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.

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

by

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I N D E X .

I N T R O D U C T O R Y .

PART I. TECHNIQUE OF A CLINICAL INVESTIGATION WITH SAPONIN.

I N D E X   C O N T D .

INTRODUCTORY

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_/

corpore 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 sensitiveness 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/

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 in-
vestigate the pathology of human erythrocytes, in re-
spect 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 hypoton-
ic 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
wasnow 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 repro-
duced the conditions of toxicity of body fluids, and,
in so far, was a closer approximation to the conditions
of haemolysis in corpore.

SAPONIN/

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/

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* Ransom's work was confirmed and extended by Bashford. (6)

laboratory mammals, the guineapig, rabbit, cat and dog.

THE TECHNIQUE OF A CLINICAL INVESTIGATION
WITH SAPONIN.

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 technical/

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
than washed free of serum by repeated centrifugation,
removal of the supernatant fluid, and addition of
saline solution. To a measured quantity of the sedi-
mented corpuscles, a measured quantity of saline
solution is added. Thus a suspension of washed cor-
puscles, 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/

All the results in the following research were obtain-
ed 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
Saponin, and for the suspensions of red blood cor-
puscles.

(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 Haemocyto-
meter, 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 (·85%),   There was thus a bulk of
2c.c.of blood-suspension in each tube.

The/

The suspension having been thoroughly mixed by vigor-
ous rotation, the tubes were centrifuged, 1·5c.c. of
supernatant fluid was withdrawn by pipette from each,
and the bulk again restored to 2c.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 20c.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 2c.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 present-
ed itself.   It is evident that in conditions of
oligocythaemia, the corpuscular content of 20c.mm. of
blood, would be less than in health or polycythaemia.
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 hae-
molytic 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 cor-
puscle 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 sus-
pension/

suspension.   Of this, 1 cc. was removed.   The re-
maining 1 cc., therefore, contained the erythrocyte
content of 10 c.mm. of my blood.   Erthrocyte count
of my blood was 5,090,000 per c.mm.   Complete
Haemolytic dose, for 100 million of my washed ery-
throcytes, was previously found to be 0.60 of Saponin
solution .004 grms per 100 cc.

Incubator 37° C. Saponin Solution .004%.

Tubes	Blood Suspension	Sap.Soln.	Interval	Haemolysis	No. of red cells in Tube
1	1.4 cc.+	0.60 cc	2 hours	Complete	100 mlns.
2	1.7 cc.+	0.30 cc	"	"	50 mlns.

This experiment confirms Bashford's state-
ment, 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 ascer-
tained.   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 por-
tions of 20c.mm. which I obtained for the suspension
samples.   Secondly, in the frequent manipulations
and measurements during the course of washing, an ac-
cumulation of experimental error might produce some
defect, or excess of the bulk of fluid.   The follow-
ing modification removes both these sources of error.
A quantity of blood, 80 to 100 c.mm. was
taken from the patient, and placed in a tube con-
taining from 4 to 5cc.   of Saline solu-
tion/

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 ery-
throcyte 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 0·5c.c.
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.

	50	47
	51	39
(1)	40	52
	49	50
	45	41
	235	229

(2) appears between the columns in the third row.

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.

$\therefore$ No.of erythrocyte per 1c.mm.of original

suspension = $\dfrac{464 \times 4000 \times 8}{160}$

"   " 0:5cc.   "

$\therefore$ "   = $\dfrac{464 \times 4000 \times 8 \times 500}{160}$

$\therefore$ "   "

= 46,400,000.

In this above example, one obtained a series

of/

of 0·5cc. 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 as-
certained 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 consider- .
ed/

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 contrifugation, 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 in-
vestigations on healthy blood, where the Haemoglobin-
value of a fixed amount of blood is constant.   But
they are cumbrous and difficult to work, and they have
not been employed in the great bulk of recent research-
es 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 diseas-
es, 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 in-
dubitably 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 hae-
molysis 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 ·02grms. per 100cc.

Incubator 37°C. Incubation period 2 hours.

Tubes	Entire Blood.	Sal.Soln.	Sap.Soln.	Haemolysis.
1	20c.mm.+	1·78cc.	+0·20cc.	20-30%.
2	" +	1·76cc.	+0·22cc.	50-60%.
3	" +	1·74cc.	+0·24cc.	80-90%.
4	" +	1·72cc.	+0·26cc.	100% .
5	" +	1·48cc.	+0·50cc.	100% .
6	" +	1·98cc.	+0· 0cc.	0% .

In the above Table, Tube 6 is the control.

Tube/

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 hae-
molysis; 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 in-
vestigation 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 re-
cent researches on Haemolysis, is the perfect trans-
parence 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/

I had devised, and by the usual gauge of transparence.
But, after centrifugation it was not difficult to ob-
serve 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 haemoly-
sis 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 deter-
mination of the end point of haemolysis, which is the
foundation on which their results are built, is ob-
tained 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 sedi-
ment 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 sus-
pension 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 percent-
age/

percentage of the original number of cells. exposed to
haemolysis.

The following example will illustrate.

Entire blood of myself used.

Sap.Soln. ·02grms.per 100cc.

Incubator 37^0C.

Incubation-period 2 hours.

Tube.	Entire Blood.	Sal.Soln.	Sap.Soln.	Haemolysis by Enumeration.
1	20c.mm	1·72cc.	0·26cc.	
2	"	1·70cc.	0·28cc.	98%
3	"	1·68cc.	0·30cc.	99·23%
4	"	1·44cc.	0·54cc.	100%
5	"	1·98cc.	0 ·0cc.	0%

I now give the materials from which these
figures were obtained

ZEISS COUNTING-CHAMBER   PREPARATIONS.

Tube 2.	Tube 3.	Tube 4.
105 red-cells in 400 squares.	41 cells per 400 squares.	0 cells in 1200 squares.
∴Total number of cells in tube.	∴Total number of red-cells in tube	
$= \dfrac{105 \times 4000 \times 2000}{400}$	$= \dfrac{41 \times 4000 \times 2000}{400}$	
= 2,100,000.	= 82,000.	

The/

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 cal-
culation gives as percentages, 2% of unhaemolysed
cells in Tube 2, and 0·77% in Tube 3.   The percent-
ages of haemolysed cells in these tubes were, there-
fore, 98%, and 99·23% respectively, as determined by
this method of enumeration.

I next proceeded to carry out the Colori-
meter 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 omit-
ted.

HAEMOLYSIS

Tubes	Sap.Soln.	Enumerative Method.	Color Method.	Sediment after Centrifugation.
1	0·26 cc.		90-100%	Distinct red spot
2	0·28 cc.	98%	?100%	Faint spot
3	0·30 cc.	99·23%	100%	Faint powder
4	0·54 cc.	100%	100%	None

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 im-
portant 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 protoplasmic envelope, might be expect-
ed 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 sim-
ultaneous 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 de-
vised.   Thus, if, by enumeration, I found a disappear-
ance 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 ·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/

Tubes	Dose of Sap. Soln.	Haemolysis by Enumeration	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%	
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 incompar-
ably 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 corrobora-
tive, 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 trans-
parent 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 fur-
ther shown that the error introduced was not small,
but great.   For example when a certain dose of Saponin
produced in 2 hours a haemolysis of 99%, the additional
Saponin/

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 con-
sequences, 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 Incuba-
tor temperature was 37°C, 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.

TUBES	BLOOD SUSPENSION.	Sal.Soln.	Sap.Soln.	Haemolysis
1	0·5cc.+	1·34cc. +	0·16cc.	98·62%
2	" +	1·26cc. +	0·24cc.	99·95%
3	" +	1 18cc. +	0·32cc.	100%

2.   Blood of A.T.M.   Health.
0·5cc.of blood-suspension contains
57,800,000 erythrocytes.

TUBES	BLOOD SUSPENSION.	Sal.Soln.	Sap.Soln.	Haemolysis
1.	0·5cc.+	1·34cc. +	0·16cc.	99·62%
2.	" +	1·30cc. +	0·20cc.	99·96%
3	" +	1·26cc. +	0·24cc.	100%

3./

3. Blood of J.P.M.   Health.

0·5cc. of Blood-suspension, contains

74,000,000 erythrocytes.

TUBES	BLOOD SUSPENS.	SAL. SOLn.	SAP. SOLn.	HAEMOLYSIS.
1	0·5cc.+	1·32cc	+0·18cc	93.52%
2	" +	1·28cc	+0·22cc	98.03%
3	" +	1·24cc	+0.26cc	99.97%
4	" +	1·20cc	+0.30cc	100%

4. W.P. aet.54   Pernicious Anaemia.

0·5cc.of Blood-suspension contains

34,700,000 erythrocytes.

TUBES	BLOOD SUSPENS.	SAL. SOLn.	SAP. SOLn.	HAEMOLYSIS.
1	0·5cc+	1.38cc	+0.12cc	93.46%
2	" +	1.34cc	+0.16cc	99.86%
3	" +	1.30cc	+0.20cc	99.96%
4	" +	1.22cc	+0.28cc	100%

5. Mrs H.L.aet.59   Pernicious Anaemia.

0.5cc of Blood-suspension contains

29,200,000 erythrocytes.

TUBES/

Tubes.	Blood Suspension.	Sal.Soln.	Sap.Soln.	Haemolysis.
1	0·5cc.+	1·44cc.	+0.06cc.	30·8%.
2	" +	1·40cc.	+0·10cc.	90·9%.
3	" +	1·36cc.	+0·14cc.	98·98%.
4	" +	1·32cc.	+0·18cc.	99·92%.
5	" +	1·28cc.	+0·22cc.	100%.

6. Mrs H.M.aet 56.   Pernicious Anaemia.

0·5cc. of Blood-Suspension contains

40,600,000 erythrocytes.

Tubes.	Blood Suspension.	Sal.Soln.	Sap.Soln.	Haemolysis.
1	0·5cc.+	1·34cc.	+0·16cc.	99·81%.
2	" +	1·30cc.	+0·20cc.	99·97%.
3	" +	1·26cc.	+0·24cc.	100%.

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 erythro-
cytes by Saponin approaches completion, the increment
of dose required, to complete haemolysis, is out of
all proportion to the number of cells remaining unhae-
molysed.

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 hae-
molysis i.e. double the amount that produces 90% hae-
molysis.

These tables further show, that this in-
crement of dose to complete haemolysis is proportion-
ally greatest between 99%, and 100% or Complete Hae-
molysis.   Thus in Table 4 the amount of Saponin has
to be increased from 0·16cc. to 0·28cc. to carry hae-
molysis from 99·86% to 100% – an increment of 1/3 of
the total haemolytic lose.   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 hae-
molytic dose.

INADEQUACY   OF   TEST   OF   TRANSPARENCE.
_____

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 hae- molysis, 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 hae- molytic 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 haemoly- tic 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 hae-molysis, 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 incub-ation, I determined the degree of haemolysis by enumeration at half-hour intervals.

T.B.aet. 38.   Jaundice.

0·5cc.Blood-Suspension contains

50,000,000 erythrocytes

Blood Suspension.	Sal.Soln.	Sap.Soln.	Haemolysis after ½hr.			
0·5cc.+	1·38cc.	+0·12cc.	94·88%	99·28%	99·88%	99·96%

The above result demonstrates, in another way/

way, the same fact, the great retardation that takes
place in haemolysis as it very nearly approaches com-
pletion.   The former tables, showed the great in-
crement 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 hae-
molysis to completion.

In view of this established fact I deter-
mined to adopt as the end-point, not Complete Hae-
molysis 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 com-
parative purposes.

