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>1.48cc</td>
<td>0.12</td>
<td>1.47  60%</td>
<td>90%</td>
<td>97%</td>
<td>99.4%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>1.38cc</td>
<td>0.16</td>
<td>2.20</td>
<td>-</td>
<td>-</td>
<td>99.9%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>1.50cc</td>
<td>0.00</td>
<td>2.20</td>
<td>Control</td>
<td>-</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

I now give the enumerations that furnished these data of haemolysis. These were made as before in the Zeiss counting-chamber, and the results calculated from them.

Tube/
Tube 1.  2 hours.

<table>
<thead>
<tr>
<th></th>
<th>Fields of 16 Squares</th>
<th>Fields of 16 Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Prep.</td>
<td>35 red-cells</td>
<td>36 red-cells</td>
</tr>
<tr>
<td>2nd Prep.</td>
<td>34 &quot;</td>
<td>40 &quot;</td>
</tr>
<tr>
<td>35 &quot;</td>
<td>35 40 &quot;</td>
<td></td>
</tr>
</tbody>
</table>

139 "per 64 sq. 150 " per 64 sq.

There is thus a total of 139×150, or 289 red-cells for 128 squares or an average of 36.1 red-cells per 16 squares.

From this last figure we get the total in the tube of 2cc. to be,

\[
\frac{36.1 \times 4000 \times 2000}{16}, \text{or } 18,050,000 \text{ red-cells.}
\]

That number remains unhaemolysed cut of 50 millions exposed to haemolysis. Expressed as a percentage, that will be 36.1%.

So that the haemolysis in Tube 1 is 100-36.1 or 63.9%

It will be seen from the above that the average/
average number of cells for 16 squares, remains, after calculation, the percentage of unhaemolysed red-cells. So that the percentage of haemolysis can immediately be obtained from it, and without necessity of calculation.

**TUBE 2.**

<table>
<thead>
<tr>
<th>1/2 hour.</th>
<th>1 hour.</th>
</tr>
</thead>
<tbody>
<tr>
<td>43 per 16 sq.</td>
<td>(1) 61 per 100 sq.</td>
</tr>
<tr>
<td>39 &quot; &quot;</td>
<td>(2) 61 &quot; &quot;</td>
</tr>
<tr>
<td>33 &quot; &quot;</td>
<td>average 61 per 100 sq.</td>
</tr>
<tr>
<td>40 &quot; &quot;</td>
<td>. . in the whole tube there are now $\frac{61 \times 4000 \times 2000}{100}$</td>
</tr>
<tr>
<td>41 &quot; &quot;</td>
<td>i.e. 4,880,000 red-cells.</td>
</tr>
<tr>
<td>196 per 80 sq.</td>
<td>or 9.76% unhaemolysed cells.</td>
</tr>
<tr>
<td>=average 39.2 per 16 sq.</td>
<td>i.e. 90.24% haemolysis.</td>
</tr>
<tr>
<td>i.e. 100-39.2</td>
<td>or 60.8% haemolysis.</td>
</tr>
</tbody>
</table>

**TUBE 2 (Contd.)**

1½/
**TUBE 2 (Contd.)**

<table>
<thead>
<tr>
<th>1½ hours</th>
<th>2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 per 200 sq.</td>
<td>37 per 400 squares</td>
</tr>
<tr>
<td>33 &quot; &quot;</td>
<td>42 &quot; &quot;</td>
</tr>
<tr>
<td>63 per 400 sq.</td>
<td>39.5 average 400 sqrs.</td>
</tr>
<tr>
<td>. . in the whole tube there are now</td>
<td>. . in the whole tube there are now</td>
</tr>
<tr>
<td>63 x 4000 x 2000</td>
<td>39.5 x 4000 x 2000</td>
</tr>
<tr>
<td>i.e. 1260,000 red-cells.</td>
<td>i.e. 790,000 red-cells.</td>
</tr>
<tr>
<td>as a percentage these are</td>
<td>as a percentage this is</td>
</tr>
<tr>
<td>2.52% . . there is</td>
<td>1.58% . . there is</td>
</tr>
<tr>
<td>97.48% haemolysis</td>
<td>98.42% haemolysis</td>
</tr>
</tbody>
</table>

**TUBE 3. 2 hours.**

8 cells per 1200 squares

. . in the whole tube there are now

8 x 4000 x 2000

1200

or 53,300 red-cells.

This, as a percentage of the original total is 0.1%. . . there is 99.9% haemolysis.

**CONTROL/**
CONTROL:

Tube 4 is the control. Saline solution alone is added to the sample: and at the end of 2 hours' incubation, the absence of haemolysis is proved by the colourless supernatant fluid. Such a control was, of course, employed in every case.

The technique outlined above is, tedious and laborious, but is not complicated. In every respect it is novel, however, & differs from that employed in haemolytic research. It comprises:

(1) a special method of preparing equal washed-blood-suspensions: from blood-fluids of widely different corpuscular content.

(2) a special method of determining haemolysis, the enumerative method.

(3) Special standards of haemolysis - namely, that effected by a dose that will haemolyse a large majority of healthy red-cells, and that effected by a dose haemolytic for a small minority of healthy red-cells.

This last point, the standard of haemolysis, is not merely a modification of the usual standard employed/
employed - complete haemolysis; it is an entire departure from it. The divergence was, at first, slight. The first step was the selection of 99.9% haemolysis. A farther one was a circa 99.5% haemolysis. But, finally, a complete breach with the existing standard was made in the way last described.

It will be appropriate, at this stage, to deal with two points.

(1) Efficiency of Washing of Erythrocytes.

Saponin has been shown by Ransom and Hedon (loc.cit.) to attach itself to some element in the plasma. That it does so, in large amount in human plasma, is shown from the following experiment.

Blood of myself. Entire-blood-suspension used. 0.5cc samples of this suspension used, containing 50 million red-cells. Each tube brought by addition of saline and saponin solutions to 2 cc.

<table>
<thead>
<tr>
<th>TUBE</th>
<th>SAP.SOL.</th>
<th>2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7cc.</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>0.8cc.</td>
<td>0%</td>
</tr>
<tr>
<td>3</td>
<td>0.9cc.</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>1.0cc.</td>
<td>36%</td>
</tr>
</tbody>
</table>
On p. 84. No 7 of the Table given, there showed that 0.12 cc caused 99.8% haemolysis of 50 millions of my washed red-cells. The enormous protection against Saponin, possessed by the plasma in which human erythrocytes are suspended, is, therefore, demonstrated. Is it certain that three washings will entirely remove this protective fluid, especially in oligocytahaemia where the proportion of plasma to corpuscles is increased?

The washing was carried out in tapered centrifuge tubes, and the bulk of the saline suspension was 5 cc. After centrifugation, one could, with certainty remove all the supernatant fluid, except 0.05 cc. That is, after each washing, 1/100 of the original bulk of fluid was left. Therefore, after 3 washings (1/100) or 1000000 of the original plasma-accompaniment of the corpuscles was left behind. Even when one considers the relatively large attraction of Saponin for serum, demonstrated above, the error caused by so extremely small a quantity of serum, can be neglected with perfect confidence.

(2) STABILITY OF SAPONIN SOLUTIONS.

In my early work with Saponin, there was evidence/
evidence of a slow deterioration of activity, in solutions kept for some time. But by rigid experiment, I determined that solutions of the strength used, if kept in the dark, and in a cool place, showed no loss of strength whatever, after 12 days. This was done by comparing the action of a solution of that age, with a fresh one, upon equal samples of blood of the same individual, in doses that produced both a great, and a slight haemolysis in 2 hours. The differences in haemolysis recorded, were very small, and they oscillated indifferently between the old and the fresh solutions. In fact they were due to the unavoidable error of experiment. But in all subsequent results, the Saponin solution was freshly made every week. And, further, in the different tables that will be shown, the results in any one table whether of health and disease, were not obtained in series and with a single solution of Saponin, but singly and at intervals, and with many different solutions. Before proceeding to give results by the final method of investigation it will be well to give in summary the more important conclusions established during the development of the technique.

SUMMARY/
SUMMARY of RESULTS and CONCLUSIONS, as to the SPECIAL TECHNIQUE of a CLINICAL INVESTIGATION; & as to some SPECIAL PHENOMENA of SAPONIN-HAEMOLYSIS.

1. In measuring the haemolytic activity of Saponin for human erythrocytes, the latter must be washed free of serum.

2. In the technique of this research, the basis of preparation of samples of washed-blood-suspension is the enumeration of the red-cells in these suspensions, and not in the entire blood from which they are obtained.

3. The Colorimetric method devised, is suitable for the determination of degrees of Incomplete Haemolysis. It is superior to the methods commonly employed in haemolytic research, which adopt such indefinite terms as a "slight," "moderate," or such equally indefinite symbols as +, ++.

4. The Colorimetric Method is inaccurate in determining Complete Haemolysis: but not more so than the test of transparence commonly employed.
5. The Enumerative Method of determining degrees of Haemolysis, is superior to any other method, both for Incomplete and for Complete Haemolysis.

6. The Enumerative Method consists in counting, at any stage, the remaining cells suspended uniformly in the fluid, in obtaining by subtraction the number of cells destroyed, and in expressing the latter number, as a percentage of the original total exposed to haemolysis.

7. This method is proved reliable by the correspondence of results, obtained by it, and by the Colorimetric Method.

8. The correspondence of results by these two methods establishes the fact, that when a number of red-cells are exposed to haemolysis by Saponin, Haemoglobin is released totally from cell by cell, and not partially and simultaneously from all the cells.

9. This fact may be corroborative of the view that, in mammalian erythrocytes, the Haemoglobin lies free within the cell-envelope, and is not entangled in a stroma.
10. The Enumerative Method proves that, for Saponin, transparence is a faulty test of Complete Haemolysis.

11. It shows that the increment of dose required to carry haemolysis from 99% to 100% is disproportionately large, and is often one third of the total haemolytic dose.

12. In consequence of conclusion 11, the error introduced by the use of the Transparence test of Complete Haemolysis may not be small, but large.

13. The retardation at the close of haemolysis (no. 11) can also be expressed in terms of time. - a complete haemolytic dose effecting 99% haemolysis rapidly, and the remainder of haemolysis very slowly.

14. This retardation of haemolysis operates from an early period of the action, progressively increases, and becomes extreme at its close.

15. The explanation of this phenomenon lies in the fact that an erythrocyte, after its haemolysis, absorbs far more Saponin than suffices to effect that haemolysis.
16. The consequence of this fact is, that as soon as haemolysis begins to occur, in a collection of erythrocytes exposed to the action of Saponin, there is a deviation of unfixed Saponin to the "cell-remnants" of the destroyed cells, and therefore a diminution of Saponin available for the envelopes of Haemoglobin containing cells.

17. This deviation of Saponin must therefore begin with the first onset of haemolysis, must increase with its progress, and must be greatest just before its termination.

18. The amount of deviation possible may be gauged from a comparison of the total amount of Saponin fixed by a red-cell before and after haemolysis, with that sufficient to haemolyse it,—a proportion of at least 10 to 1.

19. Deviation of Saponin is greatest with doses that produce Complete Haemolysis in 2 hours, because in such cases haemolysis begins early and proceeds rapidly, so that the greater part of the available unfixed Saponin is seized by the "cell-remnants" of the haemolysed erythrocytes, and is, therefore, diverted/
diverted from the envelopes of the few remaining erythrocytes.

20. Conclusion 19 explains the disproportionate increment of Saponin required to carry haemolysis from 99% to 100%. And Conclusion 11; and from another point of view, the disproportionate increment of time required to effect the same result - Conclusion 13.

21. Deviation of Saponin is least with doses that will produce a very slight haemolysis in 2 hours, because in such cases haemolysis begins late and proceeds slowly, with the result that when it does occur the bulk of the available Saponin is already fixed by the envelopes of unhaemolysed erythrocytes.

22. In the above explanation this feature of the end-point of Saponin haemolysis depends, not upon the action of Saponin, but upon the peculiar nature and structure of the erythrocyte.

23. If this explanation is correct, it is probable that this phenomenon of haemolysis with Saponin is not peculiar to it, but also accompanies the action of other haemolytic substances.
substances—the snake-venoms, and haemolytic sera, which have a similar chemical action upon an identical material.

24. But the vast majority of researches upon the action of these latter substances determine complete haemolysis by the test of transparency, and found their results upon that determination.

25. The use of the Transparence Test in Saponin-haemolysis having been shown to introduce gross error, and grounds having been shown for analogy between the action of Saponin and that of these other substances there is a probability that the use of the same test in investigation of the latter is equally inaccurate.

26. Variation in bulk of the fluid in which Saponin-erythrocyte interaction takes place, has a profound effect upon the haemolysis, though the amount of Saponin, and the number of red-cells remain unaltered.

27. Increase in bulk of fluid with the other factors unchanged, delays haemolysis if the observations are confined to a short period: but in/
in addition diminishes it, if the observations are prolonged.

28. Decrease in bulk of fluid, with the other factors unchanged, accelerates haemolysis.

29. This influence of the bulk of fluid upon haemolysis shows Bashford's statement, "A multiple of the solvent dose of glucoside (saponin, etc), will dissolve the same multiple of the standard quantity of blood", to be inadequate.

30. Corrected and amplified, that statement would be, "A multiple of the solvent dose of glucoside will dissolve the same multiple of the standard quantity of blood, in the same time, provided that the bulk of fluid in which interaction is taking place is proportionally adjusted.

31. The explanation of the influence of bulk of fluid upon haemolysis is partly due to effect of dilution upon chemical interaction, and partly to the effect of the deviation of Saponin already described.

32. The haemolytic action of Saponin is not completed within 2 hours, but continues for a period of at least 20 hours.

33./
33. With a dose of Saponin that effects a great amount of haemolysis in 2 hours, the subsequent haemolysis is small and slow.

34. With a dose of Saponin that effects a small amount of haemolysis in 2 hours, the subsequent haemolysis is greater and more rapid.

35. Where haemolysis in 2 hours is not complete, it never becomes so within a period of 6 hours at 37°C, or within a period of 22 hrs. at 18°C.

36. To prevent the unequal operation of the factor of bulk of fluid it is necessary in a clinical investigation to prepare an equal suspension number of washed erythrocytes, in an equal bulk of fluid in all cases, independent of the corpuscular content of the entire blood. As the latter varies widely in disease, this requirement introduces a difficulty into a clinical investigation.

37. This requirement was attained by an exact enumeration of the washed blood-suspension prepared from each case, by the measurement of an amount of it that will just contain a selected standard number of erythrocytes, and by the adjustment of the mixture of blood suspension/
suspension and Saponin-solution to an equal bulk in all cases by addition of saline solution.

38. Complete Haemolysis being a stage that is liable to be over reached without detection, the standard end-point of haemolysis was at first taken as 99.9% or thereby.

39. Later, the standard end-point of haemolysis was brought back to between 99.2% and 99.8%, because of the disproportionate increment of doses already shown to occur between 99% and 100%.

40. Results obtained with this modified standard end-point - No 39 in a representative number of cases, showed that the dose of Saponin required to produce such a haemolysis of an equal number of washed red-cells, in the same time was practically the same in health, and in various diseases in which some divergence might have been expected.