CLINICAL   RESULTS.   FIRST   SERIES.
_____

With this specialised technique, in the pre-
paration of equal samples of washed erythrocytes. in
the method of determining haemolysis, and in the end-
point of haemolysis selected, I began to collect re-
sults/

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 $37^{\circ}C$. 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 $37^{\circ}C$. And here the sample of blood-suspension contained 79,400,000 erythrocytes. Therefore the haemolytic dose for 100, million erythrocytes would be

$$\frac{\cdot24 \times 100,000,000}{79.\ 400,000} \quad \text{i.e.} \quad 0\cdot30cc.$$

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

Name.	No.of erythrocytes in sample.	Sap.Sol.	Haemolysis	Haemolytic Dose for standard No. of Erythro.
C.Mc.N.	79,400,000	+0·24cc.	99·95%	0·30cc.
A.T.M.	57,800,000	+0·20cc.	99·96%	0·34cc.
J.P.M.	74,000,000	+0·26cc.	99·97%	0·35cc.

Tables/

Tables 4, 5, & 6 p.   show the results ob-
tained in three cases of Pernicious Anaemia.   Their
conversion was as follows:-

Name.	No.of Erythrocytes in sample.	Sap.Sol.	Haemolysis.	Haemolytic Dose for standard No. of Erythrocytes.
W.P.	34,700,000	0·20cc.	99·96%	0·57cc.
Mrs.H.L.	29,200,000	0.18cc.	99·92%	0·61cc.
Mrs H.M.	40,600,000	0·20cc.	99·97%	0·49cc

These cases of Pernicious Anaemia, therefore.
show a considerable increase in the resistance of
their erythrocytes to destruction by Saponin, as com-
pared 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, accord=
ing 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 ·004grms.
per 100cc.   Incubator temperature 37°C.   Incubation
period 2 hours.   These three factors continue con-
stant hereafter, unless where specially mentioned.

H.D. aet.20.   Exophthalmic Goitre.
0·5cc. washed blood-suspension contains
44,400,000 erythrocytes.

Tubes./

Tubes.	Blood Suspension	Sal.Soln.	Sap.Soln.	Bulk of Tube Cont	Haemol.
1	0·5cc.+	1·38cc.	+0·12cc.	2cc.	96·85%
2	0·25cc.+	1·69cc.	+0·06cc.	2cc.	55·95%

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/

Blood of myself.

0·5cc. of Blood suspension contains

54,300,000 erythrocytes.

TUBE.	BLOOD SUSPENSION.	SAL.SOL.	SAP. SOLN.	BULK OF TUBE CONTS.	HAEMOLYSIS.
1	0·5cc.+	0·42cc.	+0·08	1cc.	85·55%
2	0·5cc.+	3·42cc.	+0·08	4cc.	44:07%
3	0·5cc.+	0·34cc.	+0·16	1cc.	99.93%
4	0·5cc.+	3·34cc.	+0·16	4cc.	95:74%

At first sight it would appear that the
approximation of haemolysis in Tubes 3 & 4, viz.,
99.93% & 95.9% 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 inconsis-
tency.

This last Table confirms what the other
had suggested, the importance of the bulk of fluid
on which the cells are suspended, upon Saponin hae-
molysis.   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/

Blood of myself.

0·5cc.Blood suspension contains 50 millions of erythrocytes.

Tubes.	Blood Suspension.	Sal. Soln.	Sap. Soln.	Bulk.	Haemolysis in 2hrs.
1(a)	0·5cc.+	1·38cc	+0·12cc	2cc	99·8%
2(a)	0·25cc+	1·69cc	+0·06cc	2cc	40%
3(a)	0·25cc+	0·69cc	+0·06cc.	1cc	99·24%
1(b)	0·5cc+	1·26cc	+0.24cc	2cc	100%
2(b)	0·25cc+	1·63cc	+0.12cc	2cc	100%
3(b)	0·25cc+	0·63cc.	+0.12cc	1cc	100%

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 ex-
act and is obtained by an amount of Saponin, which ex-
ceeds 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 prev-
ious Table p.   where the difference in bulk is bet-
ween 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 hae-
molysis 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 follow-
ing 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.

Tubes	Blood Suspension	Sal. Soln.	Sap. Soln.	Bulk.	Haemolysis after ½ hour	1 hour
1	0·5cc.+	1.28cc + 0·22		2cc.	63%	97.88%
2	" +	0.28cc + 0·22		1cc.	98.92%	

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 98.92% 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/

The above series of experiments on the inter-
play 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 er-
ythrocytes 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 gluco-
sides.   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 ac-
tion 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 adopt-
ed this standard of practically instantaneous hae-
molysis. At any rate, in his tables he does not
mention any period at which readings of complete hae-
molysis were made, and it can only be assumed that
they were made shortly after the doses of Saponin
were added .

Throughout my investigations, the deter-
minations 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 re-
searches 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-tempera-
ture 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 up-
on 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.

Tubes/

TABLE f. & 7. TUBES 5+6.

TUBE 5. 50 mhrs R.B.C. SAP. SOL 0.12cc. BULK Rea
"   6. 25 "   "   "   0.06cc " 4cc.
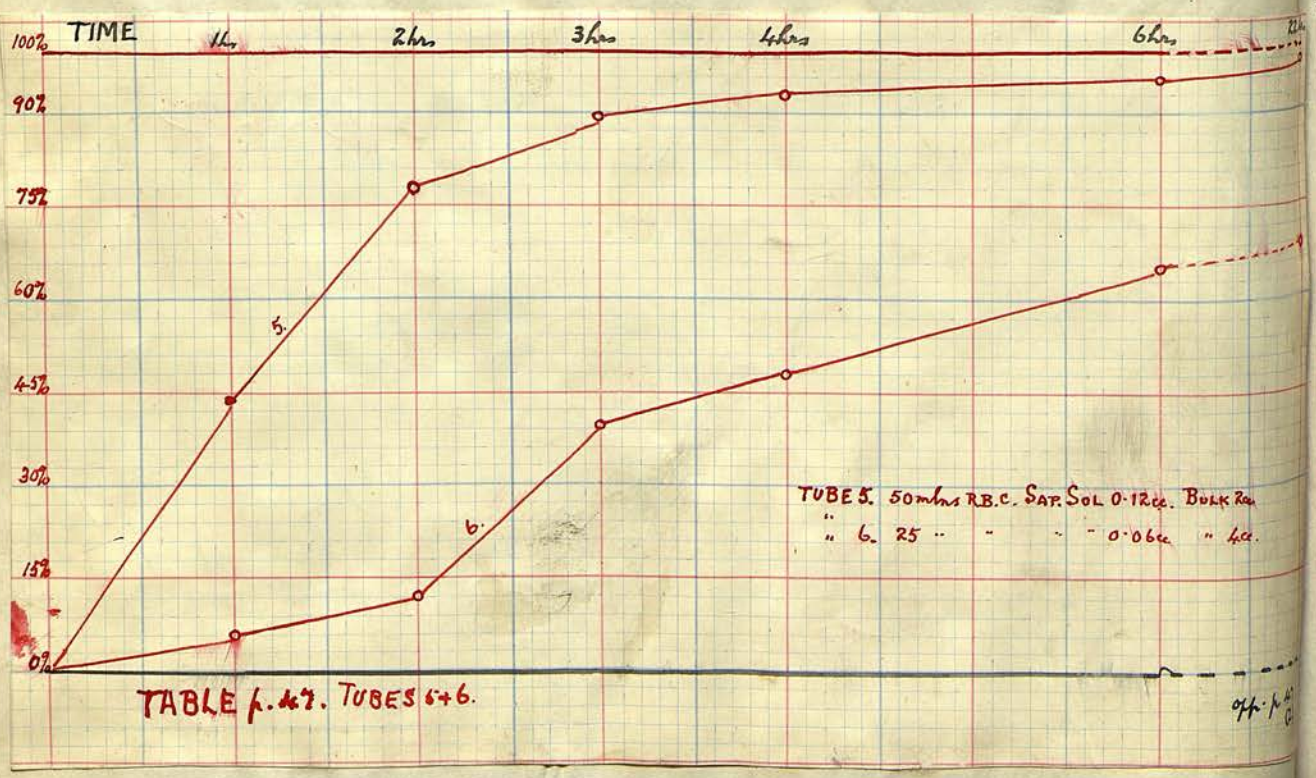
TBS.	BLOOD SUSP.	SAL. SOL.	SAP. SOL.	BULK	HAEMOLYSIS after 1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	22 hrs.
	0·5 cc +	1·28cc +	0·22cc	2cc	97·88%	99·88%	99·88%	99·88%	99·92%	----
	0·5 cc +	3·28cc +	0·22cc	4cc	57·44%	89·12%	94·56%	96·72%	98·24%	98·24%
	0·25cc +	1·39cc +	0·11cc	2cc	53·92%	84·64%	91·68%	92·16%	95·20%	98·16%
	0·25cc +	3·39cc +	0·11cc	4cc	0·00%	0·00%	0·00%	0·00%	20·00%	60·32%
	0·5 cc +	1·38cc +	0·12cc	2cc	44·00%	78·00%	89·28%	93·22%	95·24%	98·40%
	0·25cc +	1·44cc +	0·06cc	4cc	6·00%	12·00%	40·00%	48·00%	65·44%	68·32%

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
6 showing the same effect with a smaller dose. This division has been
made in the graphic charts.

TABLE p.47.
TUBES 1-4.
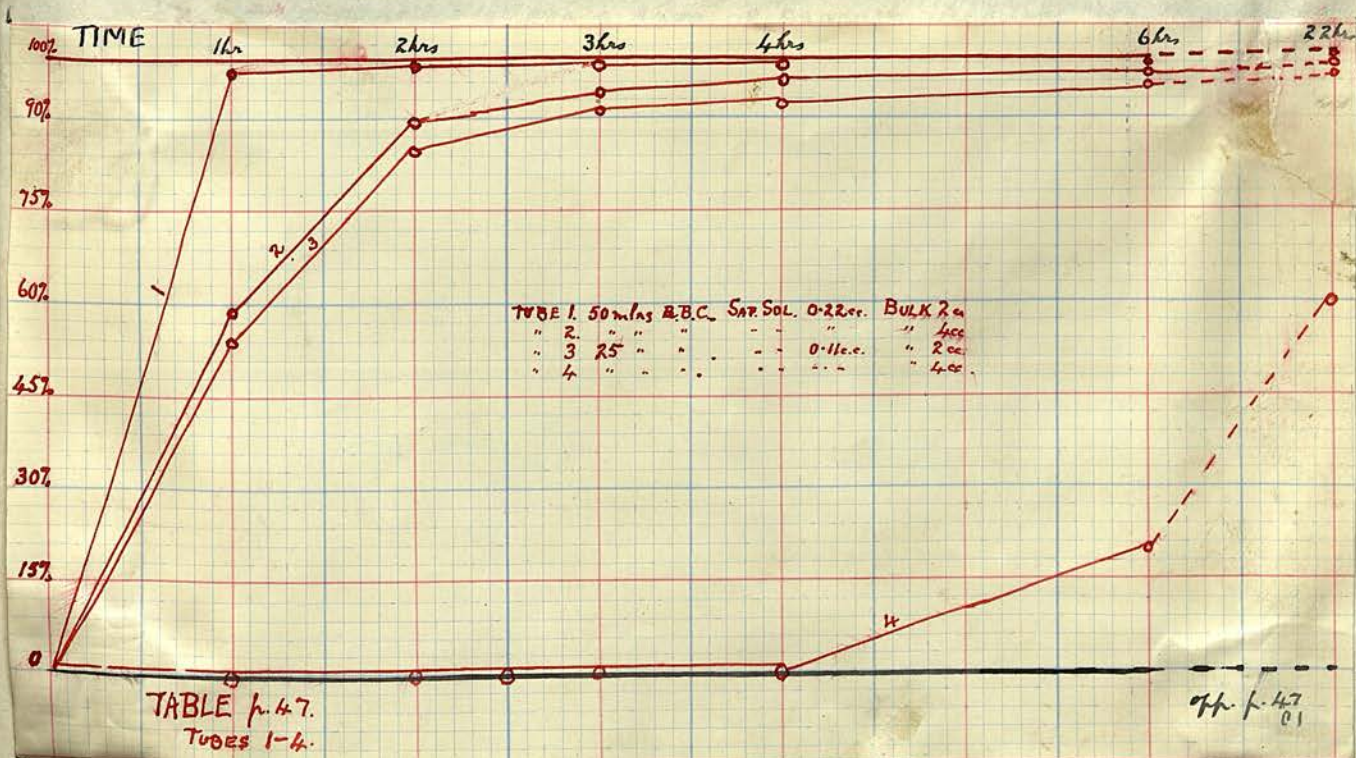
It establishes some new results, and confirms and ex-
tends others already shown.