41. On consideration it was seen that the result was due again to the influence of deviation of Saponin, to the cell remnants of haemolysed erythrocytes.
42. The dose of Saponin that will produce such a haemolysis in 2 hours is a large one and effects an early and rapid haemolysis.

Conclusion 19 showed that the deviation of Saponin was greatest in such a case. This large deviation, therefore, would easily efface variations in the resistance of the cell envelopes that might exist in different individuals. For the total haemolysis-resistance of the cell being small in comparison with its total absorption of Saponin, variations in the amount of the former would be a still smaller proportion.

43. The standard of haemolysis was, therefore, changed to one in which the factor of deviation of Saponin would operate in a much smaller degree.

44. The object was to find a dose of Saponin which would effect a haemolysis of a majority of the standard number of healthy erythrocytes in 2 hours; but one which would do so slowly, and with a considerable latent period, thus giving time for a greater fixation of Saponin by the cell envelopes & reducing the/
the amount of deviation.

45. In addition to the above dose, it was proposed to give a very small dose, that would produce a very slight haemolysis of the standard number of healthy red-cells in 2 hours. In this case, deviation of Saponin would be reduced to a minimum, - Conclusion 21. and as fair a separation as possible of the low-resistance group of cells would be made.
PART II.

Section I.

CLINICAL RESULTS. FINAL SERIES.

HEALTH. TABLE I.

In this and the following tables, Saponin solution is 0.004 grm. per 100 cc.
All the preparations are kept in the Incubator at 37° C.

(m) = male. (f) = female.

The Colour Index per corpuscle is given: its standard is 1.
It was not thought necessary to insert in addition the Haemoglobin-value of 20 cmm. of blood, from which the Colour Index was obtained.

Table/
### HAEMOLYSIS.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Sex</th>
<th>Age</th>
<th>Erythrocytes</th>
<th>Colour-Leucocytes</th>
<th>Sap.Sol. 0.12 cc.</th>
<th>0.08 0.06 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/4 hr. 1 hr. 1 1/2 hrs. 2 hrs. 2 hrs.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>J.G.(m)</td>
<td>19</td>
<td>5,610,000</td>
<td>0.98</td>
<td>7000</td>
<td>14%</td>
<td>58%</td>
</tr>
<tr>
<td>2</td>
<td>J.S.(m)</td>
<td>22</td>
<td>5,030,000</td>
<td>1.06</td>
<td>6400</td>
<td>14%</td>
<td>57%</td>
</tr>
<tr>
<td>3</td>
<td>D.L.M.(m)</td>
<td>23</td>
<td>4,960,000</td>
<td>0.95</td>
<td>5800</td>
<td>14%</td>
<td>55%</td>
</tr>
<tr>
<td>4</td>
<td>C.S.M.(m)</td>
<td>24</td>
<td>5,800,000</td>
<td>0.94</td>
<td>6800</td>
<td>25%</td>
<td>70%</td>
</tr>
<tr>
<td>5</td>
<td>W.M.S.(m)</td>
<td>25</td>
<td>5,200,000</td>
<td>1.1</td>
<td>4800</td>
<td>23%</td>
<td>55%</td>
</tr>
<tr>
<td>6</td>
<td>C.M.(m)</td>
<td>26</td>
<td>5,500,000</td>
<td>0.98</td>
<td>5000</td>
<td>7%</td>
<td>66%</td>
</tr>
<tr>
<td>7</td>
<td>J.A.G.(m)</td>
<td>26</td>
<td>5,650,000</td>
<td>1.03</td>
<td>6200</td>
<td>23%</td>
<td>56%</td>
</tr>
<tr>
<td>8</td>
<td>M.A.(f)</td>
<td>27</td>
<td>4,800,000</td>
<td>1.1</td>
<td>7800</td>
<td>26%</td>
<td>65%</td>
</tr>
<tr>
<td>9</td>
<td>J.M.(m)</td>
<td>28</td>
<td>4,800,000</td>
<td>1.02</td>
<td>4600</td>
<td>11%</td>
<td>68%</td>
</tr>
<tr>
<td>10</td>
<td>A.N.(m)</td>
<td>35</td>
<td>4,650,000</td>
<td>0.94</td>
<td>6200</td>
<td>35%</td>
<td>73%</td>
</tr>
<tr>
<td>11</td>
<td>J.T.(f)</td>
<td>35</td>
<td>5,250,000</td>
<td>0.99</td>
<td>8800</td>
<td>25%</td>
<td>66%</td>
</tr>
<tr>
<td>12</td>
<td>W.S.(m)</td>
<td>36</td>
<td>5,050,000</td>
<td>1.0</td>
<td>6000</td>
<td>7%</td>
<td>59%</td>
</tr>
<tr>
<td>13</td>
<td>C.H.(m)</td>
<td>41</td>
<td>6,100,000</td>
<td>0.88</td>
<td>6200</td>
<td>24%</td>
<td>70%</td>
</tr>
<tr>
<td>14</td>
<td>C.L.(f)</td>
<td>53</td>
<td>4,900,000</td>
<td>0.94</td>
<td>7200</td>
<td>23%</td>
<td>63%</td>
</tr>
<tr>
<td>15</td>
<td>J.D.(m)</td>
<td>60</td>
<td>5,150,000</td>
<td>0.97</td>
<td>6000</td>
<td>32%</td>
<td>74%</td>
</tr>
</tbody>
</table>

The operation of the 0.12 cc. dose of Saponin in No. 5 is illustrated.
RESULTS OF TABLE I. HEALTH.

This table comprises investigations on the blood of 15 healthy persons; 12 of these were males, and 3 females. The ages range from 19 to 60.
Where a dose of 0.12 cc. of Saponin solution is given, the degree of haemolysis produced in 2 hours ranges from 84% to 93%. That seems a surprisingly small fluctuation; but in view of all that has been said of the retardation in Saponin-haemolysis, this variation is somewhat larger than the figures show.

The degree of haemolysis produced by the smaller doses varies between 10% and 27% with 0.08 cc. Saponin solution: and between 7% and 23% with 0.06 Saponin solution. This alternation between two small doses was tentative with a view to determine which would give the more marked differentiation in health and disease. Experience showed that in cases of diminished resistance to Saponin, the smaller dose of 0.06 cc gave the more striking distinction.

The above table emphasises a further point. It shows a correspondence between half an hour's haemolysis by the larger dose and 2 hours' haemolysis by the smaller dose. That is shown in No.10 where with 0.12 cc. of Saponin solution there is 35% haemolysis in ½ hour: and 23% haemolysis with 0.06 cc. in 2 hours, the latter being the maximum haemolysis effected by the small dose in the table. The same correspondence is well illustrated in No.5.

Interpretation/
INTERPRETATION OF VARIATIONS FROM THE STANDARD OF SAPONIN-RESISTANCE IN HEALTH.

A consistent standard of resistance to Saponin having been obtained, tables will now be given showing variation from that standard on the part of the red-cells in different diseases.

These variations, presently to be shown, undoubtedly will express a corresponding variation in the structure of the cell material acted upon. The greatest care has been taken to eliminate errors of technique, and the results in most cases are decisive enough to outweigh the error of experiment. These results will therefore express facts as to feebleness or vigour of resistance of human erythrocytes to Saponin-haemolysis. But will they allow a wider conclusion to be drawn, and give information as to how these same cells will resist the natural destruction in the blood-stream and organs of the body? That question is very difficult to answer. In the first place nothing is certainly known of the nature of blood destruction in corpore. The view of Quincke is that it proceeds by an ingestion of old erythrocytes by leucocytes and connective-tissue cells in the blood stream/
stream, and in such organs as the spleen, liver and bone marrow. Hunter on the other hand deduces from his elaborate researches, that there is in addition a much more active haemolysis, that this is confined to the portal circulation; that it is a twofold process, consisting in the release of Haemoglobin from the erythrocyte in the spleen and in the gastro-intestinal capillaries, and in the interception of this free Haemoglobin in the liver cells where it is converted into the bile-pigment.

But Hunter's experiments are not decisive, and his deductions are not generally accepted as conclusive by physiologists. In the standard text books of physiology the question is regarded as quite unsettled and one of which we remain still in almost complete ignorance.

It is therefore apparent that great caution must be exercised in applying the results of Saponin haemolysis in vitro to haemolysis in corpore, normal or abnormal. Saponin indeed is a general protoplastic poison not limited in its action to red-cells, and therefore presumably will test some important common element in protoplasasm. In this research it is used in an isotonic solution and its action measured upon/
upon the red-cells suspended in a similar physiological fluid. It might therefore be expected when thus employed, to indicate abnormal metabolism in the protoplasmic envelope of red cells, either in the direction of undue wastage, or excessive building-up.

But until more is known of the nature of the haemolytic action of different substances, and especially of the nature of haemolysis in corpore, it will be wiser to limit the conclusions to Saponin, and if a wider application is given, to remember that at the best it is only a probability.

The few existing data on red-cell resistance obtained by the hypotonic salt-solution method have been confidently transferred to in corpore conditions. This is a reckless application of conclusions further than the facts permit. There is no evidence to show that red-blood corpuscles become haemolysed in corpore by entering a hypotonic medium. Their behaviour in a hypotonic solution in vitro is no index to their resistance or friability to a different haemolytic agent acting in isotonic fluids in corpore. The results obtained by this method, when properly employed so as to eliminate the numerous serious fallacies that attend it, no doubt express some facts of physical condition/
condition: But it is exceptionally difficult to interpret them: and it is quite unjustifiable to assume as has been done, that they determine the resistance of red-cells in their natural medium. The method departs from a cardinal principle of experiment in investigating the behaviour of a tissue in unnatural conditions, and errs more seriously still in immediately applying the results to the behaviour of the tissue in its normal condition. These considerations will be more fully discussed later, but it is appropriate to mention them at this stage, as they bear on some of the results in disease obtained by Saponin-haemolysis.

DISEASE.

Tables will now be shown illustrating the reaction of human red-cells in various diseases to Saponin-haemolysis.

Appended to them, a short-description of the cases investigated will be found. As an endeavour was made to select clear and unmistakeable examples of the disease, or pathological condition being investigated, these notes are made very brief: They only establish/
establish the fact of the diagnosis, and indicate roughly the extent of the condition. In a few cases, where special conclusions are drawn, the description is somewhat extended to allow this to be done.

In all these tables as before, Saponin-solution is 0.004 grms. per 100 cc. Incubator temperature is 37° C.

At the foot of each table an average case of health is given, to facilitate the appreciation of any variation from health shown in the table above it.
### TABLE II. JAUNDICE, OBSTRUCTIVE.

**Group 1. Deep Persisting Jaundice.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sex</th>
<th>Age</th>
<th>No. of Red Cells</th>
<th>Colour I.</th>
<th>Leucocytes</th>
<th>Sap. 0.12cc.</th>
<th>0.08 0.06 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 1 1 1/2 2 2 2</td>
<td></td>
</tr>
<tr>
<td>E.K. (m)</td>
<td>67</td>
<td>4,580,000</td>
<td>0.9</td>
<td>8,600</td>
<td>79%</td>
<td>90%</td>
<td>98.7%</td>
</tr>
<tr>
<td>T.B. (m)</td>
<td>38</td>
<td>3,800,000</td>
<td>0.8</td>
<td>11,600</td>
<td>95%</td>
<td>99.2%</td>
<td>99.8%</td>
</tr>
<tr>
<td>T.D. (m)</td>
<td>40</td>
<td>3,880,000</td>
<td>1.0</td>
<td>8,600</td>
<td>95%</td>
<td>99.2%</td>
<td>99.8%</td>
</tr>
<tr>
<td>J.B. (f)</td>
<td>46</td>
<td>4,250,000</td>
<td>1.03</td>
<td>10,600</td>
<td>84%</td>
<td>93%</td>
<td>99%</td>
</tr>
<tr>
<td>E.G. (f)</td>
<td>53</td>
<td>3,750,000</td>
<td>0.93</td>
<td>16,000</td>
<td>91%</td>
<td>98%</td>
<td>99.7%</td>
</tr>
</tbody>
</table>

**Group 2. Slight or Disappearing Jaundice.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sex</th>
<th>Age</th>
<th>No. of Red Cells</th>
<th>Colour I.</th>
<th>Leucocytes</th>
<th>Sap. 0.12cc.</th>
<th>0.08 0.06 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 1 1 1/2 2 2 2</td>
<td></td>
</tr>
<tr>
<td>W.P. (m)</td>
<td>47</td>
<td>4,360,000</td>
<td>0.9</td>
<td>4,600</td>
<td>47%</td>
<td>84%</td>
<td>96%</td>
</tr>
<tr>
<td>T.M. (m)</td>
<td>62</td>
<td>4,930,000</td>
<td>1.03</td>
<td>6,600</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F.A. (m)</td>
<td>24</td>
<td>5,320,000</td>
<td>1.0</td>
<td>5,600</td>
<td>11%</td>
<td>76%</td>
<td>89%</td>
</tr>
</tbody>
</table>

Health (average):

- 23% 55% 75% 86% 18% 10%
No.1. E.K. (aet.63), Malignant Disease of Liver, Deep jaundice of skin and conjunctivae: began 2 months ago; without pain or sickness: stools greyish-white urine loaded with bile-pigment, no bile acids. Liver much enlarged: vertical diameter in right nipple/
nipple-line is 10½ inches: flat round elevations, without umbilication, palpable on its abdominal surface.

No.2. T.B. (aet.38. m.). Chronic Pancreatitis. Deep jaundice. Intermittent attacks of epigastric pain, followed by transient jaundice, for 18 months. Since last attack a month ago, jaundice has persisted. Stools greyish-white: no gall-stones. Urine has much bile-pigment, no bile-acids: shows pancreatic crystals to Carmidge’s Test.


No. 5. Mrs E.G. (aet.53). Catarrhal Jaundice (?)
Deep jaundice of 7 weeks' duration: came on with
t rigor, and pain in right hypochondrium has per-
sisted. Stools greyish white: no gall-stones.
Urine has much bile-pigment, no bile-acids.

No. 6. W.P. (aet.47, m.). Chronic venous Congestion
of Liver. Has had chronic bronchitis and asthma
for 6 years. Admitted 10 days ago with high-
coloured urine with traces of bile-pigment. Bile-
pigment disappeared from urine 2 days ago, but it
is still high-coloured. Abundant bile-pigment in
stools. Liver slightly enlarged: vertical dia-
meter in right nipple-line 6½ inches. Skin has a
muddy sallow tinge.

No. 7. T.M. (aet.62, m.). Recurrent catarrhal Jaun-
dice (disappearing). First attack caused by
drinking bout, 3 weeks ago. Second attack, slight,
a week ago. At present skin & conjunctivae have
slight yellowish tinge. Urine has traces of bile-
pigment. Stools have abundant bile-pigment.

No. 8/

RESULTS OF TABLE II.

The above table has been divided into two groups, the first including cases of deep and persistent jaundice, the second, cases where jaundice is slight and is disappearing.

In the first group, the red blood corpuscles show a very feeble resistance to Saponin. This is very clearly marked in the operation of the large dose, but this diminished resistance is still more clearly accentuated in the operation of the smaller dose. Thus in No. 1, Table II., 0.06 cc Saponin solution produces 36% haemolysis, as compared with 10% haemolysis effected by a similar number of red cells of a healthy person.