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-tempera-
   ture, 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 res-
pectively 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 maxi-
mum is still short of the degree of haemolysis at-
tained 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 ampli-
fication 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 cor-
puscles is the same, but in Tube 1 there is $99 \cdot 88\%$
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 ob-
jected 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 ad-
    justment of the factors of bulk and time in haemo-
    lysis 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 ex-
tending 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 incre- ment 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 haemo- lysis must also be transferred to other groups of haemolytic substances, which act by a solution of the red-cell envelope.   These facts, interesting in them- selves, will thus obtain a much wider significance.

The/

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 ery- throcytes, and some time after complete Haemolysis had occurred, to add a fresh quantity of washed erythro- cytes.   These latter were found to sink to the bottom of the tube unchanged.   It was shown that the Haemo- globin 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 con- tinued 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.

TUBES/

TUBES	BLOOD SUSPENS.	SAL. SOL^n.	SAP. SOL^n.	HAEMOLYSIS IN TWO HOURS.
1	0·5cc +	1·44cc	+ 0·06cc	46·0 %
2	0·5cc +	1·38cc	+ 0·12cc	99·6 %
3	0·5cc +	0·70cc	+ 0·80cc	Complete Haemolysis.

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:-

TUBE	BLOOD SUSPENS.	SAL. SOL^n.	FLUIDS OF TUBE I.	HAEMOLYSIS IN TWO HOURS.
4	0·5cc +	0·5cc	+ 1 cc	0 %

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.

C.H.   aet. 41.   Health.

0.5cc. of washed blood suspension con-
tains 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' in-cubation at 37°C. 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:-

TUBE	BLOOD SUSPENS.	SAL. SOL^{n}.	FLUID OF TUBE I.	HAEMOLYSIS IN TWO HOURS.
2	0.5cc.+	0.5cc. +	1 cc.	61.25%

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 approxi-mation to say that 1 cc. of the fluid of Tube 2 after 2 hours' incubation at 37ºC 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 1·3cc.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 Sapon-
in solution.   The conclusion is, that 1·3cc of Sap-
onin solution was fixed by 50 million red-cells of
C.H. in 2 hours, although 0·24cc 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 Com-
plete Haemolysis in the same time.   But this fact can
be stated in a much more illuminating way.   As ex-
pressed 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 suffi-
cient 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 pro-
gress of haemolysis during 2 hours, where the dose of
Saponin effects almost Complete, or Complete Haemoly-
sis.   Such an example has already been given, but
may now be repeated to give clearness to the argument.

    T.B. aet.38   Jaundice.

BLOOD SUSPENS.	SAL. SOL^n.	SAP. Sol^n.	HAEMOLYSIS AFTER			
			$\frac{1}{2}$hr.	1 hr.	$1\frac{1}{2}$ hrs.	2 hrs.
0.	5cc +	1.38cc + 0.12cc	94.88%	99.28%	99.88%	99.96%

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 illustrat-
ed.   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 ex-
planation.   It is due primarily, to the fact that an
erythrocyte entirely releases its Haemoglobin long
before it has satisfied its affinity for the haemolyt-
ic 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 pro-
portion 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 ab-
sorption 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 hae-
molysis approaches completion.

It should be stated that the term "cell rem-
nant" is provisional, and does not express any defin-
ite idea as to physical condition.   These "cell-rem-
nants" 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 hae-
molysis of a number of erythrocytes by Saponin,
material still remains which can absorb far more Sap-
onin than is sufficient to entirely release their Hae-
moglobin.

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 Haemo-
globin, 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 ex-
posed to haemolysis, there must be variations in re-
sistance 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 it-
self 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 hae-
molysis by Saponin compared with its total affinity
for Saponin before and after haemolysis, it is evi-
dent, 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 sub-
stance.   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 pro-
longed 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 con-
centration.   We, therefore, obtain an explanation of
the fact already noted, that, in dilution, the re-
tardation 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 Com-
plete Haemolysis by the test of Transpar-
ence 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 Sapon-
in-haemolysis may be relevant to the action
of other haemolytic substances.

The/

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 de-
tected by so delicate a means as the
Enumerative Method: and was not detected
by the test of transparence, which is uni-
versally employed in researches on snake
venoms and haemolytic sera.

REMEDY OF ERROR OF TECHNIQUE.

The discovery of the error of technique in-
troduced by the use of different numbers of erythro-
cytes in the same bulk of fluid, led a good deal
further than was expected, and brought to light in-
teresting 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 investi-
gations, the corpuscular content of the samples of
blood-suspension varied from 10 millions to 80millions
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 con-
tent 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-cor-
puscle-suspension so that in every case,
and independant of the original erythro-
cyte 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 pro-
blem 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 erythro-
cytes per 0·5cc sample.

To obtain 5 such samples, and some in ad-
dition 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 oligocythaemia, 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-
ed/

collected in a tube containing about 5cc. of 0·85
saline.   Washing(shaking up, centrifugation and pipet-
ting 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 suspen-
sion 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.

	FIELDS OF 16 SQUARES.		FIELDS OF 16 SQUARES.
	67		66
	57		57
1st.Prep.	60	2nd.Prep.	50
	58		68
	49		46
	291		287

The/

The total for 10 fields is 578 erythrocytes.

The average of 1 field is 57·8 or approx. 58.

∴ in 0·5cc of the undiluted suspension hours will

be:- $\dfrac{58 \times 4000 \times 8 \times 500}{16}$ or 58mln.erythroc.

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 -

$\dfrac{0·5cc \times 24}{50}$ i.e. 0·24cc.

Having then measured off 3 cc of the un-
diluted 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

$$\frac{58 \times 4000 \times 8}{16} \text{ per cmm.}$$ i.e. 116,000 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 $\frac{1 \times 50,000,000}{116,000}$ c.mm.

or 43 cmm. or ·43cc.

The amount of blood-suspension whose cor-
puscular 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.

TUBE.	BLOOD SUSPENSION	SAL. SOL^n.	SAP. SOL^n.	BULK.
1	0·43cc. +	1·45cc.	+0·12cc.	2cc.
2	" +	1·39cc.	+0·18cc.	"
3	" +	1·33cc.	+0·24cc.	"

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 manipulatons 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 al-
most 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, deter-
mined 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 as-
certain the dose of Saponin that produced a degree of
haemolysis somewhere within this range.

RESULTS/

RESULTS OBTAINED BY THE TECHNIQUE IN CONDI-
TIONS OF HEALTH AND DISEASE.

---

2 nd. Series.

I now proceed to give a table of results.
In all cases the Saponin Solution was .004 grms.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.

NO.	NAME.	AGE.	NO. OF RED CELLS PER cmm.	COLOUR INDEX.	SAP. SOL.	HAEMOLYSIS IN 2 HRS.
1.	W.M.S.	25	5,090,000	1.03	0.14cc.	99.63%.
2.	J.P.M.	28	5,300,000	0.97	0.14cc.	99.39%.
3.	A.T.M.	26	4,830,000	0.98	0.16cc.	99.64%.
4.	J.N.	25	5,100,000	1.06	0.16cc.	99.4% .
5.	J.A.G.	26	5,400,000	1.01	0.16cc.	99.84%.
6.	M.S.	22	4,890,000	1.04	0.16cc.	99.52%.
7.	C.M.	26	5,200,000	1.0	0.16cc.	99.8%.

All the above cases are males.

These results were so remarkably uniform,
that I considered their number in the meantime suffi-
cient/

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.

NO.	NAME.	AGE.	NO. OF RED CELLS PER cmm.	COLOUR INDEX.	DISEASE.	SAP. SOL.	HAEMOLYSIS IN 2 HRS.
1.	M.R. (f)		1,170,000	1.2	Pernicious Anaemia	0.16cc	99.44%
2.	G.J. (m)	25.	1,170,000	1.3	"	0.14cc	99.90%
3.	P.C. (m)	37.	1,970,000	1.01	Splenic Anaemia	0.16cc	99.64%
4.	W.S. (m)	14.	2,790,000	0.6	Simple Anaemia	0.18cc	99.8%
5.	E.M'c (f)	20.	2,330,000	0.7	"	0.16cc	99.88%
6.	M.D. (f)	20.	4,130,000	0.58	"	0.16cc	99.2%
7.	Mrs.A S. (f)	37.	3,290,000	0.6	Secondary Anaemia	0.16cc	99.84%
8.	W.H. (m)	37.	3,520,000	0.8	Malaria	0.12cc	99.36%
9.	Mrs.G (f)	44.	3,500,000	1.0	Exopthalmic Goitre	0.16cc	99.76%

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 0.16cc; 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 pos-
sibility.   The gauge of an equal resis-
tance 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 hae-
molysis 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 specu-
lation. It is a hypothesis for which preceding
pages show a sound experimental basis. It has al-
ready been shown that as haemolysis approaches its
close, the destruction of the remaining cells pro-
ceeds 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, hae-
molysis in the early stages of the incubation period
may have proceeded rapidly and to a considerable de-
gree; but that subsequent haemolysis may have pro-
gressed much more slowly, because of the partial ab-
sorption 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 con-
siderable 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 de-
viation of Saponin to "cell-remants" than in the for-
mer 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 re-
tardation.

This/

FINAL DEVELOPMENT OF TECHNIQUE.

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 interest-
ing grouping of the cells of the sample ac-
cording to their resistance.

(2)   But in addition to a dose of Saponin that
would haemolyse 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 sam-
ple.

That minimal haemolytic dose would com-
pare with and confirm the early stage of haemolysis
in the larger dose: Indeed. as a test of the feeb-
lest 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 smal-
ler dose.   The group of feebly resistant cells sep-
arated 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 investi-
gation of a case by the method outlined above.

Jan. 31st, 1908. G.J. Male: aet 25 Pernicious Anaemia.

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 in-
vestigation.

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 cent-
rifuge/

centrifuge-tube, shaken up, centrifuged, the super-
natant 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 solu-
tion 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:-

	FIELDS OF 16 SQUARES.		FIELDS OF 16 SQUARES.	
	56 red-cells		56,red-cells	
	51	"	51	"
1st Prep.	53	" 2nd Prep.	53	"
	54	"	60	"
	214	"	220	"

These confirmatory counts make a total of

434 red-cells in 128 squares.

$$\therefore \frac{434 \times 4000 \times 8}{128} \text{ or } 108,000 \text{ red-cells are}$$

contained in 1 cmm. of the original blood-

suspension.

$$\therefore \text{ 50 million red-cells are contained in}$$

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

or 0.46cc.

Samples of the blood-suspension, 0·46cc were

measured off, and haemolytic preparations were pre-

pared as under.