In the second group where jaundice is disappearing, and bile-pigments have again appeared in the/
the faeces, this feebleness of resistance is much less marked, but is still decidedly below the standard of health. With regard to No.7, T.M., the table shows no record of use of a large dose of Saponin. Here a dose, 0.14 cc., was given, but could not be incorporated in the table. The result of its operation can now be shown separately, and compared with the action of a similar dose on an equal number of red-cells of health.

<table>
<thead>
<tr>
<th>Name &amp; age</th>
<th>Condition</th>
<th>Sap.</th>
<th>1/2 hr.</th>
<th>1 hr.</th>
<th>1 1/2 hrs.</th>
<th>2 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.N. (62)</td>
<td>Jaundice (slight)</td>
<td>0·14 cc</td>
<td>83%</td>
<td>99.8%</td>
<td>99.9%</td>
<td>99.9%</td>
</tr>
<tr>
<td>M.B.S. (22)</td>
<td>Health</td>
<td>0·14 cc</td>
<td>21%</td>
<td>94·0%</td>
<td>97·0%</td>
<td>98·0%</td>
</tr>
</tbody>
</table>

The difference is best seen at the end of a half-hour's haemolysis. That result shows that in the operation of a large dose of Saponin, No.7, Table II., is concordant with the rest of the table.

No. 6 is especially interesting, for only an icteric tinge was present in the skin, and there were no bile-pigments in the urine.

Two/
Two other points in this table may be noted. In group 1 there is a slight diminution of red-cells, with a slight increase of leucocytes. In group 2 there is no diminution of red-cells, and the number of leucocytes is within the range of health.

INTERPRETATION OF RESULTS OF TABLE II.

OSTRUCTIVE JAUNDICE.

These results are clear. They show that in obstructive jaundice the red blood corpuscles are much diminished in resistance to Saponin-haemolysis, and that the deeper the jaundice, the feebler does that resistance become.

This conclusion is interesting, because it exactly contradicts the existing data on the resistance of the red-cells in jaundice. It is very important to note that these data were obtained by a different method, namely, that of hypotonic salt solutions. This divergence of results obtained by a different method will be discussed later. It is sufficient in the meantime to state that von Limbeck, Chanel/
Chanel, and Viola, using the method of hypotonic salt solutions, have agreed in finding the resistance of the red-cells in jaundice increased, and the more so, the deeper the jaundice.

The results obtained by these two methods, Saponin haemolysis, and hypotonic salt solution haemolysis, are therefore concordant for each, though opposed to each other. Presumably each tests a different factor of cell-resistance. But the question is - which of them more approximately tests that factor of cell-resistance which determines haemolysis in corpore?

Now in the first place, Saponin is a general protoplasmic poison, not limited in its action to haemolysis. Hedon (7) has shown that clearly in his researches, where he studied its action upon the bronchial epithelium of tadpoles and small fishes; and Ransom (5) has shown its affinity for cholesterin, which is an element of all tissues, and an important constituent of red blood corpuscles.

Secondly, it is agreed that the specific gravity of the blood in jaundice is increased. The blood-fluid in that condition is therefore in a condition unfavourable to haemolysis of its cells by a process analogous to that which occurs in a hypotonic salt/
salt solution. Therefore the use of the 'hypotonic' method in jaundice will gauge a species of resistance of the red blood corpuscles which will not be tested in corpore. On the other hand, we know nothing to contra-indicate the possibility that Saponin-haemolysis - the action of a general protoplastic poison may test the resistance of the red-cell in a way analogous to its haemolysis in corpore in jaundice.

THE BILE-ACIDS IN JAUNDICE.

Considered by themselves, these results of Saponin-haemolysis in jaundice are very suggestive. They throw a quite new light on an unsettled and much discussed question, namely, the fate of the bile-acids in jaundice. The toxic action of the bile-acids upon the heart, the central nervous system, and the blood corpuscles is well known, and yet it is equally well known that in jaundice there is surprising absence of the toxic phenomena that might be expected to accompany the retention in the blood of these general protoplastic poisons. The slowing of the heart, indeed, and the itchiness of the skin have been attributed to the action of the bile acids; but this is only/
only a minor degree of poisoning. Oligocythaemia indicating a destruction of red-cells does not occur, and the increased resistance of the cells which the 'hypotonic' method shows is quoted as additional proof that there is no haemolytic interaction between the bile acids and the erythrocytes (13).

But it is exactly at this point that the results of Saponin-haemolysis in the red-cells of jaundice become relevant and important. They show that to Saponin, which, like the salts of the bile-acids, is a general protoplasmic poison, the red-cells in jaundice offer a much enfeebled resistance. That indicates the action of some toxic material upon the erythrocytes in jaundice, and the bile-acids are naturally suggested. Now, von Limbeck (4) has already made that suggestion of interaction of bile-acids and red-cells, and in applying it to the facts of increased corpuscular resistance obtained by the 'hypotonic' method, has offered the explanation that the weaker red-cells having been destroyed by the bile-acids, only the strongest and most resistant cells remain. But having made that explanation, he immediately withdraws it, because of two facts that are inconsistent with it, namely, the absence of oligocythaemia indicating erythrocytolysis, and the presence of/
of a hypertonic blood plasma. His citation of the latter is interesting and indicates a curious confusion in his mind between the use of the "hypotonic" method in a test tube experiment, and the operation of a haemolytic substance like Sodium Glycocholate in corpore. He seems to believe that the salt of the bile acid will only exert its property of haemolysis in a hypotonic medium in corpore, and will be prevented from doing so in a hypertonic one.

But the possibility of interaction between the bile acid and erythrocytes in Jaundice, which occurred to me independently of von Limbeck's suggestion, becomes a much more feasible one, when the red-cells are shown to be feebly resistant to Saponin haemolysis.

The results of Table II show experimental evidence in favour of such interaction, and offer a fresh standpoint for the discussion of very important problems.

Assuming this hypothesis of interaction between the bile-acids and the red-cells in Jaundice, there is a preliminary consideration which will aid the subsequent discussion. In Jaundice the circulation of the bile-acids is disturbed. In health there is/
is an intestino-hepatic circulation of their salts, which are formed in the liver-cells, are excreted with the rest of the bile, and are re-absorbed from the intestine. The important point is that in health they make their first entrance into the blood stream by the gastro intestinal capillaries, in the portal area of the circulation. Croftan (14) indeed has demonstrated small traces of bile acids in the general circulation in health, but they do not appear in anything more than traces outside this intestino-portal circle. But in Obstructive Jaundice their exit by the bile-ducts is barred, and they can now enter the systemic circulation by the capillaries of the hepatic vein.

All this has been recognised before, but its bearing is completely changed by these facts of Saponin-haemolysis in Jaundice. These facts of course do not prove an interaction between the salts of the bile-acids and the red-cells in Jaundice, but they strongly suggest it. Yet if interaction takes place between these haemolytic salts and the red corpuscles, a question immediately occurs, "How then in Jaundice is there not decided evidence of haemolysis?" Most observers agree that in Jaundice any considerable haemolysis/
haemolysis does not occur, on the ground that there is not marked oligocythaemia (1) and Table II confirms the absence of decided oligocythaemia.

The question might be answered in 2 ways:—

1. An abnormal destruction of red-cells, due to haemolysis by the chelates, might occur, and might be made good by an abnormal production of fresh cells by the bone-marrow. But this explanation is hardly consistent with the results of Saponin haemolysis in Jaundice.

2. There might be interaction between the bile-acids and red-cells, and yet no increase, or only slight increase of erythroclytosis. That seems at first an unreasonable suggestion; but when we consider the normal circulation of the bile-acids, and their disturbed circulation in Jaundice it is not so. And it leads to an interesting conclusion, namely, that in health the cholate salts may take part in the function of haemolysis in corpore: that in Jaundice the seat of their operation is transferred from the portal to the systemic circulation, and that the condition./
condition of the red-cells in that condition, as tested by Saponin, is due to this transference. Very little is known of the function of the bile-acids. They are poisons; but they are not waste poisons, for only a small proportion of the quantity contained in the bile is passed in the faeces. They are strongly haemolytic substances. I roughly standardised the haemolytic activity of a solution of Sodium Glycocholate 0.02 grm. per 100 cc. of 0.85 Saline for 50 millions of my washed erythrocytes. 0.52 cc. of this solution produced 99.28% haemolysis in 2 hours at 37° C. Several facts as to their nature and action can be grouped suggestively together. Their proved haemolytic activity, the fact of their intestine-hepatic circulation, and their close association with that product of haemolysis the bile, all support the idea that their prime function in corpore is that of haemolysis. And that suggestion would not oppose but would harmonise with Hunter's conclusions(12) that haemolysis in corpore takes/
takes place in the spleen and in the gastrointestinal capillaries. His researches were used by him to indicate the sites of haemolysis in corpus. This further suggestion indicates the agent of haemolysis and it is quite compatible with Hunter's conclusions as to the site of haemolytic activity. But if that were the function of the bile acids in health, the absence of oligocytthaemia in Jaundice would receive an explanation perfectly consistent with their interaction with the erythrocytes and diminished red-cell destruction would be an equally natural result of the disturbed functions of the liver in that condition.

It is admitted that much of this discussion is theoretical: but it originated from a fair hypothesis built on an experimental fact. That fact is the much enfeebled resistance of red-cells in Jaundice to Jaundice to haemolysis by Saponin. That fact justified the hypothesis that interaction takes place in Jaundice between the haemolytic salts of the bile acids and the red-cells. But it remains in the meantime/
meantime merely a hypothesis. It requires a full and exact study of the action of the chelate salts, both in vitro and in corpore upon erythrocytes, and that does not exist. But it may be fairly claimed that the above discussion is as pertinent to the problems of Jaundice as other conjectures and theories grounded on experiments which are equally indecisive. The fact on which it is based. - the friability of red-cells in Jaundice to Saponin is a new one, and though it is contradicted by the data obtained by the hypotonic method, it is given by a method which approximates far more closely to the conditions of haemolysis in corpore. It offers a new standpoint from which the pathology, and the pathogenesis of Jaundice appear in a fresh light. It also offers a new starting point for further investigations of these problems, along the line of an exact study of the haemolytic action of the chelate salts.

NO HAEMOLYTIC PROPERTY OF BLOOD PLASMA IN JAUNDICE.

In view of the above hypothesis that the feeble resistance of red-cells in Jaundice to Saponin-haemolysis is due to interaction between them and the salts/
salts of the bile-acids, it was important to determine whether the serum or plasma of blood in Jaundice was actively haemolytic.

In Table II No.1, about 80ccm. of entire blood having been collected and placed in 5cc. saline, the supernatant fluid after the first centrifugation was kept in a sterile vessel. It was a greenish-yellow opalescent fluid, and it was added to a sample of washed erythrocytes as follows.

<table>
<thead>
<tr>
<th>O.M. HEALTH.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5cc. of washed blood-suspension contains 50 million red-cells.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BLOOD SUSPENSION</th>
<th>DILUTED JAUNDICE SERUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5cc. + 1.5cc.</td>
<td></td>
</tr>
</tbody>
</table>

This preparation was incubated at 37°C for 41/2 hours, when there was no haemolysis. It was then kept at room-temperature, 18°C, for 24 hours, and still there was no haemolysis. The same experiment with the diluted sera obtained from Nos. 2 and 3 Table II/
II, and with the same negative result at the end of 24 hours.

Thus in three cases of Deep Jaundice no haemolytic action was found in their sera under those conditions. That result seems to support the assumption that in Jaundice the salts of the bile acids are either produced in much less quantity or are in some way neutralised in the body or blood (13). But taken in conjunction with the other fact, demonstrated in Table II, of the feeble resistance to Saponin shown by the red-cells in Jaundice, it rather lends further support to the hypothesis that this lowered resistance is due to interaction of the salts of the bile-acids with the red-cells.

Table/
TABLE III.

FEVER. EXOPHTHALMIC GOITRE.

FEVER GROUP I.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Age</th>
<th>No. of Red-</th>
<th>Colour-</th>
<th>Leuco-</th>
<th>Sap. Sol. 0.12cc.</th>
<th>0.08cc.</th>
<th>0.06cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>J.H. (f)</td>
<td>28</td>
<td>5,010,000</td>
<td>0.88</td>
<td>11.200</td>
<td>9% 44% 72% 78%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A.C. (m)</td>
<td>21</td>
<td>6,360,000</td>
<td>0.83</td>
<td>11.000</td>
<td>6% 35% 66% 79%</td>
<td>35%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>J.M. (m)</td>
<td>27</td>
<td>5,200,000</td>
<td>0.96</td>
<td>10.600</td>
<td>14% 48% 67% 78% 27%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>W.T. (m)</td>
<td>40</td>
<td>6,500,000</td>
<td>0.92</td>
<td>14.400</td>
<td>18% 45% 58% 75% 26%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M.C. (f)</td>
<td>21</td>
<td>5,000,000</td>
<td>0.95</td>
<td>8.000</td>
<td>7% 57% 71% 84%</td>
<td>32%</td>
<td></td>
</tr>
</tbody>
</table>

HAEMOLYSIS

FEVER GROUP 2.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Age</th>
<th>No. of Red-</th>
<th>Colour-</th>
<th>Leuco-</th>
<th>Sap. Sol. 0.12cc.</th>
<th>0.08cc.</th>
<th>0.06cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>M.F. (m)</td>
<td>28</td>
<td>4,320,000</td>
<td>0.99</td>
<td>21.800</td>
<td>49% 91% 97% 98% 35%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>S.L. (f)</td>
<td>33</td>
<td>5,010,000</td>
<td>0.92</td>
<td>36.400</td>
<td>26% 81% 96% 98% 21%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>B.A. (f)</td>
<td>22</td>
<td>4,100,000</td>
<td>0.89</td>
<td>9.200</td>
<td>73% 98% 99% 99% 98%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>E.H. (f)</td>
<td>18</td>
<td>4,450,000</td>
<td>0.9</td>
<td>25.600</td>
<td>81% 99% 100% 100% 76%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EXOPHTHALMIC GOITRE.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Age</th>
<th>No. of Red-</th>
<th>Colour-</th>
<th>Leuco-</th>
<th>Sap. Sol. 0.12cc.</th>
<th>0.08cc.</th>
<th>0.06cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H.D. (f)</td>
<td>21</td>
<td>5,180,000</td>
<td>1.0</td>
<td>4.800</td>
<td>22% 46% 71% 88% 55%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>J.F. (m)</td>
<td>24</td>
<td>5,200,000</td>
<td>0.9</td>
<td>8.400</td>
<td>16% 71% 86% 92% 41%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mrs. G. (f)</td>
<td>44</td>
<td>3,500,000</td>
<td>1.0</td>
<td>3.800</td>
<td>88% 92% 99% 99% 46%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M.P. (f)</td>
<td>30</td>
<td>5,500,000</td>
<td>0.9</td>
<td>5.000</td>
<td>57% 94% 99% 99.6% 46%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HEALTH (average) 23% 55% 75% 86% 18% 10%

Table
TABLE III. CLINICAL NOTES.

FEVER. GROUP I.