TUBES	BLOOD SUSPENS.	SAL. SOL.	SAP. SOL.	IN- CUBATED	HAEMOLYSIS AFTER $\frac{1}{2}$hr	1hr.	1$\frac{1}{2}$hrs.	2hrs.
1	0.46cc	1.48cc	0.06cc	2.15pm	-	-	-	63.8%
2	"	1.42	0.12	1.47	60%	90%	97%	98.4%
3	"	1.38	0.16	2.20		=	-	99.9%
4	"	1.5	0.0	2.20	Control.			0%

I now give the enumerations that furnished

these data of haemolysis.   These were made as before

in the Zeiss counting-chamber, and the results cal-

culated from them.

Tube/

Tube 1.   2 hours.

FIELDS OF 16 SQUARES.		FIELDS OF 16 SQUARES	
	35 red-cells		36 red-cells
	35 "		40 "
1st Prep.	34 "	2nd Prep.	40 "
	35 "		34 "
	139 "per 64 sq.		150 " per 64 sq.

There is thus a total of 139x150, 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,

$$\frac{36 \cdot 1 \times 4000 \times 2000}{16}, \text{ or } 18,050,000 \text{ red-cells.}$$

∴ That number remains <u>unhaemolysed</u> out of 50 millions exposed to haemolysis.   Expressed as a percentage, that will be 36.1%.

So that the <u>haemolysis</u> 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 cal-
culation.

TUBE 2.

$\frac{1}{2}$ hour.	1 hour.
43 per 16sq.	(1) 61 per 100sq.
39 " "	(2) 61 " "
33 " "	
40 " "	average 61 per 100 sq.
41 " "	$\therefore$ in the whole tube there
	are now $\dfrac{61 \times 4000 \times 2000}{100}$
196 per 80 sq.	
=average 39.2 per 16 sq.	i.e. 4,880,000 red-cells.
i.e. 100-39.2	or 9.76% unhaemolysed cells.
or 60.8% haemolysis.	i.e. 90.24% haemolysis.

TUBE 2 (Contd.)

$1\frac{1}{2}/$

TUBE 2 (Contd.)

1½ hours.	2 hours.
30 per 200 sq.	37 per 400 squares
33 " "	42 " "
63 per 400 sq.	39.5 average 400sqrs.
∴ in the whole tube there	∴in the whole tube there
are now $\dfrac{63 \times 4000 \times 2000}{400}$	are $\dfrac{39.5 \times 4000 \times 2000}{400}$
i.e. 1260,000 red-cells.	i.e. 790,000 red-cells.
as a percentage these are	as a percentage this is
2.52% ∴ there is	1.58% ∴ there is
97.48% haemolysis	98.42% haemolysis

TUBE 3.   2 hours.

8 cells per 1200 squares

∴ in the whole tube there are now

$$\frac{8 \times 4000 \times 2000}{1200}$$

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 em-
ployed 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 hae-
molyse a large majority of healthy red-
cells, and that effected by a dose hae-
molytic 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 depart-
ure 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, con-
      taining 50 million red-cells.   Each tube
      brought by addition of saline and saponin
      solutions to 2 cc.

TUBE	SAP.SOL.	HAEMOLYSIS IN 2 hours.
1	0.7cc.	0%
2	0.8cc.	0%
3	0.9cc.	0%
4	1.0cc.	36%

On/

On p.64. No 7 of the Table given, there showed that <u>0.12cc</u> 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 oligocythaemia 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 5cc. After contrifugation, one could, with certainty remove all the supernatant fluid, except 0.05cc. That is, after each washing, $\frac{1}{100}$ of the original bulk of fluid was left. Therefore, after 3 washings $\left(\frac{1}{100}\right)^3$ or $\frac{1}{1000000}$ 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 experi-
ment, I determined that solutions of the strength
used, if kept in the dark, and in a cool place, show-
ed 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 pro-
duced both a great, and a slight haemolysis in 2hours.
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 ob-
tained in series and with a single solution of
Saponin, but singly and at intervals, and with many
different solutions.   Before proceeding to give re-
sults 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-HAE-

    MOLYSIS.

----

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-suspen-

       sion , is the enumeration of the red-cells

       in those 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 Incom-

       plete Haemolysis. It is superior to the

       methods commonly employed in haemolytic re-

       search, which adopt such indefinite terms as

       a "slight," "moderate", or such equally in-

       definite symbols as +, ++.

4. The Colorimetric Method is inaccurate in deter-

       mining Complete Haemolysis: but not more so

       than the test of transparence commonly employ-

       ed.

5./

5. The Enumerative Method of determining degrees of
   Haemolysis, is superior to any other method,
   both for Incomplete and for Complete Hae-
   molysis.

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

7. This method is proved reliable by the correspon-
   dence 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./

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/

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 destroy-
    ed cells, and therefore a diminution of
    Saponin available for the envelopes of Haemo-
    globin containing cells.

17. This deviation of Saponin must therefore begin
    with the first onset of haemolysis, must in-
    crease with its progress, and must be great-
    est 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, be-
    cause 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 hae-
    molysed erythrocytes, and is, therefore,
    diverted/

diverted from the envelopes of the few re-
maining erythrocytes.

20. Conclusion 19 explains the disproportionate in-
crement of Saponin required to carry haemoly-
sis from 99% to 100%. - and Conclusion 11;
and from another point of view, the dispro-
portionate 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 transparence, 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 obser-
vations are prolonged.

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

29. This influence of the bulk of fluid upon hae-
molysis shows Bashford's statement, "A
multiple of the solvent dose of glucoside
(saponin, etc), will dissolve the same multi-
ple 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 pro-
portionally 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 sub-
sequent haemolysis is small and slow.

34. With a dose of Saponin that effects a small
amount of haemolysis in 2 hours, the sub-
sequent 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, in-
dependent of the corpuscular content of the
entire blood .   As the latter varies wide-
ly 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 haemoly-
sis 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/

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 com-
    parison 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 de-
    viation 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 pro-
duce a very slight haemolysis of the stand-
ard 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.

P A R T   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/

No.	Name Sex	Age	Erythrocytes per c.mm.	Colour-Index	Leuco-cytes	HAEMOLYSIS. Sap.Sol. 0·12 cc.				0·08 cc.	0·06 cc.
						½ hr.	1 hr.	1½ hrs.	2 hrs.	2 hrs.	2 hrs.
1	J.G.(m)	19	5,610,000	0.98	7000	14%	58%	81%	90%		7%
2	J.S.(m)	22	5,030,000	1·06	6400	14%	57%	69%	84%	15%	
3	D.L.M.(m)	23	4,960,000	0·95	5800	14%	55%	73%	86%	23%	
4	C.S.M.(m)	24	5,800,000	0·94	6800	25%	70%	83%	90%		8%
5	W.M.S.(m)	25	5,200,000	1·1	4800	23%	55%	75%	86%	18%	
6	C.M.(m)	26	5,500,000	0·98	5000	7%	66%	90%	93%		8%
7	J.A.G.(m)	26	5,650,000	1·03	6200	23%	56%	82%	88%	25%	
8	M.A.(f)	27	4,800,000	1·1	7800	26%	65%	78%	86%	27%	
9	J.M.(m)	28	4,800,000	1·02	4600	11%	68%	82%	91%		14%
10	A.N.(m)	35	4,650,000	0·94	6200	35%	73%	88%	91%		23%
11	J.T.(f)	35	5,250,000	0·99	8800	25%	66%	82%	87%	24%	
12	W.S.(m)	36	5,050,000	1·0	6000	7%	59%	76%	87%	12%	
13	C.H.(m)	41	6,100,000	0·98	6200	24%	70%	86%	90%		13%
14	C.L.(f)	53	4,900,000	0·94	7200	23%	63%	74%	84%		12%
15	J.D.(m)	60	5,150,000	0·97	6000	32%	74%	87%	93%	10%	

The operation of the 0·12 cc. dose of Saponin in No.5 is illus-
trated/

illustrated graphically below.
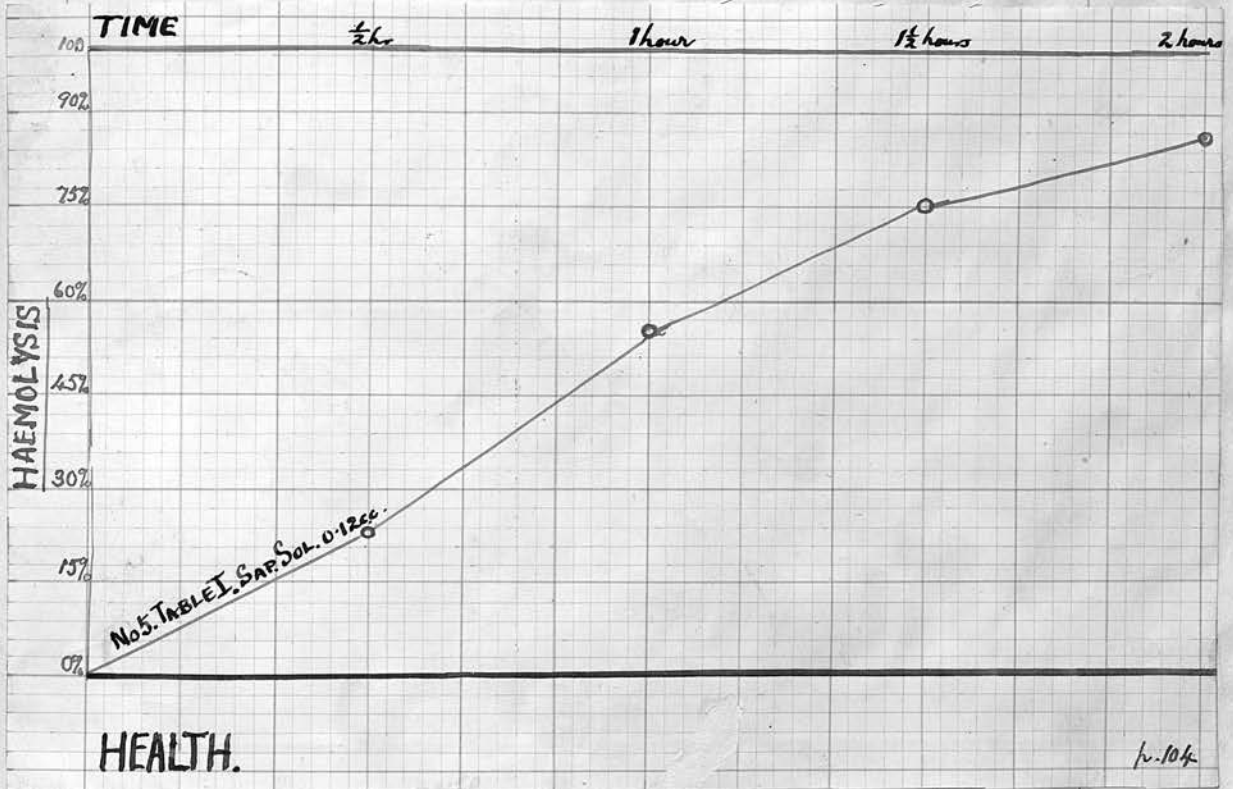
RESULTS OF TABLE I. HEALTH.

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/

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 $\frac{1}{2}$ 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 [11]. 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[12] 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 pro-
cess, consisting in the release of Haemoglobin from
the erythrocyte in the spleen and in the gastro-in-
testinal capillaries, and in the interception of this
free Haemoglobin in the liver cells where it is con-
verted into the bile-pigment.

But Hunter's experiments are not decisive,
and his deductions are not generally accepted as con-
clusive by physiologists.   In the standard text books
of physiology the question is regarded as quite un-
settled 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 protoplas-
mic poison not limited in its action to red-cells,
and therefore presumably will test some important
common element in protoplasm.   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 inter-
pret 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 de-
parts from a cardinal principle of experiment in in-
vestigating the behaviour of a tissue in unnatural
conditions, and errs more seriously still in immedi-
ately applying the results to the behaviour of the
tissue in its normal condition.   These considerations
will be more fully discussed later, but it is approp-
riate 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 in-
vestigated, 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 tem-
perature 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.