No.2. A. C. (aet. 21, m.). PYROEXIA: ACUTE LOBES PNEUMONIA. Rigor 7 days ago, followed by fever, rapid respiration, and short cough. Temperature has remained about 103° F. for last 3 days. Pulse 120. Respiration 80 per minute. Consolidation of lower and middle lobes of right lung. Rusty sputum. February 7th 1908.

No.3. J. M. (aet. 27, m.). PYROEXIA: Acute Pneumonia. Rigor 5 days ago. Temperature shows irregular fluctuations between 100° & 103° F. Irregular consolidation of lower part of right upper lobe and of middle lobe. February 27th 1908.

No.4./
No. 4. W.T. (aet. 40, m.). POST-PYROEXIAL PERIOD INFLUENZA. Yesterday afternoon had chill, and pains in back and legs: in evening temperature was 101° F. this morning it is 99° F. March 30th 1908.

No. 5. M.O. (aet. 21, f.). REMITTENT PYROEXIA.
TUBERCULOUS ENTERITIS. Diarrhoea and emaciation for 2 years. For some weeks past temperature swinging between 101° F. & 99° F. night and morning. March 23rd 1908.

FEVER GROUP 2

No. 6. V.F. (aet. 28, m.). PYROEXIA (ACUTE LOBAR PNEUMONIA) Rigor 6 days ago. For last 3 days temperature has oscillated about 102° F. Respirations 70, Pulse 124 per minute. Consolidation of left lower, and of right lower and middle lobes. March 13th 1908.

No. 7. Mrs. S.L. (aet. 33). Crisis:— Acute Lobar Pneumonia. Rigor 8 days ago. Admitted 3 days ago. Temperature since has fluctuated about 103° F. Last/
Last night it was 103.8°: this morning it is 99° F.
Pulse 100 per minute. March 26th 1908.


EXOPHTHALMIC GOITRE.

No. 1. H.D. (aet. 21, f.). Exophthalmos slight.
Goitre fairly large. Tachycardia. average pulse/
pulse rate 100 per minute. Tremor moderate. Duration of symptoms 1 year.

No.2. J.F.(aet.24,m.). Exophthalmos marked. Goitre slight. Tachycardia - average pulse rate 120 per minute. Tremor marked. Duration 5 years.


RESULTS OF TABLE III.

FEVER GROUP I.

These results seem at first difficult to interpret. Those of Fever will be considered first./
first. They have been divided into 2 groups. Group I. shows with the larger dose of Saponin a retarded haemolysis from the beginning to the end of the Incubation period, but with the smaller dose a greater haemolysis than that affected by the same dose in health. That is to say we have no longer in this series that correspondence between the early haemolysis of the large dose, and the prolonged haemolysis of the small dose that was observed in the blood of health. We have in fact in group I. a divergence in both directions from it, for while the total haemolysis effected by the large dose has diminished, that of the small dose has increased. If attention was paid only to the haemolysis of the large and small doses after 2 hours it would be easy to imagine that in Fever the group of low-resistance cells had become still feeble, while the high-resistance group had become more resistant or more numerous. But if that were the case, it is difficult to explain why there is no evidence of a group of enfeebled cells in the early stages of haemolysis of the larger dose. At the same time, the prolonged operation of a small dose of Saponin gives undoubtedly a superior test of the group of feebly resistant cells than does the early opera-
operation of a large one: and one is inclined to accept the evidence the former shows of such a group, and in the meantime to leave unexplained the early retarded haemolysis of the larger dose which is apparently inconsistent with it. Further the final retarded haemolysis with the large dose is a clear proof that the total resistance of the sample of cells in this group is increased. We thus arrive at the conclusion that in Group I. the sample of red cells contains a group of cells which are more feebly resistant than the similar group in a sample of health and at the same time a group of cells of higher resistance than in health and that the effect of the latter outweighs that of the former so that the total resistance of the sample to Saponin-haemolysis is increased. This conclusion is supported by the evidence of poly-cythaemia which is shown in Group I. and may be interpreted by it to mean that while there is a greater wastage of red-cells, this waste is countered, and indeed made good by an increased output of fresh cells by the bone marrow. That is quite in line with the known facts of increased activity of general metabolism in Fever.

FEVER/
FEVER. GROUP II.

If this interpretation of the facts of Saponin haemolysis shown in Group I is correct, it is in no way invalidated by what is shown in Group II. In Group II there is diminished resistance shown to both the large and the small doses of Saponin, and that diminution appears throughout the operation of the former. The Clinical Notes show a higher degree of pyrexia in this group, and especially in Nos. 8 and 9 in which it was extreme. In this group the correspondence between the haemolysis in 2 hours of the smaller dose and that of the larger dose in half an hour is restored. Further it is to be noted that in this group polycythaemia is absent, though marked oligocythaemia does not appear. It is fair to conclude that in these cases of severe pyrexia, there is an excessive destruction of red-cells, and an excessive production of fresh ones by the bone-marrow: So far as the number of red-cells is concerned a normal balance is almost maintained; but these cells are saturated with haemolytic material, and are being rapidly destroyed in corpore, and in vitro offer a feeble resistance. Whether this excessive destruction/
destruction indicated in Group II is due to excessive activity of the normal haemolytic function, or to presence of extraneous haemolytic agents, there are no materials for judging.

The conclusions to be drawn from both groups would be (1) that in Fever there is an increased destruction of erythrocytes. (2) That where this increased wastage is slight as in Group I, the compensation caused by increased output by the bone marrow is excessive, obliterates the effects of that waste, and produces polycythaemia, and causes a total increased resistance of a sample of red-cells to Saponin. (3) That where this wastage is excessive as in Hyperpyrexia though the fresh output from the bone marrow may prevent olycycthaemia from appearing, the total resistance of a sample of cells to Saponin is much diminished.

**TABLE III. EXOPHTHALMIC GOITRE.**

The four cases of Exophthalmic Goitre investigated have been included in Table III along with those of Fever, because their results of Saponin haemolysis correspond, and because the clinical phenomena of Exophthalmic/
Exophthalmic Goitre allow a similar interpretation of these results.

This group of 4 cases might also be subdivided like those of Fever. For Nos. 1 and 2 show that discrepancy between the haemolysis of the larger dose in $\frac{1}{2}$ hour and the smaller in 2 hours, already noted in Group I. of Fever. While in No. 4 the correspondence is restored. No. 3 was investigated before the final technique was finally settled; but though incomplete, is concordant.

The condition of Exophthalmic Goitre might fairly be described as one of Fever without pyrexia. In almost every system there is evidence of increased metabolism and increased functional activity. That is illustrated in the muscular tremor, in the cardiovascular activity and excitability, in the moisture of the skin, and in the psychical excitement. It is therefore reasonable to expect both an increased activity of the bone-marrow and an increase of the function of haemolysis. The polycythaemia shown in this table is a well established fact of the disease. It, and the surmise of increased haemolysis form with the results of Saponin haemolysis a concordant and inter-dependent group. Exactly the same interpretation may be/
be applied to these results as to those of Fever. Indeed the analogy between the two conditions just pointed out, and the analogous results of Saponin-haemolysis obtained in both make it more probable that this common interpretation is the correct one.

It should be observed that in No.3 of the Exophthalmic Goitre Table, moderate oligocytæmia is present. But the incompleteness of the investigation in this case does not justify a discussion of this fact. The single result of Saponin-haemolysis recorded is quite consistent with the others.

The conclusion to be drawn from the results of Saponin-haemolysis in Exophthalmic Goitre is that there is a moderate increase in the amount of red-cell destruction, with a compensatory activity of the bone-marrow. In 3 of the 4 cases recorded above, this compensation was more than adequate and in spite of the increased destruction of red-cells that is taking place has produced the condition of polycythaemia. In Exophthalmic Goitre then, the condition of the blood is one of polycythaemia together with increased friability of the red-cells to Saponin.
TABLE IV. ANAEMIA.

PERNICIOUS ANAEMIA.

<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>No. of Red Cells</th>
<th>Colour Leuco-</th>
<th>Sap. Sol. 0.12 cc.</th>
<th>0.08 cc.</th>
<th>0.06 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex.</td>
<td>Index.</td>
<td>cytes per cmm.</td>
<td>per cmm.</td>
<td>1/2 hr.</td>
<td>1 hr.</td>
<td>1 1/2 hrs.</td>
</tr>
<tr>
<td>G.J. (m)</td>
<td>25</td>
<td>1,090,000</td>
<td>1.3</td>
<td>4,600</td>
<td>60%</td>
<td>90%</td>
</tr>
<tr>
<td>&quot; (3 weeks later).</td>
<td>744,000</td>
<td>1.1</td>
<td>2,400</td>
<td>84%</td>
<td>98%</td>
<td>99%</td>
</tr>
<tr>
<td>J.S. (m)</td>
<td>51</td>
<td>792,000</td>
<td>1.5</td>
<td>3,300</td>
<td>95%</td>
<td>98%</td>
</tr>
<tr>
<td>&quot; (5 weeks later).</td>
<td>2,370,000</td>
<td>1.5</td>
<td>3,000</td>
<td>75%</td>
<td>93%</td>
<td>97%</td>
</tr>
<tr>
<td>M.R. (f)</td>
<td>37</td>
<td>1,170,000</td>
<td>1.1</td>
<td>2,800</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Mrs S.</td>
<td>54</td>
<td>2,550,000</td>
<td>1.8</td>
<td>4,000</td>
<td>28%</td>
<td>76%</td>
</tr>
<tr>
<td>J.M. (m)</td>
<td>51</td>
<td>1,960,000</td>
<td>1.5</td>
<td>6,800</td>
<td>40%</td>
<td>70%</td>
</tr>
<tr>
<td>A.W. (m)</td>
<td>65</td>
<td>1,760,000</td>
<td>1.13</td>
<td>4,200</td>
<td>28%</td>
<td>71%</td>
</tr>
</tbody>
</table>

Pernicious Anaemia with Spinal Cord Complications.

<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>No. of Red Cells</th>
<th>Colour Leuco-</th>
<th>Sap. Sol. 0.12 cc.</th>
<th>0.08 cc.</th>
<th>0.06 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex.</td>
<td>Index.</td>
<td>cytes per cmm.</td>
<td>per cmm.</td>
<td>1/2 hr.</td>
<td>1 hr.</td>
<td>1 1/2 hrs.</td>
</tr>
<tr>
<td>A.S. (m)</td>
<td>41</td>
<td>3,870,000</td>
<td>1.03</td>
<td>6,000</td>
<td>54%</td>
<td>82%</td>
</tr>
<tr>
<td>&quot; (10 days later).</td>
<td>4,340,000</td>
<td>0.95</td>
<td>3,200</td>
<td>54%</td>
<td>84%</td>
<td>92%</td>
</tr>
<tr>
<td>R.L. (m)</td>
<td>36</td>
<td>2,070,000</td>
<td>1.5</td>
<td>3,000</td>
<td>58%</td>
<td>76%</td>
</tr>
<tr>
<td>E.M. (f)</td>
<td>56</td>
<td>3,040,000</td>
<td>1.2</td>
<td>3,200</td>
<td>40%</td>
<td>74%</td>
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</table>

Health average, etc. | 23% | 55% | 73% | 86% | 18% | 10% |
<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>No. of Red Cells</th>
<th>Leucocytes</th>
<th>Colour</th>
<th>Sap.Sol. 0-12cc</th>
<th>0-08cc</th>
<th>0-06cc</th>
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<tr>
<td>Health (average)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>A.S. (f)</td>
<td>37</td>
<td>1,940,000</td>
<td>0.98</td>
<td>3,600</td>
<td>27%</td>
<td>78%</td>
<td>88%</td>
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<tr>
<td>Mrs R. (f)</td>
<td>29</td>
<td>2,010,000</td>
<td>0.42</td>
<td>8,000</td>
<td>54%</td>
<td>77%</td>
<td>88%</td>
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<tr>
<td>J.F. (f)</td>
<td>33</td>
<td>4,720,000</td>
<td>0.5</td>
<td>6,000</td>
<td>48%</td>
<td>62%</td>
<td>81%</td>
</tr>
<tr>
<td>SIMPLE ANAEMIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.C. (f)</td>
<td>22</td>
<td>4,340,000</td>
<td>0.48</td>
<td>6,400</td>
<td>44%</td>
<td>76%</td>
<td>93%</td>
</tr>
<tr>
<td>M.D. (f)</td>
<td>20</td>
<td>4,130,000</td>
<td>0.58</td>
<td>4,200</td>
<td>--</td>
<td>--</td>
<td>--</td>
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<tr>
<td>PURPURA</td>
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<tr>
<td>J.H. (m)</td>
<td>37</td>
<td>4,470,000</td>
<td>0.96</td>
<td>6,400</td>
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<tr>
<td>J.G. (m)</td>
<td>42</td>
<td>1,890,000</td>
<td>1.0</td>
<td>18,000</td>
<td>38%</td>
<td>77%</td>
<td>91%</td>
</tr>
<tr>
<td>SPLENIC ANAEMIA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P.C. (m)</td>
<td>37</td>
<td>1,970,000</td>
<td>1.01</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>SPLENO-MEDULLARY LEUKAEMIA</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.F. (f)</td>
<td>29</td>
<td>3,100,000</td>
<td>1.0</td>
<td>30,800</td>
<td>42%</td>
<td>68%</td>
<td>83%</td>
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<tr>
<td>HODGKIN'S DISEASE</td>
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<tr>
<td>Mrs A. (f)</td>
<td>25</td>
<td>2,610,000</td>
<td>0.84</td>
<td>37,000</td>
<td>14%</td>
<td>55%</td>
<td>68%</td>
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<tr>
<td>Health (average)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23%</td>
<td>55%</td>
<td>76%</td>
</tr>
</tbody>
</table>
TABLE IV. CLINICAL NOTES.

PERNICIOUS ANAEMIA.

No.1. G.J (age 25, m.). PERNICIOUS ANAEMIA (second attack). First attack was a year ago, but he recovered almost completely. Blood film shows numerous megalocytes, megaloblasts and normoblasts; much pochelocytosis; Jan. 31st, 1908.
No.2. The Same. Febr. 21st, 1908. Patient's condition has got steadily worse since last investigation.

No.3. J.S. (aet. 51, m.). PERNICIOUS ANAEMIA (first attack). Has felt increasing weakness for four months. Blood-film shows numerous megalocytes, and many nucleated red cells, chiefly megaloblasts. Febr. 18th, 1908.

No.4. Same as No.3. March 5th, 1908. Has improved much since last investigation; has been treated with arsenic.

No.5. M.R. (aet. 37, f.). PERNICIOUS ANAEMIA (first attack). Weakness first noticed five months ago, and has increased. No haemorrhages or other cause known. Blood-film shows megalocytes and a few nucleated red-cells.

No.6. Mrs. S. (aet. 54). PERNICIOUS ANAEMIA (chronic). Patient at present working at home; was in hospital five months ago for six weeks. Her blood-determination was then as it is now, and was not improved/
improved by treatment. At present she feels "a little weak", but otherwise is able to work.
Blood-film shows numerous and very large megalocytes; no nucleated red-cells observed.

No.7. J.M. (aet.51, m.). CHRONIC PERNICIOUS ANAEMIA. Has been "bloodless" at times for five years; is constipated, and has bleeding piles for many years. Blood-film shows numerous large megalocytes, and one or two normoblasts.

No.8. A.W. (aet.65, m.). PERNICIOUS ANAEMIA. Has felt growing weakness for six months; no cause known. Blood-film shows many megalocytes, and some nucleated reds, - both normo-, and megaloblasts. but chiefly the former.

PERNICIOUS ANAEMIA WITH SPINAL-CORD COMPLICATIONS.

No.9. A.S. (aet.41, m.). PERNICIOUS ANAEMIA (second attack). First attack of weakness came on in April 1907 without known cause. His condition got worse till August 1908, when he began to improve/
improve, and almost entirely recovered strength. Weakness returned slightly in November 1907, and with it, stiffness in the legs and difficulty in walking. He now shows well-marked sclerosis of the lateral columns of the spinal-cord. Blood-film shows almost no pochelocytosis or megalocytes. No nucleated red-cells found. March 3rd, 1908.

No.10. Same as No.9. March 13th, 1908. Blood condition almost the same as when last examined.

No.11. R.I. (aet.36, m.). CHRONIC PERNICIOUS ANAEMIA. Has noticed pallor and slight weakness for a year. Three months ago stiffness in the legs and difficulty in walking appeared. At present both knee-jerks are exaggerated, both Plantor reflexes show Babinski's sign. Blood-film shows numerous large megalocytes; two nucleated red-cells seen, both megaloblasts.

No.12. Mrs E.M. (aet.56). PERNICIOUS ANAEMIA (second attack). First noticed weakness and pallor in the beginning of 1906; was under treatment in hospital for several months of the summer/
summer, and went home in the early autumn with blood-condition healthy and strength restored. Weakness returned in summer of 1907, and with it stiffness and weakness of the legs in walking. She now shows a marked spastic paraplegia. Blood-film shows a slight increase of size of erythrocytes. No nucleated red-cells seen. Their occurrence was exceptional throughout numerous observations from the beginning of this case.

SECONDARY ANAEMIA.

No.1. Mrs. A.S. (aet. 37). SECONDARY ANAEMIA of unknown cause. Has been in poor health for two years. Spleen is enlarged and is palpable at the costal margin. Urine contains a considerable amount of blood, with trace of albumen; no casts.

No.2. Mrs. R. (aet. 28). ANAEMIA SECONDARY TO POST-PARTUM HAEMORRHAGE. Lost much blood after birth of child two months ago. Has felt weak, tired, and short of breath since.

No.3/
No. 3. J.F. (aet. 33, f.). SECONDARY ANAEMIA, cause unknown. Has been "bloodless" for eight years. She suffers severe pain over region of left ovary, but gynecological examination shows no organic change in the pelvic organs to account for this.

SIMPLE ANAEMIA.

No. 1. M.M.C. (aet. 22, f.). Has suffered from "bloodlessness" at times for three years, constipation alternating with short attacks of diarrhoea.

No. 2. M.D. (aet. 20, F.). Recurrent attacks of "bloodlessness" for four years; constipation.

PURPURA.

No. 1. J.H. (aet. 37, m.). History of occasional purpuric rashes with bleeding from the gums for seven years. Social conditions and food have been always satisfactory.

No. 2/
No. 2. J.G. (aet. 42, m.). PURPURA, with Carcinoma of Prostate. Has had vomiting and diarrhoea for fifteen weeks. Condition of Prostate caused no local symptoms. On admission a fortnight ago, there was a purpuric eruption of large haemorrhagic spots on the legs and arms. Pigmented traces of this remain at present.

SPLENIC ANAEMIA.

No. 1. P.C. (aet. 37, m.). Has noticed the enlargement of the spleen a little over a year ago; it has slowly increased in size since. At the same time he became weak, easily tired, and short of breath. Spleen now extends downwards to the anterior superior spine of the ilium, and laterally to the umbilicus. Differential leucocyte-count:—Polymorpho, 59%; Lymphocytes, 28%; large Mononuclear, 10%; Eosinophiles, 2%.

SPLENO-MEDULLARY LEUKAEMIA.

No. 1. B.F. (aet. 29, f.). Has noticed enlargement of spleen, and has felt weak, and short of breath for/
for 2½ years. Spleen now extends to the umbilicus laterally and down into the pelvis. Since exposure to X-rays, the leucocytes have fallen from 180,000 to about 30,000 per c.mm. At present the leucocytes include 50% Polymorpho-nuclear, and 30% Myelocytes.

HODGKIN'S DISEASE.

No.1. Mrs. A. (25). Has noticed onset of pallor and weakness about 5 years ago. From this date slow and progressive enlargement of glands on the left side of the neck, and in the left axilla. These now form large, fused hard masses. Spleen considerably enlarged and is palpable below the costal margin: extends to the level of the umbilicus downwards, and to 2 inches from it laterally.

RESULTS OF TABLE IV.

This table might be regarded as giving the results of Saponin-haemolysis in various conditions of oligocythaemia: Oligocythaemia is the common blood-characteristic/
characteristic of all the cases included in it with the exception of the two cases of Simple Anaemia. That common feature is a useful standpoint from which to compare the results in the different groups of the Table. The groups, Pernicious Anaemias, is the largest and most representative. It includes 12 investigations upon 9 cases of Pernicious Anaemia. Where there are repeated observations on one case, this is either because the blood-condition had much changed when the results of haemolysis proved to be different; or because the blood-condition had remained stationary when the Saponin-reaction remained unchanged.

In cases 1 - 5 the condition is severe and oligocythaemia is marked. In these the resistance of the red-cells is decidedly lowered, and it reaches its minimum in Cases 2 & 3 where the oligocythaemia is greatest. In Cases 1 & 2 the blood is from the same individual, but in Case 2, the determination, made after an interval of 3 weeks, shows a decided fall in resistance, and in that interval the number of red-cells has fallen from 1,090,000 to 744,000 per c.mm. A corresponding change is seen in Cases 3 & 4, both again from one individual: for the increased/
creased resistance evident in Case 4 is accompanied by an increase of the red-cells from 792,000 to 2,370,000 per c.mm. In Cases 6 - 12 the condition is less severe, and oligocythaemia is less marked. In these the resistance is decidedly above that of Cases 1 - 5, but is still appreciably below the standard of health. The final stage of haemolysis of the larger dose of Saponin shows little change from that of health, but if attention is paid both to the early stage of haemolysis of the larger dose, and to the haemolysis of the smaller dose in 2 hours the diminution of resistance in those cases of Pernicious Anaemia becomes apparent. In this group oligocythaemia does not march pari passu with diminished resistance, for if Cases 7 & 8 be compared with Cases 9, 10, & 12, it will be seen that in the former fewer red-cells and a higher resistance go together, while in the latter there is a diminished resistance with more red-cells. That divergence makes it difficult to draw conclusions from the factor of oligocythaemia. Case 8 is a severe anaemia so far as the blood-determination goes, and in all the essential characteristics is a classical case of Pernicious Anaemia, and yet the reaction of its red-cells/
cells to Saponin is practically that of health. In Case 12 again, the number of red-cells is higher, but the Saponin-resistance of these cells is appreciably lower. A comparison of the two factors, oligocythaemia, and Saponin-resistance in this Table of Pernicious Anaemia gives the following results. Where the oligocythaemia is below 1,500,000 per c.mm. the Saponin-resistance is lowest but where the oligocythaemia is moderate, namely between 1,500,000 and 2,500,000 per c.mm. the Saponin resistance is highest, and is greater than in cases where the red-cells number upwards of 2,500,000 per c.mm.

SAPONIN RESISTANCE & THE RATE OF PROGRESS OF THE DISEASE.

The number of red-cells, and their Saponin-resistance are therefore not concordant in their variations. But it will be found that correspondence exists between the rate of progress of the disease and the red-cell resistance. In Cases 1 - 5; the Anaemia is of short duration or acutely progressive, and in them the Saponin-resistance is low. But in Cases 6 - 12 the anaemia is of long standing.
standing and slowly progressive and in this group, irrespective of the number of cells, the Saponin-
resistance is higher.

TABLE IV. THE OTHER ANAEMIAS.

The results of Saponin-haemolysis in the other groups of Anaemia are interesting when con-
sidered along with those of Pernicious Anaemia. They are uniform and show a diminished resistance as compared with that of health. The degree of diminu-
tion both with larger and smaller doses of Saponin is almost identical with that of Cases 6 - 12 of moderate or chronic Pernicious Anaemia.

In these different groups also as in Pernicious Anaemia, the degree of oligocythaemia gives little clue to the Saponin resistance. The different groups show a wide range in the number of erythrocytes, and yet throughout there is only a narrow range of variation of Saponin-resistance. And further the two factors do not in their varia-
tions fluctuate in the same direction but rather diverge. The relation of the Colour-Index(Haemo-
globin-richness of the corpuscle) to the Saponin-
resistance/
resistance throughout the whole of Table IV. is noteworthy. If the severe cases of Pernicious Anaemia are excluded, it will be seen that an excess, defect or standard quantity of Haemoglobin has no effect upon the Saponin-resistance of the corpuscles.

DISCUSSION OF RESULTS OF

TABLE IV.

This table is chiefly an investigation into the Saponin-resistance of erythrocytes in Pernicious Anaemia. In the other groups of the table there are not many investigations in any one group and these may not be sufficiently representative of the disease. But collectively they form a fairly large group of data from widely different blood-conditions; and in considering the pathology and pathogenesis of Pernicious Anaemia from the point of view of the Saponin-resistance of its corpuscles, the similar facts from this heterogeneous group of Blood-Diseases will be very relevant to that discussion.

HYPOTONICITY IN PERNICIOUS ANAEMIA.

At/
At the outset, it may be stated that no data exist of the resistance of the red-cells in Pernicious Anaemia obtained by the hypotonic salt solution method. And yet Pernicious Anaemia is a disease more favourable than most for the Application of this method. In severe cases the specific gravity of the blood reaches a lower point than that recorded in any other diseases, though von Noorden (13.) has pointed out that the diminution in the specific gravity of the blood-serum is less than that of the entire blood. The use of hypotonic salt-solutions might, therefore be expected to test some factor of cell-resistance which is called into play in the blood in Pernicious Anaemia. The method however has not been employed, but in any case it is most unlikely, that the oligocythaemia of Pernicious Anaemia is due to laking in corpore of the red-cells in a hypotonic medium. If that took place free Haemoglobin would be found in the blood-serum in Pernicious Anaemia and such Haemoglobinaemia does not occur. In the 12 investigations on Pernicious Anaemia in Table IV., the supernatant fluid after the first washing was observed, and in no case was it tinted with free Haemoglobin in the smallest degree. A similar observation was made on 9 other cases of Pernicious Anaemia investigated during/
during the period when my technique was erroneous, and was always negative. At the same time it might be held that a lowered specific gravity in the blood of Pernicious Anaemia while not directly laking the corpuscles might assist and exaggerate the action of the normal haemolysis in corpore. But there is no experimental evidence that the action of a haemolytic solvent is increased if the red-cells are suspended in a fluid which is almost hypotonic. That is merely an assumption, though one that has been freely made.

And there is experimental evidence in the opposite direction. Sach's (15) has shown that corrosive sublimate a haemolytic substance, when added to blood-cells in \( \frac{1}{6} \) solution, prevents the laking of these cells in distilled water. That is to say, the minor action of one haemolytic agent has altogether prevented the action of another haemolytic agent - distilled water.

DIFFERENT VIEWS AS TO THE ETIOLOGY OF PERNICIOUS ANAEMIA.

It will be well to shortly state and group together the principal views as to the etiology of Pernicious/
Pernicious Anaemia: and then to adjust the facts of Saponin-haemolysis to them.

The condition of the bone marrow in Pernicious Anaemia is the basis of several theories. Pepper (16) first described it, and considered it the cause of the disease and to consist in a primary hyperplasia. Cohnheim (17) also considered it primary, and of the nature of retrogression to the embryonic condition. Rindfleisch(18) took it to be a primary failure to convert erythroblasts into erythrocytes so that the nucleated cells continued to grow and became excessively large. Muir (19) studied the bone marrow in conjunction with the pigmentary changes in the liver. He concluded from their combined evidence that there was an excessive destruction of blood in Pernicious Anaemia; and that the condition of the bone marrow was not primary, but secondary and of the nature of regeneration to compensate the excessive haemolysis.

The clinical phenomena of the disease, and especially its association with bad or insufficient food, poverty, previous haemorrhage, suggested another view. This was stated in slightly different forms by many observers, but Eichhorst's may be taken as representative/
representative of the group. He believed that
Pernicious Anaemia might be due to widely different
causes that lowered nutrition; that it was Anaemia
in its extreme degree, and differed from other forms
of Anaemia only in degree and not in kind. He
admitted the possibility of a primary form. (20).

Hunter (12) enunciated a different and
original view. From extensive observations on the
sites of storage of blood pigment in the body, and
from variations in the degree and site of that
storage produced by the injection of haemolytic
substances into the blood, he came to two main con-
clusions.

1. Normal haemolysis in corpore takes place in
   the portal circulation.

2. In Pernicious Anaemia there is abnormal
   haemolysis in the portal circulation, not an excess of the normal haemolysis,
   but an extraneous haemolysis due to the
   introduction of some haemolytic poison
   into the portal system from the gastro
   intestinal area.

Stockman (21) opposes Hunter's view, and
believes that Pernicious Anaemia is only an extreme
form/
form of Anaemia. Its apparently pathognomonic features in the liver and bone marrow are due to capillary haemorrhages, superfinal and interstitial all over the body. The loss of blood occasioned by these haemorrhages causes both the regenerative changes in the bone marrow shown by Muir and the excessive storage of pigment in the liver emphasised by Hunter. This view may be regarded as a re-statement of Eichhorst's. But it is adjusted to the most recent pathological facts as to the condition of the liver and bone marrow in Pernicious Anaemia, and it is original in the place given to capillary haemorrhages in the production of the final stage of Anaemia.

SAPONIN-HAEMOLYSIS IN PERNICIOUS ANAEMIA.

From the above summary it will be seen that the etiology of Pernicious Anaemia has been discussed from several points of view, - from the condition of the bone marrow from the study of pigment deposits in the liver and elsewhere, and from experimental production in corpore of haemolysis. But the bearing of the resistance of the red-cells in Pernicious Anaemia has/
has not received attention. Hunter indeed quotes two isolated observations on this point. The first is by Mackern and Davy, and dates back to 1876 (22). They observed in a severe case of Pernicious Anaemia, a concentration of the Haemoglobin in the periphery of the large distorted corpuscles, and concluded that there was a separation of the colouring-matter from the stroma, owing to the soft condition of the latter. This conclusion is not only very doubtful, but is also away from the question at issue which is the condition of the cell envelope with regard to its power to retain its Haemoglobin-contents.

The second is by Copeman that in Pernicious Anaemia it is unusually easy to obtain Haemoglobin crystals by taking of the blood (23). Hunter does not give the correct reference to this observation, and I have been unable to trace it. Neither of these observations were exact, and very little could be drawn from them; and they occupy no place in Hunter's elaborate arguments to prove the existence of a specific haemolytic substance in Pernicious Anaemia. But it is evident that the resistance of the red-blood cells, if it could be accurately determined, might give valuable information as to the pathology of Pernicious Anaemia. It expresses a reaction of living cells, and as such might shed more light on the/
the question than the histological examination of organs postmortem. And any facts as to such a reaction are specially relevant to William Hunter's view of Pernicious Anaemia as a specific haemolytic Anaemia.