HAEMOLYSIS.

No.	Name. Sex.	Age.	No. of Red Cells per cmn.	Colour-Index.	Leuco-cytes per cmn.	Sap.0·12cc.				0·08 cc.	0·06 cc.
						½ hr.	1 hr.	1½ hrs.	2. hrs.	2 hrs.	2 hrs.
1	E.K.(m)	67	4,580,000	0·9	8,600	79%	90%	98·7%	99·6%	–	86%
2	T.B.(m)	38	3,800,000	0·8	11,600	95%	99·28%	99·8%	99·9%	–	96%
3	T.D.(m)	40	3,880,000	1·0	8,600	95%	99·22%	99·8%	99·9%	89%	–
4	J.B.(f)	46	4,250,000	1·03	10,600	84%	93%	99%	99·8%	93·5%	–
5	E.G.(f)	53	3,750,000	0·93	16,000	91%	98%	99·7%	99·9%	–	65%
	Group 2. Slight or Disappearing Jaundice.										
6	W.P.(m)	47	4,360,000	0·9	4,600	47%	84%	96%	98%	49%	–
7	T.M.(m)	62	4,980,000	1·03	6,600	–	–	–	–	–	32%
8	F.A.(m)	24	5,320,000	1·0	5,600	11%	76%	89%	94%	–	31%
	Health (average).					23%	55%	75%	86%	18%	10%

Graphic/

Graphic Chart.
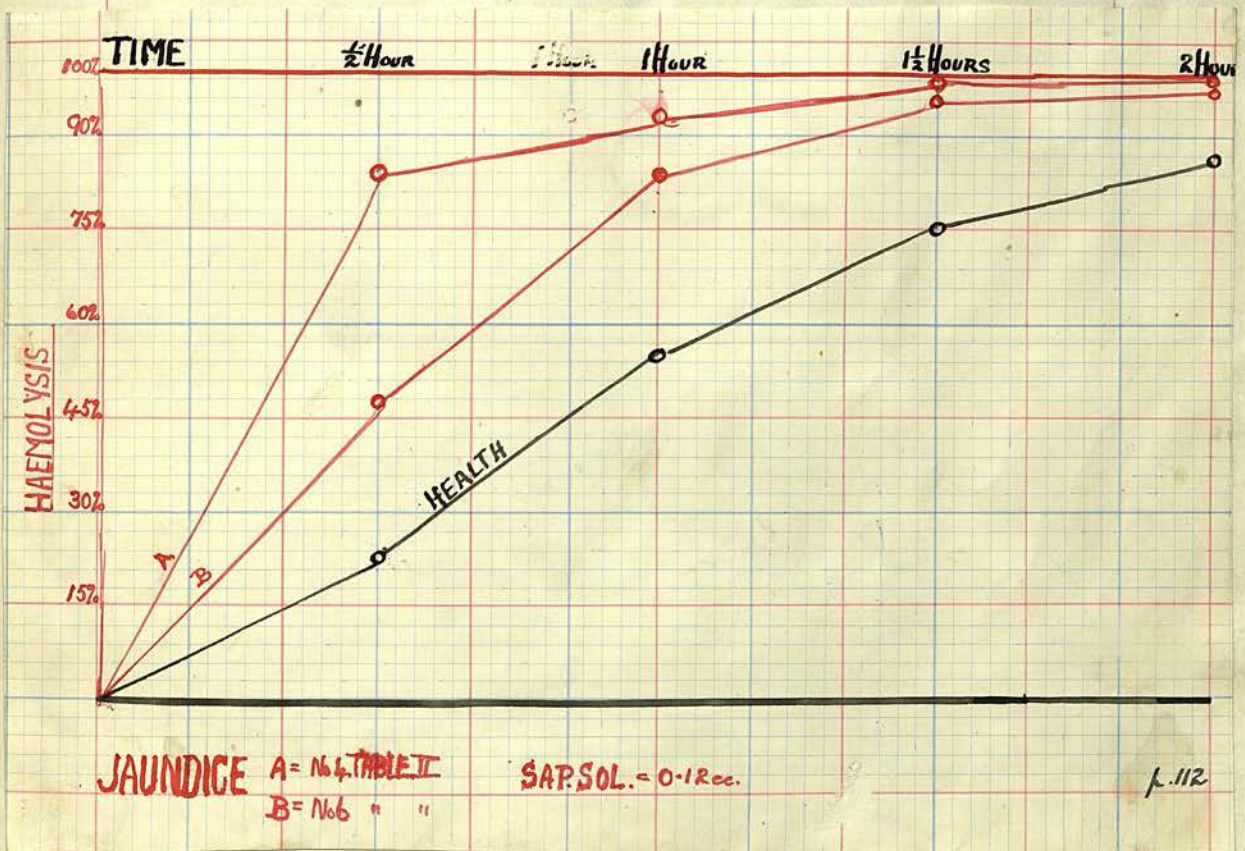
NOTES.

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 per-
sisted.   Stools greyish-white: no gall-stones.
Urine has much bile-pigment, no bile-acids: shows
pancreatic crystals to Cammidge's Test.

No.3.   T.D. (aet.40. m.).   Cause unknown.   Deep jaun-
dice: came on 2 months ago without pain or sick-
ness.   Stools greyish white: no gall-stones.
Urine has much bile-pigment, no bile-acids.   No
enlargement of liver.   Subsequent operation re-
vealed no obstruction in pancreas, duodenum, bile-
ducts or gall-bladder.

No.4.   Mrs E.B. (aet.46).   Malignant Disease of Gall-
bladder.   Deep jaundice: came on 9 weeks ago
without pain.   Stools greyish white: urine has
much bile-pigment, no bile-acids: liver not en-
larged.   Subsequent operation established above
diagnosis.

No.5/

No.5.   Mrs E.G. (aet.53).   Catarrhal Jaundice (?)
Deep jaundice of 7 weeks' duration: came on with
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 $6\frac{1}{2}$ 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/

No.8. F.A. (aet.24, m.). Catarrhal Jaundice (dis-
    appearing). Followed drinking bout a week ago.
    Liver not now enlarged: moderate jaundice of skin
    and conjunctivae. Stools contain much bile pig-
    ment. Urine has bile-pigment, no bile-acids.

RESULTS OF TABLE II.
_____

The above table has been divided into two
groups, the first including cases of deep and persis-
tent 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 pro-
duces 86% 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 dis-
appearing, 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 incorporat-
ed 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.

Name & age.	Condition	Sap. Sol.	Haemolysis after			
			$\frac{1}{2}$ hr.	1 hr.	$1\frac{1}{2}$ hrs.	2 hrs.
T.M. (62)	Jaundice (silght)	0·14cc	83%	99·8%	99·9%	99·9%
M.B.S. (22)	Health	0·14cc	21%	94·0%	97·0%	98·0%

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 con-
cordant 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.

OBSTRUCTIVE 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 haemo-
lysis, are therefore concordant for each, though op-
posed to each other.   Presumably each tests a dif-
ferent 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 con-
dition 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 protoplasmic 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 protoplasmic 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 al-
ready 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 'hypo-
tonic' method, has offered the explanation that the
weaker red-cells having been destroyed by the bile-
acids, only the strongest and most resistent cells
remain. But having made that explanation, he im-
mediately withdraws it, because of two facts that are
inconsistent with it, namely, the absence of oligocy-
thaemia indicating erythrocytolysis, and the presence
of/

of a hypertonic blood plasma. His citation of the
latter is interesting and indicates a curious con-
fusion 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 pre-
vented 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 be-
tween the bile-acids and the red-cells in Jaundice,
there is a preliminary consideration which will aid
the subsequent discussion. In Jaundice the circula-
tion 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 any-
thing 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 Sa-
ponin-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 con-
firms 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 cholates, 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 erythrocytolysis.
That seems at first an unreasonable sugges-
tion; but when we consider the normal cir-
culation of the bile-acids. and their dis-
turbed 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 con-
dition,/

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 gastro
intestinal capillaries. His researches
were used by him to indicate the sites of
haemolysis in corpore. This further sug-
gestion 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
oligocythaemia 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 hypoth-
esis 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 justi-
fied 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 cholate 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 hypo-
tonic method, it is given by a method which approxi-
mates 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 cholate salts.

<div align="center">

NO   HAEMOLYTIC   PROPERTY   OF   BLOOD

PLASMA   IN   JAUNDICE.

</div>

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 80cmm.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.

C.M.   HEALTH.
_____

0·5cc. of washed blood-suspension contains 50 million red-cells.

BLOOD SUSPENSION	DILUTED JAUNDICE SERUM
0·5cc. +	1·5cc.

This preparation was incubated at 37° C.for 4½ 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 assump-
tion 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.

HAEMOLYSIS

No.	Name Sex	Age	No. of Red-cells per cmm.	Colour-Index	Leuco-cytes per cmm.	Sap.Sol.0·12cc.				0·08cc.	0·06cc.
						½ hr.	1 hr.	1½ hrs.	2 hrs.	2 hrs.	2 hrs.
1	J.H.(f)	28	5,010,000	0·88	11,200	9%	44%	72%	78%		22%
2	A.C.(m)	21	6,360,000	0·83	11,000	6%	35%	66%	79%		35%
3	J.M.(m)	27	5,200,000	0·96	10,600	14%	48%	67%	78%	27%	
4	W.T.(m)	40	6,500,000	0·92	14,400	18%	45%	58%	75%	26%	
5	M.C.(f)	21	5,000,000	0·95	8,000	7%	57%	71%	84%		32%
					FEVER GROUP 2.						
6	M.F.(m)	28	4,320,000	0·99	21,800	49%	91%	97%	98%	35%	
7	S.L.(f)	33	5,010,000	0·92	36,400	26%	81%	96%	98%		21%
8	B.A.(f)	22	4,100,000	0·89	9,200	73%	98%	99·9%	100%	98%	
9	E.H.(f)	18	4,450,000	0·9	25,600	81%	99%	100%	100%		76%
					EXOPHTHALMIC GOITRE.						
1	H.D.(f)	21	5,180,000	1·0	4,800	22%	46%	71%	88%	55%	
2	J.F.(m)	24	5,200,000	0·9	8,400	16%	71%	86%	92%		41%
3	Mrs.G.(f)	44	3,500,000	1·0	3,800				98%		
4	M.P.(f)	30	5,500,000	0·9	5,000	57%	94%	99%	99.6%		46%
	HEALTH (average)					23%	55%	75%	86%	18%	10%

Table/

GRAPHIC CHARTS.

FEVER A = No I TABLE II
B = No II " "
C = HEALTH

SAP. SOL. = 0·12 c.c.

p. 130 (1)

EXOPHTHALMIC GOITRE A = No I TABLE III (Ex.G.)   HEALTH = C
B = No 4 " "

SAP. SOL = 0·12 c.c.

p. 130 (2)
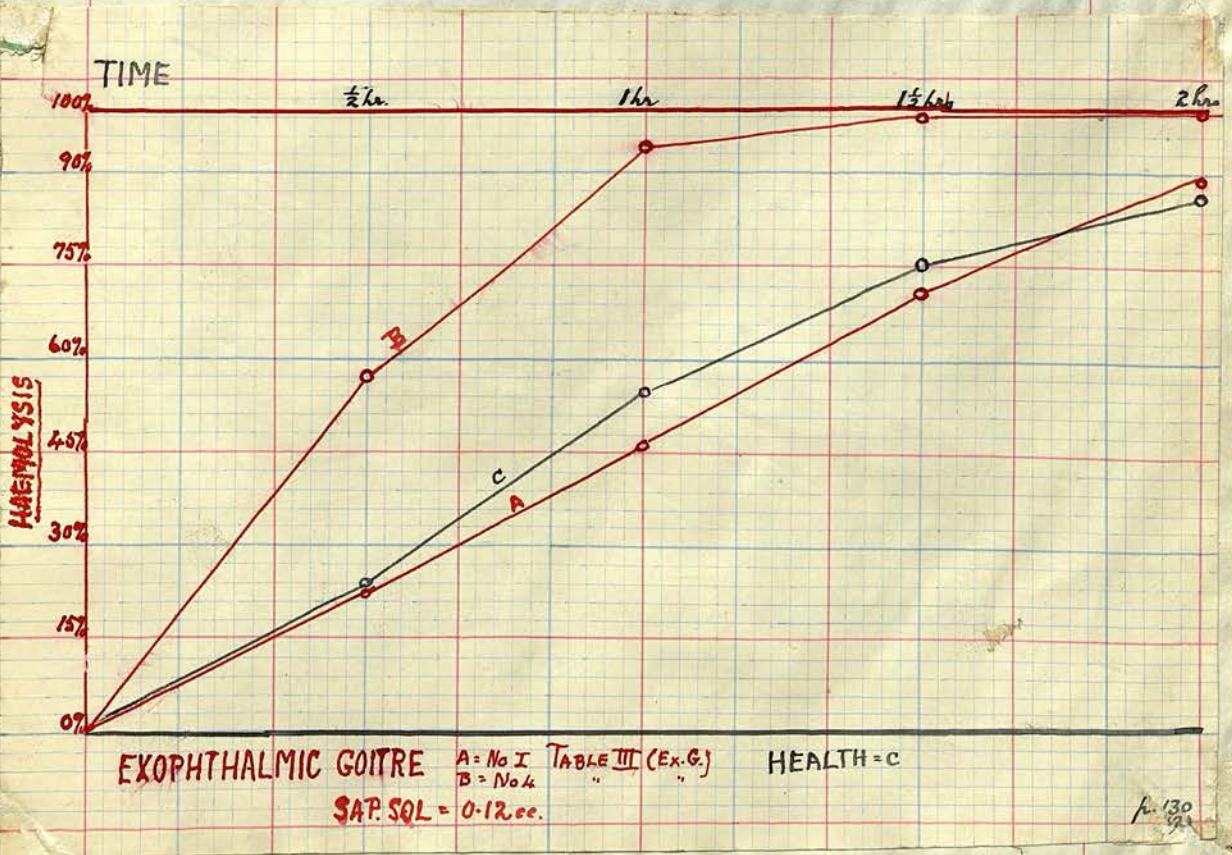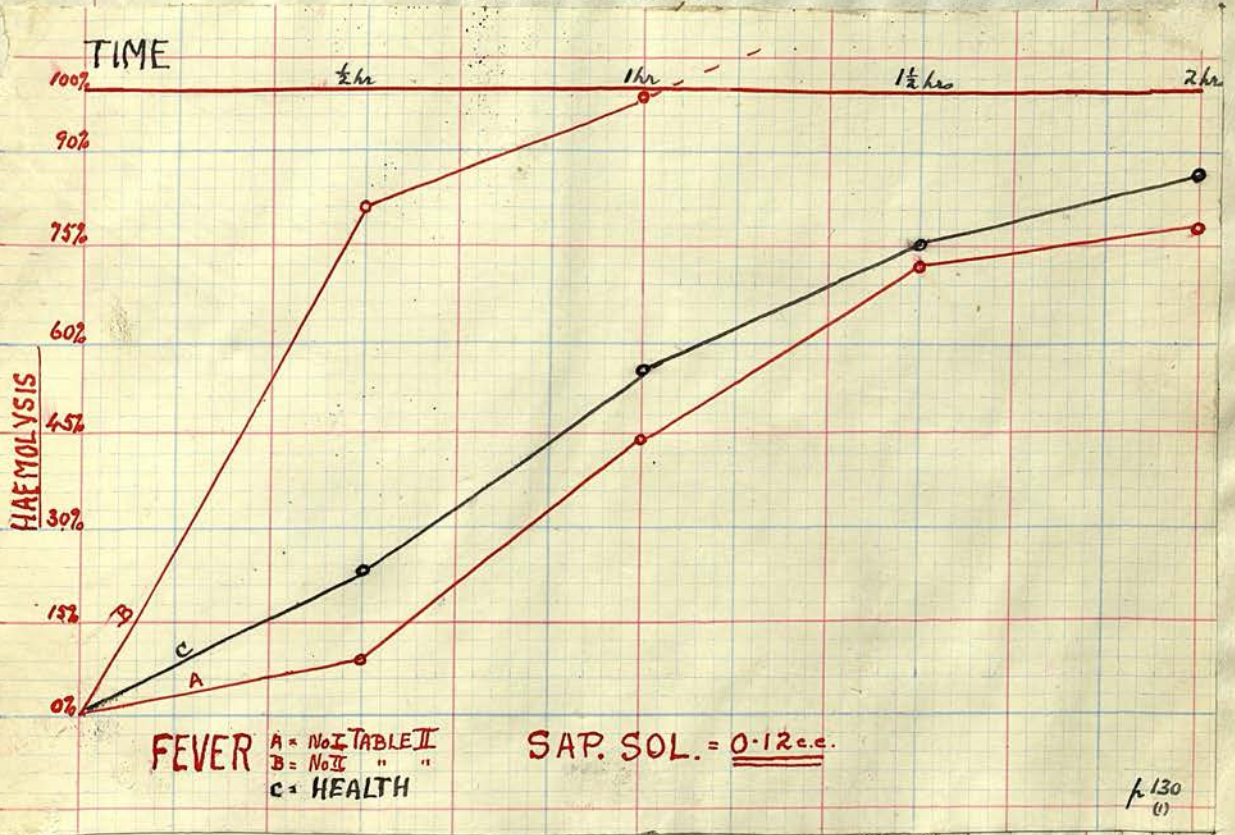
TABLE III. CLINICAL NOTES.

FEVER. GROUP I.

No.1.   Mrs.J.H.(aet.28). REMITTENT PYROEXIA
(Phthisis?).   Has had cough and fever at nights
for 15 months.   At present, temperature reaches
103° F. in the evening, with remission to 99·6° F.
in the morning.   Profuse crepitations all over
upper lobe of left lung, without consolidation.
No Tubercle Bacilli in sputum.   January 28th 1908

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.C.(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 morn-
ing.   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.   Re-
spirations 70. Pulse 124 per minute.   Consoli-
dation 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.

No.8.   B.A.(aet.22,f.).   REMITTENT PYROEXIA. Acute
Miliary Pulmonary Tuberculous.   Cough & fever for
four months.   In that period has lost $3\frac{1}{2}$ stones
weight, weighing now $4\frac{1}{2}$ stones.   Sputum contains
many Tubercle Bacilli.   No consolidation of lungs.
Temperature swings from 104° F. in the evening to
99° F.in the morning.   March 4th 1908.

No.9.   E.H.(aet.18,f.).   Hyperpyroexia.   Fulmina-
ting Typhoid Fever.   Admitted 3 days ago. with
Temperature 105° F. which has remained there
since with slight irregular remissions.   Diarr-
hoea with "pea-soup" stools.   Great meteorism.
Profuse eruption of rose-coloured spots on ab-
domen, back, shoulders and arms.   Blood gives a
slight Widal reaction.   March 20th 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.

No.3.   Mrs.G.(aet.44,f.).   Exophthalmos marked.
Goitre fairly large.   Tachycardia – average pulse
rate 110.   Tremor marked.   Duration of symptoms
9 months.

No.4.   M.P.(aet.30,f.).   Exophthalmos extreme.
Goitre very large.   Tachycardia – average pulse
rate 110.   Tremor very marked.   Duration of
symptoms 9 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 Incu-
bation 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 haemoly-
sis 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 haemoly-
sis 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 feebler, while the high-resistance group had be-
come 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 Sa-
ponin gives undoubtedly a superior test of the group
of feebly resistant cells than does the early opera-
tion/

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 re-
tarded haemolysis of the larger dose which is apparr-
ently inconsistent with it.   Further the final re-
tarded 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 resis-
tant 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 out-
weighs 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 inter-
preted 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 metabol-
ism 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 pyroexia 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 olyocythaemia does not appear.   It is fair to conclude that in these cases of severe pyroexia, 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 destruc-tion/

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 de-
struction of erythrocytes.   (2) That where this in-
creased wastage is slight as in Group I, the compensa-
tion 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 Hyperpy-
roexia though the fresh output from the bone marrow
may prevent olyocythaemia 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 in-
vestigated have been included in Table III along with
those of Fever, because their results of Saponin haemo-
lysis 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 sub-
divided like those of Fever.   For Nos.1 and 2 show
that discrepancy between the haemolysis of the larger
dose in ½ hour and the smaller in 2 hours, already
noted in Group I.of Fever.   While in No.4 the corres-
pondence 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 cardio-
vascular activity and excitability, in the moisture
of the skin, and in the psychical excitement.   It is
therefore reasonable to expect both an increased act-
ivity of the bone-marrow and an increase of the func-
tion 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 oligocythaemia
is present.   But the incompleteness of the investiga-
tion 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 polycy-
thaemia.   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.

Name. Age Sex.	No.of Red Cells per cmm.	Colour Index.	Leuco-cytes per cmm.	HAEMOLYSIS.					
				Sap.Sol. 0·12 cc.				0·08cc.	0·06cc.
				½ hr.	1 hr.	1½hrs.	2 hrs.	2 hrs.	2 hrs.
G.J.(m) 25	1,090,000	1·3	4,600	60%	90%	97%	98%	--	64%
"(3 weeks later).	744,000	1·1	2,400	84%	98%	99%	99·8%	--	89%
J.S.(m) 51	792,000	1·5	3,300	95%	98%	99%	99·9%	--	--
"(5 weeks later).	2,370,000	1·5	3,000	75%	93%	97%	98·9%	--	71%
M.R.(f) 37	1,170,000	1·1	2,800	--	--	--	98·7%	--	--
Mrs S. " 54	2,550,000	1·8	4,000	28%	76%	91%	94%	--	66%
J.M.(m) 51	1,960,000	1·5	6,800	40%	70%	81%	88%	59%	--
A.W.(m) 65	1,760,000	1·13	4,200	29%	71%	89%	90%	28%	--

Pernicious Anaemia with Spinal Cord Complications.

Name. Age Sex.	No.of Red Cells per cmm.	Colour Index.	Leuco-cytes per cmm.	½ hr.	1 hr.	1½hrs.	2 hrs.	2 hrs.	2 hrs.
A.S.(m) 41	3,870,000	1·03	6,000	54%	82%	92%	97%	44%	--
"(10 days later).	4,340,000	0·95	8,200	54%	84%	92%	95%	49%	--
R.L.(m) 36	2,070,000	1·5	3,000	58%	76%	88%	90%	--	30%
E.M.(f) 56	3,040,000	1·2	3,200	40%	74%	89%	96%	54%	--
Health average, etc.				23%	55%	73%	86%	18%	10%

SECONDARY/

SECONDARY ANAEMIA.

HAEMOLYSIS.

No.	Name. Age. Sex.	No.of Red Cells per cmm.	Colour Index.	Leuco-cytes per cmm.	Sap.Sol. 0·12cc.				0·08cc.	0·06cc.
					½ hr.	1 hr.	1½hrs	2 hrs.	2 hrs.	2 hrs.
1.	A.S.(f) 37	1,940,000	0·98	3,600	27%	78%	88%	93%	--	43%
2.	Mrs.R.(f) 29	2,010,000	0·42	8,000	54%	77%	88%	93%	55%	--
3.	J.F.(f) 33	4,720,000	0·5	6,000	42%	62%	81%	90%	34·8%	--
	Health (average)				23%	55%	75%	86%	18%	10%

SIMPLE ANAEMIA.