RESULTS OF SAFFONIN-HAEMOLYSIS IN PERNICIOUS ANAEMIA IN THEIR BEARING ON HUNTER'S THEORY.

There is a preliminary observation. On the assumption of Hunter's view of a powerful haemolytic poison located in its action to the portal circulation, we would expect to find free Haemoglobin in the systemic circulation. It is admitted that in the majority of cases that this does not occur, and Hunter explains it by stating that all the free Haemoglobin is intercepted by the liver. But his experimental evidence in support of this is not conclusive. A decisive experiment, such as the production of Haemoglobinemia within the portal circulation, either by injection of a haemolytic substance or of free Haemoglobin into that area of the blood-stream, was not performed. But even if we admit the explanation, we would expect to find evidence/
evidence of the existence of this haemolytic poison in the envelopes of the red-cells that have escaped into the general circulation. These must show some trace of contact with a powerful poison which can almost decimate their numbers, in face of extraordinary efforts on the part of the bone marrow to make good the loss. But if the cell envelopes do bear such traces of haemolytic interaction, Saponin-haemolysis does not discover them. The moderate diminution of resistance in Pernicious Anaemia which Table IV shows is not consistent with a rapid destruction of red-cells in corpus by a foreign haemolysin. That diminution no doubt is extreme in Cases 2 & 3 but there the poverty of blood is extreme. The striking fact of Table IV is that it contains a group of cases of Chronic but pronounced Pernicious Anaemia (all showing the classical blood-characters of Pernicious Anaemia), and that in these the resistance of the red-cells to Saponin-haemolysis is only slightly less than that of a varied group of other Anaemias (whose blood-characters are quite distinct from those of Pernicious Anaemia). The conclusion is that in Anaemia generally the resistance of the red-cells is lower than in Health, but/
but there is no clear line of division to be drawn in respect of Saponin haemolysis, between Pernicious Anaemias and other Anaemias. That result lends no support to Hunter's view of a specific haemolytic toxin, or indeed to the much more guarded statement of Muir that in Pernicious Anaemia an excessive destruction of erythrocytes takes place. (loc. cit.)

CONCLUSION FROM RESULTS OF SAPONIN-HAEMOLYSIS IN ANAEMIA.

Saponin has been shown by Ransom (loc. cit.) to link itself to Cholesterin in solution and also to link itself to some element in the envelopes of red-cells. It has therefore been fairly enough assumed that it attaches itself to the Cholesterin which is known to form an important element in these cell-envelopes. If that fact is applied to the results of Saponin haemolysis in Table IV, the inference is that, in the various groups of Anaemia contained in it, there is a defect of Cholesterin as compared with that present in an equal number of cell-envelopes of Health, but that differences in this/
this defect depend rather on the degree of Anaemia than on other special characters of the blood, nucleated red-cells, Haemoglobin-richness, which have been used to classify distinct groups of blood-diseases.

The term, anaemia may be taken to include two things, a reduction of the number of cells, and a defect of Haemoglobin. Table IV showed that variations in the amount of Haemoglobin did not produce corresponding variations in the resistance to Saponin-haemolysis. For in the group of Pernicious Anaemias the red-corpuscles contained excess of Haemoglobin, in the group of Simple and Secondary Anaemias a defect of it, and in the other groups of the Table a standard amount of it; and yet the variations of this factor did not affect the Saponin resistance. Within a certain range, the same thing can be said of the factor of oligocythaemia. That is to say, Table IV showed considerable variation both in the number of red-cells, and in the amount of Haemoglobin; but throughout, the diminution of resistance to Saponin was approximately equal, or at least its degree did not depend upon the other two factors. Anaemia therefore involves another departure from the standard of health, namely a defect in the protoplastic envelope that contains the/
the Haemoglobin of the cell. A red-cell, constituted mainly of two things, a protoplasmic envelop, and Haemoglobin, may be defective in one of these constituents or in both. The peculiarity of the red-cells in Pernicious Anaemia is that the supply of Haemoglobin is abundant while the materials that go to form the protoplasmic envelope are lacking. The consequence is oligocythaemia combined with a surplus of Haemoglobin in each cell. But, in Pernicious Anaemia in respect of the protoplasmic envelope, there is not a much greater defect, cell for cell, than in other Anaemias. The difference at any rate is rather in degree than in kind. But clinical facts show that in Pernicious Anaemia this defective formation of the protoplasmic envelope is permanent and progressive: while in other anaemias it is not generally so severe, may be only temporary, and is not progressive beyond a certain point. That conclusion, based on the results of Saponin-haemolysis practically restates Eichhorst's view that Pernicious Anaemia is a failure in nutrition which may be primary, but is often secondary to the many different causes that lower nutrition: and that it only differs from other forms of Anaemia in being extreme and progressive/
progressive. The affinity of Saponin for Cholesterol in an element in all protoplasm, and the results of Saponin-haemolysis in Anaemia suggest that the amount of Cholesterol is inadequate or that its metabolism is in some way deranged. And the widely spread fatty degeneration of tissues and organ that is found postmortem in Pernicious Anaemia supports this hypothesis of a break down in the intimate metabolism of the body protoplasm. It may be objected that the hypothesis of an excessive erythrocytolysis would equally well explain the fact of general fatty degeneration. But the Saponin-resistance of the red-cells in Pernicious Anaemia is inconsistent with Hunter's theory of the presence in the body of a powerful haemolytic poison. The diminution of resistance in it is too small to support that assumption: it is not nearly so extreme as in Jaundice. And in Jaundice the increased friability of the red-cells only expressed itself in the resistance to Saponin. There was not in addition a marked oligocytthaemia. In Pernicious Anaemia, therefore, where oligocytthaemia is marked, the friability of the remaining cells should be much more extreme, on the assumption of Hunter's theory but instead of that their resistance to Saponin is much greater/
greater than in Jaundice.

INCREASE OF IRON PIGMENT IN THE LIVER IN PERNICIOUS ANAEMIA.

This fact forms the basis of Hunter's theory of Pernicious Anaemia. In conjunction with the condition of the bone marrow it led Muir (19) to his conclusion of an excessive haemolysis in this disease. And it seemed to demand from Stockman (21) a special explanation, namely the occurrence of numerous capillary haemorrhages. It seems to be assumed in the above that an increase of iron-pigment in the liver must be due to an increased destruction of red-cells either intravascularly (Hunter) or extravascularly (Stockman). But it is equally reasonable that the liver should be the storehouse of excess of such pigment which the bone marrow is unable to make use of. It is necessary to deal with this point because Stockman's statement as the frequency of capillary haemorrhages in Pernicious Anaemia has not been generally accepted. But the increase of iron-pigment in the liver is perfectly consistent without that occurrence of capillary haemorrhage.
It is compatible with the view of Pernicious Anaemia as a disease where, of the two kinds of material that build up the erythrocyte, the supply for the protoplasmic envelope is deficient, but the amount of Haemoglobin is unaltered and abundant.

**SCLEROSIS OF THE SPINAL CORD IN PERNICIOUS ANAEMIA.**

This complication of Pernicious Anaemia has been attributed by Hunter to toxic action, and has been used by him to support the theory of the existence of a specific poison. But in view of the conclusions just drawn from the results of Saponin-haemolysis in Pernicious Anaemia, it is capable of another explanation.

Cholesterin is an important constituent of red-cells, and an even more important element of the medullated nerve-sheaths in the central nervous system. The replacement of the nerve sheaths by fine connective tissue in the lateral sclerosis complicating Pernicious Anaemia may be due to the reduction in the supply of Cholesterin, and to its diversion to the more urgent needs of the bone marrow.
Case 9, Table IV supports this suggestion. Here there was recovery from a very severe attack of Pernicious Anaemia, but this was followed by the appearance of lateral sclerosis of the spinal cord, although the blood-condition continued near the standard of health. The interpretation of this according to the above suggestion would be that the previous severe anaemia had diverted cholesterin from the spinal cord. The system would now require a smaller amount to repair metabolic waste, and enough would thus be available for the bone-marrow to maintain the blood near the balance of health.

The other two cases in this sub-group of Pernicious Anaemias in Table IV. are concordant with this suggestion. In them the anaemia is much more severe, but it is of a chronic and slowly progressive form.

CONCLUSION.

The results of Saponin-haemolysis in Pernicious Anaemia are not consistent with Hunter's view of a haemolytic toxin. Their approximation to similar/
similar results in other forms of Anaemia indicate that in Anaemia generally there is a lowered state of nutrition. This lowered nutrition in Pernicious Anaemia may be primary, or secondary to many debilitative conditions. It essentially differs from that in other forms of Anaemia in being progressive.

TABLE V. TWO CASES OF ANAEMIA WITH ENLARGEMENT OF SPLEEN SHOWING SPECIAL REACTION TO SAPONIN.

<table>
<thead>
<tr>
<th>No. Name</th>
<th>Age</th>
<th>No. of Red Cells</th>
<th>Colour Leuco- Index</th>
<th>Sap Sol. 0.12cc.</th>
<th>0.08cc. 0.06cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.K. (m)</td>
<td>36</td>
<td>3,900,000</td>
<td>0.93</td>
<td>1,800 88%</td>
<td>98% 99% 99.6%</td>
</tr>
<tr>
<td>&quot; (2 weeks later)</td>
<td></td>
<td>3,900,000</td>
<td>0.80</td>
<td>1,600 83%</td>
<td>97% 98% 99%</td>
</tr>
<tr>
<td>Mrs EL (f)</td>
<td>36</td>
<td>1,980,000</td>
<td>1.16</td>
<td>4,400 95%</td>
<td>98% 99.5% 99.8%</td>
</tr>
<tr>
<td>&quot; (3 weeks later)</td>
<td></td>
<td>2,400,000</td>
<td>1.00</td>
<td>2,800 91%</td>
<td>99% 99.8% 99.9%</td>
</tr>
<tr>
<td>Health (average)</td>
<td></td>
<td></td>
<td></td>
<td>23% 25% 75%</td>
<td>86% 18% 10%</td>
</tr>
</tbody>
</table>

HAEMOLYSIS

CLINICAL/
No. 1. J.K. (aet. 36, m.). Coachman; admitted to hospital in December 1907 for the purpose of operation for inguinal hernia, but he developed pyrexia, which has returned at intervals since. The pyrexic periods last two or three days, and the temperature rises to 100° or 101° F. He has never been out of Scotland, and never suffered from these intermittent attacks of fever before. Spleen is slightly enlarged - 4½ inches vertically in mid-axillary line. Has an enlarged gland, the size of a bean, firm and resilient, behind the middle of the posterior border of the sterno-mastoid.

Differential Leucocyte count:—

- Polymorphs 40%
- Lymphocytes 44%
- Large Mononuclears 13.8%
- Eosinophiles 2.2%

Film shows a few nucleated reds.

(a). February 25th, 1908.

(b). March 12th, 1908.
No. 2. Mrs E. L. (aet. 36). Has been weak, easily tired, and short of breath for ten years. Her skin from this time was always sallow, but at times became of a more jaundiced colour. Went to South Africa four years ago, when enlargement of her spleen was first pointed out to her by the doctor. Spleen now extends laterally to the umbilicus and downwards 1½ inches below it. No enlargement of superficial lymphatic glands.

Differential Leucocyte count:

<table>
<thead>
<tr>
<th>Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphs</td>
<td>43%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>45%</td>
</tr>
<tr>
<td>Large Mononuclears</td>
<td>4%</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>8%</td>
</tr>
</tbody>
</table>

Film shows very numerous nucleated reds - normoblasts.

(a). March 6th, 1908.
(b). March 27th, 1908.

These two cases showed such a feeble resistance to Saponin that I have grouped them distinctly from the other anaemias. Two observations were made at separate periods on each case; and without much change in the blood-condition, the feeble resistance was confirmed by the repeated observations.
The oligocythaemia is not marked, but this fact should be placed alongside another, the presence of nucleated red-cells – normoblasts. The latter indicates an exceptional degree of activity on the part of the bone-marrow. Both together point to an excessive destruction of red-cells. The differential leucocyte determination in each case confirms that suggestion indirectly. The total number of leucocytes is reduced in both, and in the first case to a remarkable extent. That reduction is mainly in the polymorpho-nuclear cells, which are produced in the bone-marrow. That may mean either a defective production of them in the marrow, or an excessive destruction in the blood stream.

This oligocythaemia, the evidence of increased output of red-cells, and the feeble resistance of the red-cells to Saponin-haemolysis together strongly suggest that an excessive destruction of erythrocytes is occurring in these two cases. What the nature of that exceptional haemolysis is, whether an increase of normal haemolysis, or the presence in the blood-stream of a foreign haemolytic substance, there is no material for judging.

The enlargement of the spleen is noted in both cases. Its significance is quite obscure. In several/
several of the cases in Table IV. enlargement of this organ was recorded and coexisted with a red-cell resistance little below the standard of health.

**TABLE VI. DIABETES MELLITUS.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Sex</th>
<th>Age</th>
<th>No. of Red Cells</th>
<th>Colour Leuco-Sap.</th>
<th>Sol. 0.12 cc.</th>
<th>0.08 cc.</th>
<th>0.06 cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>G.L.</td>
<td>m</td>
<td>59</td>
<td>4,600,000</td>
<td>1.0</td>
<td>6,800</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2.</td>
<td>J.L.</td>
<td>m</td>
<td>43</td>
<td>4,800,000</td>
<td>1.06</td>
<td>9,600</td>
<td>10%</td>
<td>35%</td>
</tr>
<tr>
<td>3.</td>
<td>G.W.</td>
<td>m</td>
<td>27</td>
<td>4,670,000</td>
<td>0.9</td>
<td>7,000</td>
<td>2%</td>
<td>37%</td>
</tr>
<tr>
<td>4.</td>
<td>R.M.</td>
<td>m</td>
<td>18</td>
<td>5,600,000</td>
<td>0.97</td>
<td>6,000</td>
<td>28%</td>
<td>53%</td>
</tr>
</tbody>
</table>

Health (average) | 0.98 cc. 0.06 cc. |

**CLINICAL NOTES. TABLE V.**

No.1. G.L. (aet. 59, m.). Has noticed thirst and polyuria for six months. 100 oz. of urine per diem. Sugar, 1300 grs. per diem.

No.2/
No. 2. J.L. (aet. 43, m.). Has had polyuria for two years. Urine, 100 oz. per diem; sugar, 2100 grs. per diem.

No. 3. G.W. (aet. 27, m.). Polyuria for seven months. Urine, 120 oz. per diem; sugar, 2880 grs. per diem.

No. 4. R.M. (aet. 18, m.). Thirst and polyuria for four months. Urine, 120 oz. per diem; sugar, 3000 grs. per diem.

RESULTS OF TABLE V.

In this table the results of investigation in four well-marked cases of Diabetes Mellitus are included.