No.	Name. Age. Sex.	No.of Red Cells per cmm.	Colour Index.	Leuco-cytes per cmm.	½ hr.	1 hr.	1½hrs	2 hrs.	2 hrs.	2 hrs.
1.	M.C.(f) 22	4,340,000	0·48	6,400	44%	76%	93%	95%	58%	--
2.	M.D.(f) 20	4,130,000	0·58	4,200	--	--	--	94%	--	--

PURPURA.

No.	Name. Age. Sex.	No.of Red Cells per cmm.	Colour Index.	Leuco-cytes per cmm.	½ hr.	1 hr.	1½hrs	2 hrs.	2 hrs.	2 hrs.
1.	J.H.(m) 37	4,470,000	0·96	6,400	--	--	--	--	--	51%
2.	J.G.(m) 42	1,890,000	1·0	18,000	38%	77%	91%	94%	--	54%

SPLENIC ANAEMIA.

No.	Name. Age. Sex.	No.of Red Cells per cmm.	Colour Index.	Leuco-cytes per cmm.	½ hr.	1 hr.	1½hrs	2 hrs.	2 hrs.	2 hrs.
1.	P.C.(m) 37	1,970,000	1·01	--	--	--	--	96%	38%	--

SPLENO-MEDULLARY LEUKAEMIA.

No.	Name. Age. Sex.	No.of Red Cells per cmm.	Colour Index.	Leuco-cytes per cmm.	½ hr.	1 hr.	1½hrs	2 hrs.	2 hrs.	2 hrs.
1.	B.F.(f) 29	3,100,000	1·0	30,800	42%	68%	82%	91%	46%	--

HODGKIN'S DISEASE.

No.	Name. Age. Sex.	No.of Red Cells per cmm.	Colour Index.	Leuco-cytes per cmm.	½ hr.	1 hr.	1½hrs	2 hrs.	2 hrs.	2 hrs.
1.	Mrs.A.(f) 25	2,610,000	0·84	37,000	14%	55%	68%	88%	--	31·8%
	Health (average)				23%	55%	76%	88%	18%	10%

TIME   ½ hr   1 hr   1½ hr   2 hrs

HAEMOLYSIS

100 — 90% — 75% — 60% — 45% — 30% — 15% — 0%

A   B   C   D

SAP. SOL = 0.12 c.c.

ANAEMIA   A = PERNIC. ANAEMIA No 2
   B =   "   "   " 10
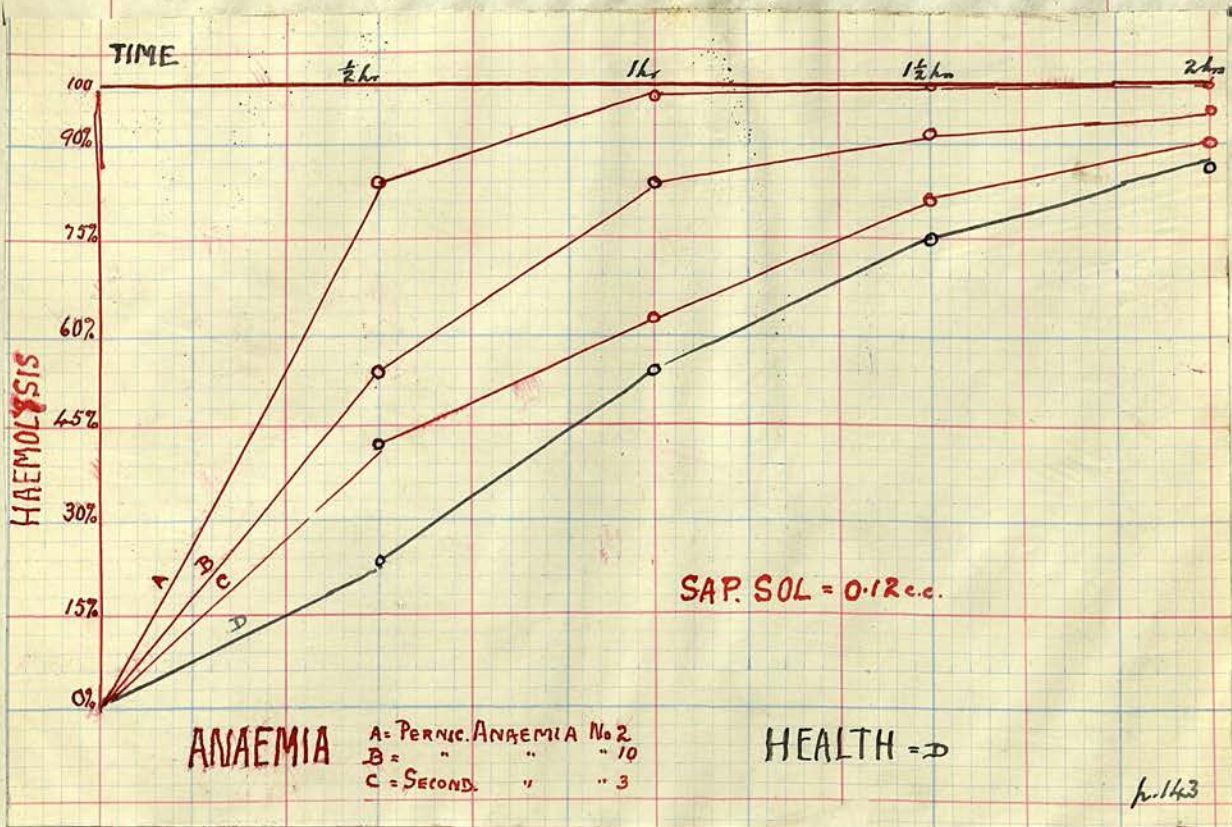   C = SECOND   "   " 3
   HEALTH = D

p. 143

TABLE IV.   CLINICAL NOTES.

PERNICIOUS ANAEMIA.

No.1.   G.J (aet.25, m.).   PERNICIOUS ANAEMIA (second
Attack).   First attack was a year ago, but he
recovered almost completely.   Blood film shows
numerous megalocytes, megaloblasts and normo-
blasts; much pockelocytosis; Jan. 31st, 1908.

No.2/

No.2. The Same. Febr. 21st, 1908. Patient's con-
dition has got steadily worse since last investi-
gation.

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 megalo-
blasts. 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 hospi-
tal 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 megalo-
cytes; 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 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 megalo-
    blasts, 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 pockelocytosis or megalo-
cytes.   No nucleated red-cells found.   March 3rd.
1908.

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

No.11.   R.L. (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 nu-
cleated 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 treat-
ment 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 consider-
able 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.C. (aet.22, f.). Has suffered from "blood-
lessness" at times for three years, constipation
alternating with short attacks of diarrhoea.

No.2. M.D. (aet.20, F.). Recurrent attacks of "blood-
lessness" 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 haemorr-
hagic 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 enlarge-
ment 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 later-
ally to the umbilicus.   Differential leucocyte-
count:- Polymorpho, 59%; Lymphocytes, 38%; 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 $2\frac{1}{2}$ years.   Spleen now extends to the umbilicus
laterally and down into the pelvis.   Since ex-
posure 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 in-
vestigations 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 re-
mained 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 com-
pared 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 diminish-
ed 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,cmm.
the Saponin-resistance is lowest but where the oligo-
cythaemia is moderate, namely between 1,500,000 and
2,500,000 per c.mm. the Saponin resistance is high-
est, and is greater than in cases where the red-cells
number upwards of 2,500,000 per c.mm.

<center>SAPONIN RESISTANCE & THE RATE OF</center>
<center>PROGRESS OF THE DISEASE.</center>

The number of red-cells, and their Saponin-
resistance are therefore not concordant in their
variations. But it will be found that correspond-
ence 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 note-
worthy.   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 1V.,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 mere-
ly 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 $\frac{1}{4}$% solution, prevents the laking of these
cells in distilled water.   That is to say, the minor
action of one haemolytic agent has altogether prevent-
ed 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 Per-
nicious 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 pig-
mentary 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 pathegnomonic
features in the liver and bone marrow are due to
capillary haemorrhages, superfinal and interestitial
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 ex-
cessive 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 con-
dition 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 produc-
tion 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 laking 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 re-
action are specially relevant to William Hunter's
view of Pernicious Anaemia as a specific haemolytic
Anaemia.

RESULTS OF SAPONIN-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 hae-
molytic 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 con-
clusive.   A decisive experiment, such as the pro-
duction of Haemoglobinaemia within the portal cir-
culation, either by injection of a haemolytic sub-
stance 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 too 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 corpore 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 pronoun-
ced 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.citat)
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 infer-
ence is that, in the various groups of Anaemia con-
tained 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, nuclea -
ted 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 Per-
nicious 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 vari-
ations 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 protoplasmic envelope that contains
the/

the Haemoglobin of the cell. A red-cell, constitut-
ed mainly of two things, a protoplasmic envelop, and
Haemoglobin, may be defective in one of these con-
stituents 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 per-
manent 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 Cholesterin
an element in all protoplasm, and the results of
Saponin-haemolysis in Anaemia suggest that the amount
of Cholesterin 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 hypo-
thesis 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 Jaun-
dice.   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
oligocythaemia.   In Pernicious Anaemia, therefore,
where oligocythaemia 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 numer-
ous 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 intravescularly (Hunter) or ex-
travascularly (Stockman).   But it is equally reason-
able that the liver should be the storehouse of ex-
cess 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/

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/

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
appearence of lateral sclerosis of the spinal cord,
although the blood-condition continued near the stan-
dard of health.   The interpretation of this accord-
ing to the above suggestion would be that the pre-
vious 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 main-
tain 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 Per-
nicious 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 debili-
tating 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.

| No. | Name. Sex. | Age | No.of Red Cells per cmm. | Colour Index. | Leuco-cytes per cmm. | HAEMOLYSIS Sap Sol. 0·12cc. | | | | 0·08cc. | 0·06cc. |
						½hr.	1hr.	1½hrs.	2hrs.	2hrs.	2hrs.
1a.	J.K. (m)	36	3,000,000	0·93	1,800	88%	98%	99%	99·6%	63%	--
1b.	"(2 weeks later).		3,900,000	0·80	1,600	83%	97%	98%	99%	45%	--
2a.	Mrs EL (f)	36	1,980,000	1·16	4,400	95%	98%	99.5%	99·8%	95%	--
2b.	"(3 weeks later).		2,400,000	1·00	2,800	91%	99%	99·8%	99·9%	--	48%.
			Health (average)			23%	25%	75%	86%	18%	10%.

CLINICAL/

CLINICAL NOTES.

No.1.   J.K. (aet.36, m.). Coachman; admitted to hos-
pital in December 1907 for the purpose of opera-
tion for inguinal hernia, but he developed
pyroexia, which has returned at intervals since.
The pyroexic 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 —
$4\frac{1}{2}$ inches vertically in mid-axillary line.
Has an enlarged gland, the size of a bean, firm
and resilient, behind the middle of the pos-
terior border of the sterno-mastoid.
Differential Leucocyte count:-

Polymorphs	40%
Lymphocytes	44%
Large Monomuclears	13·8%
Eosinophiles	2·2%

Film shows a few nucleated reds.

(a). February 25th, 1908.

(b). March 12th, 1908.

No.2/

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 $1\frac{1}{2}$ inches below it.

No enlargement of superficial lymphatic glands.

Differential Leucocyte count:-

Polymorphs	43%
Lymphocytes	45%
Large Mononuclears	4%
Eosinophiles	8%

Film shows very numerous nucleated reds — normo-

blasts.

(a). March 6th, 1908.

(b). March 27th, 1908.