In No. 1, the larger dose of Saponin given was 0.14cc., and its operation can now be separately given and compared with the action of a similar dose upon erythrocytes of health.
CONDITION

<table>
<thead>
<tr>
<th>Name</th>
<th>Age</th>
<th>Sex.</th>
<th>G.L.(ra)</th>
<th>Diabetes- Mellitus</th>
<th>M.S.(m)</th>
<th>Health</th>
<th>Sap. Sol.</th>
<th>½hr.</th>
<th>1 hr.</th>
<th>1½ hrs</th>
<th>2hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.L.(m)</td>
<td>59</td>
<td>0.14cc.</td>
<td>24%</td>
<td>36%</td>
<td>95%</td>
<td>97%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.S.(m)</td>
<td>22</td>
<td>0.14cc.</td>
<td>21%</td>
<td>94%</td>
<td>97%</td>
<td>98%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

That result is concordant with that of the small dose of Saponin recorded in Table V. for this case.

These four cases show an increased resistance on the part of the red-cells to Saponin-haemolysis. This is hardly appreciable in No.1, but in the remaining cases it is quite marked. This increase of resistance is present both throughout the larger dose, and at the termination of the smaller one. In the latter respect it differs from the results of Fever, Table III., Group I., where there was a discordance, very difficult to explain, but consistent throughout the group, between the early haemolysis of the larger dose, and the final stage of the smaller one.

These cases of Diabetes therefore show an increased resistance of a sample of their erythrocytes from the beginning to the end of haemolysis.
THE RESISTANCE OF RED BLOOD-CORPUSCLES TO HYPO-TONIC SALT SOLUTIONS:

All the exact data on the resistance of human red-cells in health and disease have been obtained by this method. They are few in number. Chanel (25) found their resistance increased in Jaundice, and proportionately to the severity of the Jaundice. This was confirmed by v. Limbeck (4) and by Viola (24).

Janowsky (25) found the resistance increased in Infectious Diseases and especially in Typhus Fever; also in Cancer of internal organs. Lang (25) studied the red-cell resistance in malignant and non-malignant conditions of the stomach, and found a great increase in the former and only a slight increase in the latter. Chanel's investigations entitled, "Recherches sur la resistance des Haematies." These Doct. Lyons, 1886, and Janowsky's several papers published in the Klinische Wochenschrift (St. Petersburg) were inaccessible to me. Fortunately Lang, in a long paper, gives a full account/
account not only of the results of these observers, but also of the technique by which they were obtained. Chanel and Janowsky both employed the principle of enumeration in determining haemolysis: and adopted it independently of each other. As the method of enumeration is the basis of the technique of the present investigation, it is necessary to state that it was conceived and developed by me early in this research, and independently of Chanel and Janowsky. It was only at a late period that I obtained from Lang's paper the clue to its previous employment by the French and Russian observers. My application of the principle was entirely different. One common fact about the results obtained by this method is noteworthy. They show in every one of the few diseases investigated an increased resistance of the red-cells as compared with health. That is significant in view of the fact that, in Saponin-haemolysis, the resistance is in some cases increased, and in others diminished, but the departure from the standard of health is much greater in the direction of diminution than in that of increase.

It has already been pointed out that results obtained in Jaundice by the one method are exactly/
exactly contradicted by those of the other. In that condition the divergence of results obtained by two different methods was most striking. I therefore employed both methods in a few cases of Jaundice, and in other conditions of disease in which I had obtained definite results with Saponin-haemolysis.

The technique of the hypotonic method I employed was very simple. In the usual way I prepared suspensions of washed red-cells, and measured from the samples that contained 50 million cells. These samples were brought in every case to a bulk of 1 cc. by addition of saline solution. In every case there was thus a suspension of 50 million washed cells in an equal bulk of 0.85 saline solution. To this was added 1 cc. of Distilled Water. These red-cells were now bathed in 0.42% saline solution. The degree of haemolysis resulting was determined by enumeration of the remaining cells in the suspension.

TIME AT WHICH HAEMOLYSIS WAS RECORDED.

A hypotonic saline suspension having been prepared, it was necessary to fix a period at which to record haemolysis. This was easy, for the results/
results given under, show that the full degree of haemolysis is reached very soon after the preparation of the hypotonic fluid.

In the following 2 tubes there is a bulk of 2 cc. of 0.42 saline solution, containing 50 mlns. washed red cells, in each tube.

HAEMOLYSIS AFTER.

<table>
<thead>
<tr>
<th>NAME</th>
<th>CONDITION</th>
<th>20 MIN.</th>
<th>1¼ hrs.</th>
<th>3 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.M.</td>
<td>Health.</td>
<td>73%</td>
<td>-</td>
<td>72%</td>
</tr>
<tr>
<td></td>
<td>Secondary.</td>
<td>83%</td>
<td>81%</td>
<td>82%</td>
</tr>
<tr>
<td>A.S.</td>
<td>Anaemia.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In each of these cases the haemolysis was followed in the same tube. The slight variations shown are due to the error of calculation. The conclusion given by these results is that haemolysis by a hypotonic salt solution is completed very quickly. The above results were obtained at room-temperature 18°C. Repetition of the experiments at incubator-temperature (37°C) gave the same result, namely that the full degree of haemolysis was reached within a few minutes of the preparation of the experiment. In the subsequent tables, haemolysis was/
was always recorded 1 hour after preparation of the tubes. The tubes were kept at room-temperature 18°C. for that period.

I now give a table which compares the results of Saponin-haemolysis, with those of hypotonic saline solution (0.42% NaCl) in a few cases of Health, of Jaundice, and some other conditions. Only enough results will be given to demonstrate that the two methods are divergent and test different elements of cell-resistance. That purpose will be served without the introduction of details of the blood-count, etc., as in the former tables of Saponin-haemolysis.

HAEMOLYSIS/
<table>
<thead>
<tr>
<th>NAME &amp; SEX</th>
<th>AGE</th>
<th>CONDITION</th>
<th>SAP 0.12cc 2 hours</th>
<th>SAL 0.42% 1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.M.(m)</td>
<td>26</td>
<td>Health</td>
<td>93%</td>
<td>72%</td>
</tr>
<tr>
<td>J.G.(m)</td>
<td>19</td>
<td></td>
<td>90%</td>
<td>80%</td>
</tr>
<tr>
<td>W.S.(m)</td>
<td>36</td>
<td></td>
<td>87%</td>
<td>77%</td>
</tr>
<tr>
<td>J.D.(m)</td>
<td>60</td>
<td></td>
<td>93%</td>
<td>75%</td>
</tr>
<tr>
<td>E.K.(m)</td>
<td>67</td>
<td>Jaundice</td>
<td>99.6%</td>
<td>47%</td>
</tr>
<tr>
<td>T.R.(m)</td>
<td>38</td>
<td></td>
<td>99.8%</td>
<td>35%</td>
</tr>
<tr>
<td>T.D.(m)</td>
<td>40</td>
<td></td>
<td>99.9%</td>
<td>55%</td>
</tr>
<tr>
<td>Mrs. E.B.</td>
<td>46</td>
<td></td>
<td>99.9%</td>
<td>25%</td>
</tr>
<tr>
<td>Mrs. G.</td>
<td>53</td>
<td></td>
<td>99.9%</td>
<td>45%</td>
</tr>
<tr>
<td>G.J.(m)</td>
<td>25</td>
<td>Pern. Anaemia</td>
<td>98%</td>
<td>85%</td>
</tr>
<tr>
<td>M.R.(f)</td>
<td>37</td>
<td></td>
<td>98%</td>
<td>96%</td>
</tr>
<tr>
<td>Mrs. S.</td>
<td>54</td>
<td></td>
<td>94%</td>
<td>55%</td>
</tr>
<tr>
<td>A.S.(m)</td>
<td>41</td>
<td></td>
<td>97%</td>
<td>80.2%</td>
</tr>
<tr>
<td>Mrs. S.</td>
<td>37</td>
<td>Second Anaemia</td>
<td>93%</td>
<td>81%</td>
</tr>
<tr>
<td>Mrs. R.</td>
<td>29</td>
<td></td>
<td>93%</td>
<td>77%</td>
</tr>
<tr>
<td>J.K.(m)</td>
<td>36</td>
<td>Anae. Table V.</td>
<td>99%</td>
<td>33%</td>
</tr>
<tr>
<td>B.A.(f)</td>
<td>22</td>
<td>Fever acute miliary</td>
<td>100%</td>
<td>98%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tuberculosis.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.F.(m)</td>
<td>28</td>
<td>Fever Pneumon.</td>
<td>98%</td>
<td>31%</td>
</tr>
<tr>
<td>Mrs. J.H.</td>
<td>28</td>
<td>Phthisis</td>
<td>78%</td>
<td>62%</td>
</tr>
<tr>
<td>A.C.(m)</td>
<td>21</td>
<td>Pneumon.</td>
<td>79%</td>
<td>90%</td>
</tr>
</tbody>
</table>
THE DIVERGENCE OF RESULTS IN TWO DIFFERENT FORMS OF HAEMOLYSIS

This table shows a remarkable divergence of the results of the two methods in the cases of Jaundice. In the cases of Pernicious Anaemia and Secondary Anaemia a diminished resistance to Saponin is accompanied by a diminished resistance to the hypotonic saline solution. In the cases of Fever, there is a correspondence of results in the first case, where by both methods a diminished resistance is recorded. In the others divergence again appears, the second case showing a diminished resistance to Saponin and increased resistance in the saline solution, the third and fourth showing increased resistance to Saponin, and a standard or diminished resistance in the saline solution.

The case of Anaemia recorded in Table V also shows a divergence of results. But attention may be concentrated on the divergence of results revealed in the cases of Jaundice. Chanel and v.Limbbeck (loc.cit.) have already established the fact of the increased resistance of the red-cells in that condition.
condition to hypotonic saline solution. The above table is a further confirmation of the fact, but it equally emphatically demonstrates the much diminished resistance of the cells to Saponin-haemolysis. Jaundice, therefore, may be taken as a test case: for in it the divergence of results appears in the most extreme degree. It must be concluded from it not only that a red-cell possesses more than one element of resistance to haemolysis, but also that it may be very deficient in one form of resistance and simultaneously possess a very high resistance of another form. It becomes, therefore, an important matter to determine which method approximates more closely to in corpore haemolysis, so that experimental data as to resistance of red-cells may be applicable to their condition in the body. Unfortunately nothing is certainly known of the nature of in corpore haemolysis. But there is a probability that Saponin-haemolysis more closely approximates to it, for with it the action on the red-cells in an isotonic suspension; while with a hypotonic saline solution the red-cells are subjected to a test which is not applied to them in the body. But the divergence of results of the two methods in Jaundice is specially interesting/
interesting. The feebleness of cell-resistance to Saponin in that condition has already been discussed and has been attributed to interaction between the salts of the bile-acids and the red-cells. That possibility suggests that a red-cell under the minor action of one haemolytic agent—sodium glycocholate, may become more resistant to another haemolytic agent distilled water.

This suggestion was tested by the following experiment. A dose of 0.06 cc. Saponin-solution acting upon 50 millions washed erythrocytes (my own) in a bulk of 2 cc. saline solution (0.85% produced in 2 hours 8% haemolysis. To this tube was now added 2 cc. distilled water, making 4 cc. of a hypotonic saline solution (0.42%). The total haemolysis in the tube after another hour was 57%. But an equal sample of fresh cells in a similar hypotonic solution at the beginning of the experiment showed a haemolysis of 72%. It seemed fair to conclude that the fixation of Saponin by the envelopes of the red-cells had increased their resistance in a hypotonic saline solution.
DURATION OF CONTACT OF RED-CELLS WITH AN ISOTONIC FLUID: ITS EFFECT UPON HYPOTONIC SALT-SOLUTION HAEMOLYSIS.

But at the same time I obtained another result which made this conclusion doubtful. On keeping a suspension of red-cells in 0.85% saline solution for many hours, I found that their resistance in a hypotonic saline solution became much increased. The following example will illustrate.

C.J. (m) aet. 25. Pernicious Anaemia.

0.59cc. blood-suspension contains 50 mlns. red-cells.

IN TUBE 1, 0.59 cc. of blood-suspension was brought to 1 cc. bulk with 0.85 saline solution: 1 cc. distilled water immediately added: and haemolysis determined in 1 hour.

IN TUBE 2, 0.59 cc. blood-suspension was brought to 1 cc. bulk with 0.85 saline solution: kept for 24 hours at room-temperature, after the period, 1 cc distilled water added, and haemolysis determined in 1 hour.

<table>
<thead>
<tr>
<th>DISTILLED-TUBES</th>
<th>BLOOD DRAWN</th>
<th>WATER ADDED</th>
<th>INTERVAL</th>
<th>HAEMOLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 11p.m. Feb. 20</td>
<td>2p.m. Feb. 20</td>
<td>3 hours</td>
<td>82%</td>
<td></td>
</tr>
<tr>
<td>2 &quot;</td>
<td>2p.m. Feb. 21</td>
<td>27 hours</td>
<td>14%</td>
<td></td>
</tr>
</tbody>
</table>

The/
The result shows that red-cells kept in an isotonic fluid for a long period develop a much increased resistance to the action of distilled water. In the table 82% haemolysis occurs in the hypotonic saline solution prepared on Feb. 20th. But an equal sample of the same red-cells kept for a day in 0.85 saline solution, and then placed in the same hypotonic medium now show only 14% haemolysis. Their resistance to this form of haemolysis has been very greatly increased by their remaining for a considerable period in the isotonic saline fluid. It became at once necessary to determine whether this change of resistance, which in 24 hours was so considerably developed early or late. The following examples show that it developed soon and progressed very rapidly. In them as before, washed suspensions were prepared from the blood, and from these, hypotonic saline solution of bulk 2 cc. and containing 50 million red-cells were prepared. The hypotonic solutions were prepared at increasing intervals from the time of withdrawal of blood from the patient, the isotonic saline suspensions being kept at room-temperature 18°C. and the hypotonic suspensions being prepared from them at the times indicated.

The table shows results obtained from the red-cells of 3 healthy women.
These results are very clear and striking. They show that the great change in resistance to hypotonic solutions already indicated takes place early, and that its maximum action is between 3 and 4 hours after withdrawal of blood from the patient.

In the results of Chanel, v.Limbeck, Janowsky and Lang (loc.cit) this factor has not time to influence their results; for entire blood was used in their determinations, and immediately on withdrawal from the patient was placed in the hypotonic medium. But in the present investigation, washed blood-corpuscles were used, and the processes of washing and counting the suspensions occupied about 2 hours. By that time changes in resistance to hypotonic saline solution were beginning. Great accuracy cannot therefore be claimed for the results in the table p.183, but the fact that the results in the case of jaundice confirm those obtained by other technical/
technical methods where this error does not operate, shows that they are roughly accurate. But these results made it impossible to draw any conclusion from the experiment on p.186. The increase of resistance to the hypotonic solution of the Saponin-fixed red-cells might be and probably was, entirely due to this increase of resistance of red-cells remaining a longer time in the isotonic fluid. In the following experiment, this factor was properly controlled.


Blood drawn 11.30 a.m.

Tube 1, 50 million washed red-cells, treated with 0.04cc Saponin Solution brought with 0.85% saline solution to 2 cc: incubated at 37°C for 1 hour: then made a hypotonic solution by addition of 2 cc distilled water.