              These two cases showed such a feeble

resistance to Saponin that I have grouped them dis-

tinctly 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 ob-

servations.

                    The/

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.

No.	Name. Sex.		Age	No. of Red Cells per cmm.	Colour Index.	Leuco- cytes	HAEMOLYSIS				0·08cc.	0·06cc
							Sap.Sol. 0·12cc.					
							½hr.	1hr.	1½hrs.	2hrs.	2hrs.	2hrs.
1.	G.L.	(m)	59	4,600,000	1·0	6,800	--	--	--	--	--	6%
2.	J.L.	(m)	43	4,800,000	1·06	9,600	10%	35%	65%	78%	--	0%
3.	G.W.	(m)	27	4,670,000	0·9	7,000	2%	37%	58%	75%	--	0%
4.	R.M.	(m)	18	5,600,000	0·97	6,000	28%	53%	74%	77%	24%	--
				Health (average)			23%	55%	75%	86%	18%	10%

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/

Name Age Sex.	CONDITION .Sap.Sol.	HAEMOLYSIS $\frac{1}{2}$hr.	1 hr.	1$\frac{1}{2}$hrs	2hrs.
G.L.(m) 59	Diabetes-Mellitus 0·14cc.	24%	86%	95%	97%
M.S.(m) 22	Health 0·14cc.	21%	94%	97%	98%

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 ob-
tained 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 increas-
ed in Infectious Diseases and especially in Typhus
Fever; also in Cancer of internal organs.
Lang (25) studied the red-cell resistance in malig-
nant 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 investi-
gations entitled, "Recherches sur la resistance des
Haematies."   These Doct.Lyons, 1886, and Janowsky's
several papers published in the Klinische Wochen-
schrift (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 diminshed, 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 re-
sults obtained in Jaundice by the one method are ex-
actly/

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 obtain-
ed definite results with Saponin-haemolysis.

The technique of the hypotonic method I
employed was very simple.   In the usual way I pre-
pared 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 $0 \cdot 85$ saline solution -1 cc.
To this was added 1 cc. of Distilled Water.   These
red-cells were now bathed in $0 \cdot 42\%$ 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 re-
sults/

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 mins.
washed red cells, in each tube.

HAEMOLYSIS AFTER.

NAME	CONDITION	20 MIN.	$1\frac{1}{2}$ hrs.	3 hrs.
C.M.	Health.	73%	-	72%
A.S.	Secondary. Anaemia.	83%	81%	82%

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 con-
clusion given by these results is that haemolysis
by a hypotonic salt solution is completed very quick-
ly.   The above results were obtained at room-tempera-
ture 18^0C.   Repetition of the experiments at In-
cubator-temperature (37^0C) 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 re-
sults of Saponin-haemolysis, with those of hypotonic
saline solution (0·42% Na.Cl) 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-hae-
molysis.

HAEMOLYSIS/

HAEMOLYSIS.

NAME & SEX.	AGE.	CONDITION	BY SAP. SOL. 0·12cc. 2 hours.	SAL. SOL.0·42% 1 hour.	
C.M.(m)	26	Health	93%	72%	
J.G.(m)	19	"	90%	80%	
W.S.(m)	36	"	87%	77%	
J.D.(m)	60	"	93%	75%	
E.K.(m)	67	Jaundice	99.6%	47%	
T.B.(m)	38	"	99.8%	35%	
T.D.(m)	40	"	99.9%	55%	
Mrs.E.B.	46	"	99.9%	25%	
Mrs.G.	53	"	99.9%	45%	
G.J.(m)	25	Pern.Anaemia	98%	85%	
M.R.(f)	37	" "	98%	96%	
Mrs S.	54	" "	94%	55%	
A.S.(m)	41	" "	97%	80.2%	
Mrs. S.	37	Second Anaem.	93%	81%	
Mrs. R.	29	" "	93%	77%	
J.K.(m)	36	Anae.Table V.	99%	33%	
B.A.(f)	22	Fever acute miliary tuberculosis.	100%	98%	
V.F.(m)	28	Fever Pneumon.	98%	31%	
Mrs J.H.	28	" Phthisis	78%	62%	
A.C.(m)	21	" Pneumon.	79%	90%	

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 solu-
tion, the third and fourth showing increased re-
sistance 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 re-
vealed in the cases of Jaundice.   Chanel and v.Lim-
beck (loc.cit.) have already established the fact of
the increased resistance of the red-cells in that
condtion/

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 simul-
taneously 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 hae-
molysis.   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 sus-
pension; 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 hypo-tonic 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 hypo-tonic saline solution.

DURATION/

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.

G.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.

TUBES	BLOOD DRAWN.	DISTILLED-WATER ADDED.	INTERVAL.	HAE-MOLYSIS.
1	11p.m.Feb.20	2p.m.Feb.20	3 hours.	82%
2	"	2p.m.Feb.21	27 hours.	14%.

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.

NAME/

NAME & AGE.	BLOOD OBTAINED.	HAEMOLYSIS IN HYPOTONIC SOLUTIONS PREPARED AFTER REMAINING IN ISOTONIC SOLUTION FOR			
		2 hrs.	3 hrs.	4 hrs.	5 hrs.
Mrs.W.(22)	10.20am.	-	81%	9%	-
Mrs.A.B(22)	10.10am.	70%	68%	16%	-
M.B. (29)	10.30am.	71%	68%	27%	13%

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 with-
drawal 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 re-
sistance 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 re-
maining a longer time in the isotonic fluid.   In
the following experiment, this factor was properly
controlled.

     E.H.   aet. 18.   Hyperpyrexia Typhoid Fever.
        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 hypo-
        tonic 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.

TUBE	BLOOD DRAWN.	HYPOTONIC SOL.PREPARED	HAEMOLYSIS
1	11.30 a.m.	2.30 p.m.	97%
2	"	"	95%

Sap.Soln.

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 diminu-
tion or increase.   It leaves unexplained the undoubt-
ed divergence of results between these two methods of
estimating resistance to haemolysis.   That diver-
gence 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 possi-
bility..

M.B.(f). aet. 29.   Health.

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 add-
ed; incubated for 2 hours at 37°C. and
haemolysis then recorded.

TUBE.	BLOOD DRAWN.	SAP.SOL. ADDED & INCUBATED	INTERVAL.	HAEMOLYSIS. 2 hours.
1	10.30a.m.	12.30 a.m.	2 hours	90.9%
2	"	2.30 a.m.	4 hours.	91.4%

The conclusion is that between an interval

of/

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-hae-
molysis were obtained.   The above experiment there-
fore 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 pro-
duce alterations in resistance to Saponin-haemolysis.
In the following experiment exactly the same pro-
cedure was adopted, but one sample of washed red-
cells was kept for more than 24 hours at room tempera-
ture before subjection to the same dose of Saponin
Solution.

M.B.S.(m). aet.22.   Health.

TUBE.	BLOOD DRAWN.	SAP.SOLN. ADDED: INCUBATED.	INTERVAL.	HAEMOLYSIS IN 2 hours.
1	11a.m.Jan 16.	1p.m.Jan.16.	2 hrs.	99.52%
2	" "	3p.m.Jan.17.	28 hrs.	99.72%

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 resis-
tance 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 enor-
mously 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 hae-
molytic 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 in-
creased/

increased resistance to haemolysis by hypotonic salt-
solution is now shown to be compatible with an un-
changed resistance to Saponin. In the results of hae-
molysis in Jaundice the increase of resistance is
also obtained by the hypotonic salt-solution method,
and the diminution of resistance by Saponin-haemoly-
sis.   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-re-
            sistance which does not determine hae-
            molysis 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 in-
crease of resistance to such a haemolytic agent in-
dicates 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 hae-
molytic 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 hae-
molysis, 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 haemoly-
sis 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 hae-
molysis which makes difficulty in expressing the re-
sults of Saponin-haemolysis in terms of physiological
and pathological activity in the body.   At the same
time Saponin is a general protoplasmic poison, and
the measurement of its haemolytic action upon red-
cells undoubtedly gauges the condition of the envelope-
protoplasm.   The results of Saponin-haemolysis will
therefore indicate the condition of the cell-envelope
apart from the question of its resistance to hae-
molysis 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 pro-
visional.

It would have been possible to have stated
the results by themselves, without attempting to ad-
just 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.

C O N C L U S I O N S/

C O N C L U S I O N S.

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.

FEVER/

FEVER.

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 resist-
        ance of the whole sample of cells is in-
        creased.   In high Fever the total resist-
        ance 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, pro-
        ducing polycythaemia, and increasing the
        total resistance of a sample of cells.   In
        high Fever, it is generally adequate, and
        prevents oligocythaemia, but the total re-
        sistance of the sample is diminished.   Brief-
        ly, in moderate Fever the destruction of
        red-cells is increased; in high Fever it is
        excessively increased.

EXOPHTHALMIC/

EXOPHTHALMIC GOITRE.

9. In Exophthalmic Goitre the conditions are ex-
actly 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 re-
sistance.

ANAEMIA.

10. In Anaemia there is a diminished red-cell re-
sistance 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 oligocythae-
mia: 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 Pernic-
ious 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 progress-
       ive, its effects are expressed by oligocythae-
       mia, 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 Oligo-
       cythaemia, 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 Per-
       nicious Anaemia is explained by the hypo-
       thesis of inferior or insufficient envelope-
       protoplasm, and an abundant supply of Hae-
       moglobin.

22.   The excess of iron-pigment in the liver in
       Pernicious Anaemia may equally well be ex-
       plained by a storage of surplus Haemoglobin,
       as by an excessive destruction of red-cells.

23/

205.

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 con-
stituent 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   oligocy-
thaemia in these two cases of Anaemia
would/

27. would indicate that in them there is an ex-
cessive destruction of red-cells.

DIABETES.

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

S E C T I O N   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 Obstruct-
ive Jaundice, where the red-cells are feeb-
ly resistant to Saponin but highly resist-
ant to hypotonic saline solutions.

31. The conclusion is that the two haemolytic pro-
cesses 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
a/

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 experi-
        mental 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 Physic-
ians 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 in-
vestigation, and for all the facilities I have en-
joyed in his laboratory.

BIBLIOGRAPHY/

BIBLIOGRAPHY.

1. MARAGLIANO. Berlin Klinisch.Wochensch. 1888.Bd. XXIV.

2. HAMBURGER. Osmotischer Druck. I.

3. BUFFA. Archiv.internat. de Pharmacolodyn.et Therap.1901.

4. v. LIMBECK. Clinical Pathology of the Blood.

5. RANSOM. Deutsch.med.Wochenshr. March 1901.

6. BASHFORD. Journ. of Path. 1902-1903.

7. HEDON. Archiv. internat.Pharmacodyn.et Therap. 1901.

8. MEONI. Annal.Poste.

9. HENRI. Compt.rend.de Soc. de Biolog. 1905.

10. LAMB. Scientific Memoirs of the Government of India. New Series No.4.

11. QUINCKE. Deutsch.Archiv.f.klin.med.1883.XXXII.

12. HUNTER. "Pernicious Anaemia". 1901.

13. V. NOORDEN. Pathology of Metabolism. Vol.II.

14. CROFTAN. Archiv.f.d.gesamm.Physiol.d.Mensch. (Pluger) Bd.90.

15. SACHS. Collected Studies in Immunity. (Ehrlich).

16. PEPPER. Amer.Journ.of Med.Sc. 1875.

17. COHNHEIM. Virchow. Archiv. 1876.Bd.68.

18. RINDFLEISCH. Virchow Archiv.Bd.121.

19. MUIR. Journ.of Path. 1894.Vol.II.

20. EICHHORST. Die progressive perniciöse anaemie. 1878.

21. STOCKMAN. British Medical Journal 1895. Vol.I.

22./