Tube 2, At the same time, 50 million washed red-cells brought with 0.85% Sal.Soln. to 2 cc, incubated for 1 hour at 37°C, then made a hypotonic solution by addition of 2 cc distilled water.

<table>
<thead>
<tr>
<th>TUBE</th>
<th>BLOOD DRAWN.</th>
<th>SOL. PREPARED</th>
<th>HAEMOLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.30 a.m.</td>
<td>2.30 p.m.</td>
<td>97%</td>
</tr>
<tr>
<td>Sap.Soln.</td>
<td>2</td>
<td></td>
<td>95%</td>
</tr>
</tbody>
</table>

The/
The conclusion is that the interaction of a small dose of Saponin with red-cells under those conditions does not alter the resistance of the cells to distilled water either in the direction of diminution or increase. It leaves unexplained the undoubted divergence of results between these two methods of estimating resistance to haemolysis. That divergence is best illustrated in jaundice but it exists in other conditions as well.

DURATION OF CONTACT OF RED-CELLS WITH AN ISOTONIC FLUID: ITS EFFECT UPON SAPONIN-HAEMOLYSIS.

But if red-cells withdrawn from the blood, and kept in isotonic saline fluids, rapidly alter in their resistance to haemolysis by hypotonic salt solutions, it was equally possible that the Saponin-resistance of red-cells might vary with the length of the interval between the withdrawal of the red-cells and their exposure to the action of Saponin. It was imperative that this possibility should be tested, for it threatened all the results of/
of Saponin-haemolysis already obtained.

The following experiment tests this possibility:


2 Tubes were prepared each containing 50 millions washed red-cells, and 0.12 cc. Saponin Solution, made up to a bulk of 2 cc. with 0.85 saline.

Tube 1, prepared and incubated at 37°C. for 2 hrs. and the haemolysis recorded.

Tube 2, was allowed to remain for 2 hours more at room-temperature before addition of Saponin: 0.12 Saponin solution then added; incubated for 2 hours at 37°C. and haemolysis then recorded.

<table>
<thead>
<tr>
<th>TUBE</th>
<th>BLOOD DRAWN</th>
<th>INCUBATED INTERVAL</th>
<th>SAP. SOL. ADDED &amp; HAEMOLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.30 a.m.</td>
<td>12.30 a.m. 2 hours</td>
<td>90.9%</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>2.30 a.m. 4 hours.</td>
<td>91.4%</td>
</tr>
</tbody>
</table>

The conclusion is that between an interval
of 2 hours and one of 4 hours from the drawing of blood, red-cells kept in an isotonic fluid do not change in their resistance to Saponin. The trifling difference in haemolysis of the above tubes is simply the error of experiment and calculation. But it was just in that period of from 2 to 4 hours from the drawing of blood that all my results of Saponin-haemolysis were obtained. The above experiment therefore entirely clears them from suspicion of the fallacy that was hypothetically possible.

It was interesting to determine if a longer period of contact with an isotonic fluid would produce alterations in resistance to Saponin-haemolysis. In the following experiment exactly the same procedure was adopted, but one sample of washed red-cells was kept for more than 24 hours at room temperature before subjection to the same dose of Saponin Solution.


<table>
<thead>
<tr>
<th>Tube</th>
<th>Blood Drawn</th>
<th>Incubated</th>
<th>Interval</th>
<th>Haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11 a.m. Jan 16</td>
<td>1 p.m. Jan 16</td>
<td>2 hrs.</td>
<td>2 hours.</td>
</tr>
<tr>
<td>2</td>
<td>&quot; &quot;</td>
<td>3 p.m. Jan 17</td>
<td>28 hrs.</td>
<td></td>
</tr>
</tbody>
</table>

The/
The conclusion is that red-cells kept in an isotonic fluid for 28 hours from their withdrawal from the blood do not within that period undergo any change in their resistance to Saponin.

**CONTRAST BETWEEN SAPONIN —, & HYPOTONIC SALT-SOLUTION — HAEMOLYSIS.**

The duration of contact of red-cells with an isotonic fluid therefore has a very different effect upon these two haemolytic agents, Saponin and a hypotonic salt-solution. After a period of 24 hrs, the action of Saponin is unchanged, and the resistance of the red-cells to it is unaltered. After the same period the resistance of the same sample of cells to a hypotonic salt solution has become enormously increased. That remarkable result shows the danger of applying the results obtained from the hypotonic salt-solution method. For it is proved that a red-cell may become enormously increased in its resistance to this form of haemolysis, and yet remain unchanged in its resistance to such a haemolytic agent as Saponin. But this fact may also throw light on the divergence of results obtained by the two methods in Jaundice. For a greatly increased/
increased resistance to haemolysis by hypotonic salt-solution is now shown to be compatible with an unchanged resistance to Saponin. In the results of haemolysis in Jaundice the increase of resistance is also obtained by the hypotonic salt-solution method, and the diminution of resistance by Saponin-haemolysis. But experiment having shown that outside the body a red-cell may increase in its resistance to a hypotonic salt-solution, and yet remain unchanged in its resistance to Saponin, it is equally possible that an increase of the former resistance in corpore may be accompanied by a diminution of resistance to another kind of haemolysis in corpore.

The critical question is, "which of these two haemolytic agents more closely approximates to the haemolytic process in the body?"

In the first place haemolysis in corpore cannot take place by laking of the red-cells in a hypotonic medium. It is therefore very unsafe to transfer the data of red-cell resistance in hypotonic solutions to red-cell resistance within the body, and chiefly for two reasons:

(1)/
(1). The method tests an element of cell-resistance which does not determine haemolysis in the body.

(2). A great increase in the resistance of red-cells to hypotonic saline solutions may take place without any change, and with even a great diminution, in the resistance to Saponin. That has been shown clinically in the condition of Jaundice, and also experimentally.

It is certain of course that accurate data as to the resistance of red-cells to hypotonic saline solutions indicate some physical change in the cell-envelope. But it is impossible to infer that increase of resistance to such a haemolytic agent indicates increased resistance to haemolysis in corpore. For it has been shown experimentally that increase of resistance to the action of distilled water may co-exist with unaltered resistance to another haemolytic agent Saponin. And in Jaundice increased resistance to a hypotonic saline solution co-exists with a much diminished resistance to Saponin. There are, therefore, experimental grounds for believing that/
that the changes in the red-cells in the body which produces an increased resistance to hypotonic saline solution are quite independent of the process of haemolysis, and that the data obtained by such a method give no indication of the nature of haemolysis in the body and of the resistance of the red-cells to that haemolysis.

The action of Saponin as employed in the present investigation approximates more closely to haemolysis in corpore. At the least, the action of its haemolysis is carried on in an isotonic fluid, but the approximation cannot be carried much further with certainty, for the nature of in corpore haemolysis and the element of the red-cells that determines resistance to that haemolysis are still obscure. It is this gap in our knowledge of in corpore haemolysis which makes difficulty in expressing the results of Saponin-haemolysis in terms of physiological and pathological activity in the body. At the same time Saponin is a general protoplastic poison, and the measurement of its haemolytic action upon red-cells undoubtedly gauges the condition of the envelope-protoplas. The results of Saponin-haemolysis will therefore indicate the condition of the cell-envelope apart from the question of its resistance to haemolysis in corpore and will give information either as/
to abnormal formation by the bone marrow or abnormal metabolic waste.

The acceptance of Hunter's view of an active *in corpore* haemolysis - a bio-chemical process of the nature of a solution of the red-cells within the portal circulation, would be very convenient, and would allow a more confident interpretation of these results of Saponin-haemolysis. But Hunter's very able and careful researches though they strongly suggest his conclusions, do not give them decisive proof. The interpretation of the results of Saponin-haemolysis in this investigation is therefore provisional.

It would have been possible to have stated the results by themselves, without attempting to adjust them to conditions in the body, or to discuss their bearing upon pathology and pathogenesis. But in some cases these results were so suggestive that a reasonable discussion could not be avoided. This was notably so in the case of Jaundice, where the results of Saponin-haemolysis not only seemed to shed light upon the disposal of the salts of the bile-acids, but further suggested that these salts had in health/
health an important part in haemolysis in the body.

In Pernicious Anaemia and other Anaemias the results obtained were equally suggestive. Indeed, they were more relevant to the Etiology of Pernicious Anaemia than other experimental data, and it was quite proper and useful to adjust them to the different prevailing views of that disease.

These data of Saponin-haemolysis in health and disease therefore accentuate the need for fresh research into the physiology of haemolysis within the body. If such knowledge were forthcoming, accurate data as to the condition of the erythrocyte-envelope in various conditions of disease would have decisive value in determining the pathology and pathogenesis of those diseases.

I now proceed to summarise the results and conclusions obtained from Saponin-haemolysis in disease: and the critical examination of the method of testing red-cell resistance by hypotonic salt-solutions.

CONCLUSIONS/
CONCLUSIONS.

JAUNDICE.

1. In Obstructive Jaundice the resistance of human red-cells to haemolysis by Saponin is much diminished; and the degree of diminution is proportionate to the severity of the Jaundice.

2. The explanation of this fact may be that in Jaundice there is interaction between the red-cells and the haemolytic salts of the bile-acids.

3. At the same time, the absence of oligocythaemia in Jaundice indicates that the destruction of red-cells, though it may be increased, is not excessively increased.

4. If such interaction takes place in Jaundice, it may also occur in Health but in different degree, and at a different site, namely in the portal circulation.

5. The condition of the red-cells in Jaundice, as tested by Saponin would thus be explained by the derangement of the circulation of the bile-salts in that condition.
6. In Fever there is a diminished resistance of the group of low-resistance cells in a sample.

7. In moderate degrees of Fever the total resistance of the whole sample of cells is increased. In high Fever the total resistance is diminished.

8. The explanation of these results is that in Fever there is increased destruction of red-cells, and an increased production of fresh cells by the bone-marrow. In moderate Fever this compensation is excessive, producing polycythaemia, and increasing the total resistance of a sample of cells. In high Fever, it is generally adequate, and prevents oligocythaemia, but the total resistance of the sample is diminished. Briefly, in moderate Fever the destruction of red-cells is increased; in high Fever it is excessively increased.
EXOPHTHALMIC GOITRE.

9. In Exophthalmic Goitre the conditions are exactly as in Fever,—increased destruction and increased production of red-cells. There is always a diminished resistance of the group of low-resistance cells. There may be either a standard total resistance of a sample of cells, or a diminished total resistance.

ANAEMIA.

10. In Anaemia there is a diminished red-cell resistance to Saponin.

11. The diminution of resistance is greatest in advanced, or in rapidly advancing cases of Pernicious Anaemia.

12. In moderate or slowly advancing Pernicious Anaemia, the diminution of resistance closely approximates to that in Simple and Secondary Anaemia.

13. The degree of diminished resistance in Anaemia does/
13. does not depend on the amount of oligocythaemia: nor on the poverty or richness of the red-cell in Haemoglobin.

14. The diminution of resistance depends on the rate of progress of the Anaemia. This is best illustrated in Pernicious Anaemia.

15. The Saponin-resistance of the red-cells in Pernicious Anaemia does not support Hunter's view of its etiology.

16. The fact that cases of Chronic but pronounced Pernicious Anaemia show a resistance equal to and sometimes greater than that in other forms of Anaemia neither "Pernicious" nor progressive, is inconsistent with the action of a powerful haemolytic toxin causing an excessive destruction of red-cells.

17. Whereas, that fact supports the view of Pernicious Anaemia as a disease where there is a defect in the metabolism that forms the envelopes of red-cells.

18. The distinction between Pernicious Anaemia, and/
18. and other forms of Anaemia is that in the former this defect of metabolism is permanent and progressive; while in the latter it is temporary.

19. So long as Pernicious Anaemia is slowly progressive, its effects are expressed by oligocythaemia, and not by a pronounced diminution of the Saponin-resistance of its red-cells.

20. When Pernicious Anaemia is rapidly progressive, its effects are expressed both by Oligocythaemia, and by a pronounced diminution of the resistance of the red-cells.

21. The association of oligocythaemia and a high Haemoglobin-Index of the red-cell in Pernicious Anaemia is explained by the hypothesis of inferior or insufficient envelope-protoplasm, and an abundant supply of Haemoglobin.

22. The excess of iron-pigment in the liver in Pernicious Anaemia may equally well be explained by a storage of surplus Haemoglobin, as by an excessive destruction of red-cells.
23. The Saponin-resistance of the red-cells in Pernicious Anaemia does not support the view of an excessive destruction of red-cells in that disease.

24. The widely-spread fatty degeneration in tissues and organs in Pernicious Anaemia supports the hypothesis of a progressive defect in the metabolism of cell-protoplasm.

25. The occurrence of sclerosis of the spinal cord as a complication is in line with the above hypothesis.

26. The affinity of Saponin for Cholesterin, and the presence of the latter as an important constituent of the envelope of red-cells and of the medullated nerve sheaths, and as an element in all protoplasm, would indicate that in Pernicious Anaemia the supply of Cholesterin has become defective or that its metabolism is deranged.

TABLE V. SPECIAL ANAEMIAS.

27. The much diminished Saponin-resistance of the red-cells together with oligocythaemia in these two cases of Anaemia would/
27. would indicate that in them there is an excessive destruction of red-cells.

DIABETES

28. In Diabetes the resistance of the red-cells is of the standard of Health, or is slightly increased.

SECTION II.

HAEMOLYSIS BY HYPOTONIC SALT-SOLUTION.

29. In various pathological conditions the data of red-cells resistance to Saponin-haemolysis, and to laking by hypotonic saline solution are opposed to each other.

30. This divergence is best illustrated in Obstructive Jaundice, where the red-cells are feebly resistant to Saponin but highly resistant to hypotonic saline solutions.

31. The conclusion is that the two haemolytic processes are different, and that each tests a different factor of cell-resistance.

32. Red-cells kept for a time in an isotonic saline solution develop an increased resistance to
32. a hypotonic saline solution. The total increase of resistance is very great, and the major part of it is effected in a period of from 2 to 5 hours from the withdrawal of the red-cells from the circulation.

33. Red-cells, kept in an isotonic saline fluid for 24 hours at 18°C. remain unchanged in their resistance to Saponin-haemolysis.

34. Conclusions 32 and 33 make a complete experimental analogy for the divergence of results obtained by the two methods of haemolysis in Jaundice.

35. In Jaundice increased resistance to a hypotonic saline solution co-exists with a greater feebleness of the cells to another form of haemolysis.

36. Saponin-haemolysis may test that form of cell-resistance that determines haemolysis in corpore.

37. Haemolysis by a hypotonic salt-solution tests a form of cell-resistance which does not determine/
37. determine haemolysis in corpore. Its data expresses some physical change in the cell: but they cannot be applied to conditions in the body. They convey no information as to the physiology and pathology of blood destruction in the body.

In conclusion, I beg to thank the Physicians of the Royal Edinburgh Infirmary for their kind permission to use results obtained from patients in their Wards.

Finally, I would express my thanks to Sir Thomas Fraser, for his suggestion of the subject, for his interest and encouragement throughout the investigation, and for all the facilities I have enjoyed in his laboratory.

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