THE ERYTHROCYTE SEDIMENTATION RATE.

AN EXPERIMENTAL STUDY.

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1.

For a year or two previous to this investigation I had been making use of the Erythrocyte Sedimentation Rate, chiefly in case of rheumatism.

In the autumn of 1941 there came into my possession a copy of a reprint of an article in the Edinburgh Medical Journal by W.F.Harvey and T.D.Hamilton.

In this article they dealt with the use of ordinary clear glass capillary vaccine lymph tubes in the estimation of the Erythrocyte Sedimentation Rate.

The work on this had been done by making use of their own blood only, and it occurred to me that it might be interesting, making use of the material to be found in general practice, to do a series of cases comparing the be haviour of these tubes with that of the standard Westergren method.

The result of this and some remarks on the use of micro-bore tubes are discussed later.

In the course of this work I came on a striking case of haem-agglutination. I was using a Lovibond Comparator for the haemoglobin estimation. The method utilised a film of actual blood 0·0045 in. thick in a special blood slide and haem-agglutination occurred so quickly that it was difficult/

difficult to get a satisfactory reading. By any other method this might have been missed.

This was accomplished by a high sedimentation rate and my interest was aroused in the mechanism of what happened in abnormal rates. Hence this investigation.

The interpretation of the test I have not touched upon. This has been the source of lengthy and sometimes heated controversy; a controversy difficult to understand when it is remembered that the fundamental elements involved in the mechanism of varying sedimentation are not yet fully elucidated.

In blood which has been prevented from clotting the erythrocytes sediment at rates which though in health are wonderfully constant, yet in certain pathological conditions, may vary considerably.

Fahraeus in 1918 and 1921 studied this subject so exhaustively that little seems to have been added to the solution of the fundamentals of the problem since then.

The title of Fahraeus' original work in 1918 was "The Suspension Stability of the Blood" and as a definition this is expressive and complete.

Latterly the phenomenon has been commonly referred to as the Blood Sedimentation Rate or shortly the B.S.R.

This as a description is loose and meaningless and should be supplanted by the term now coming more into use - the Erythrocyte Sedimentation Rate or E.S.R. which as a definition is clear.

In the use of the Erythrocyte Sedimentation Rate it is generally accepted that as a test it is non-specific. No purely diagnostic value can be assigned to it.

Its usefulness lies in two directions. Firstly, in following the improvement or otherwise of certain diseases and secondly, in cases where an abnormal rate is found and there seems no very obvious cause to account for it, it suggests that further and more thorough investigation is called for.

Conditions and disease in which the rate varies from normal are many and varied.

Personally I would suggest classifying the causes simply and as follows.

I. Conditions in which toxins are present.

II. Conditions in which there is cell destruction and the products are being absorbed, as in fractures and after radiation.

III. Indeterminate conditions such as renal and heart disease.

IV. Pregnancy which might be considered as physiological.

In all of these conditions there is found one of the proximate conditions of varying rates - a disturbance of the balance of the protein constituents of the plasma as compared to the accepted mean.

The plasma protein which is regarded as having the greatest effect in this respect is fibrinogen and in this inquiry I confined myself to a consideration of this.

The phases which occur during sedimentation may be observed in the ordinary Westergren tube and most easily in bloods with a high sedimentation rate.

For a short time at first there is a slow clearing of the upper/

upper layer, then in about fifteen or twenty minutes this layer extends with a gradual shading upwards. Inspection of this part of the tube either with the naked eye or still better with a hand lens, shews that there have been aggregations of cells forming. From this stage on, sedimentation goes on more rapidly till there occurs a late stage when the rate is much slower and finally ceases when the packing of the cell content is complete. After twenty four hours or longer the packed cells give an approximate value of the cell contents of the blood.

Some observers take a reading at the end of one hour and then at the end of the second hour but for all practical clinical purposes the generally accepted interval is taken as one hour.

The aggregation of the cells is a point I should like to draw attention to and I am commenting on this in a later Section.

I have classified this investigation under several sections.

SECTIONS.

APPENDIX.

METHODS OF PERFORMING THE TEST.

The estimation of the Erythrocyte Sedimentation rate is accomplished by the use of tubes of a variety of bores and with the use of different heights of column.

An anti-coagulant is necessary to prevent the blood from clotting.

Those most commonly used are Sodium or Potassium oxalate and Sodium citrate.

The experiments done in this investigation have all been done with Sodium citrate.

The bore of the tubes may vary from about 1 mm. or less in the so-called micro-bore tubes to, in one method, the diameter of a centrifuge tube - roughly 15 mm.

The height of the column used is usually 200 mm. or 100 mm. and though less than 100 mm. may be used, less than 50 mm. is not advised.

The two methods most commonly used are those of Westergren and Wintrobe.

The Westergren method uses a tube of 2·5 mm. bore and a column of 200 mm. It is graduated from the top of the column in mms. and is used with citrated blood - one part of 3·8% Sodium citrate solution to four of blood.

The Wintrobe method uses a special tube - the Wintrobe Haematocrit tube. This tube is 2·5 mm. in bore and uses a column of 100 mm. It is closed at the bottom and is graduated in mms. reading from the bottom of the tube.

It is used with oxalated blood and requires to be filled with a fine pipette.

It has the advantage that after taking the sedimentation rate, it can be centrifuged to give the cell volume of the plasma.

In using any method that are certain precautions which have to be taken; the tubes have to be kept vertical; there must be no vibration and the temperature must be kept as constant as possible.

This investigation began with an inquiry into the relative behaviour of the standard Westergren method and a certain method using micro-bore tubes.

This method was described by W.F.Harvey and T.D.Hamilton in the Edinburgh Medical Journal, New Series (XVth.) Vol. XLIII, 1936.

It has since been published in Edinburgh Post-Graduate Lectures in Medicine, Vol. Two, 1942, as part of a lecture By W.F.Harvey, entitled. "Simplification of Blood Examination".

In this method of performing the erythrocyte sedimentation rate, the use of capillary lymph vaccine tubes is described.

This method is advocated by the authors on the following grounds.

"It necessitates no expensive, no specially graduated apparatus; it uses little blood and therefore causes practically neither inconvenience nor distress to the patient even when repeated daily; it does not necessitate any careful cleaning of apparatus, for all or most of it is thrown away when done with".

The tubes used are clear glass vaccine lymph tubes. They are stated by the authors to be remarkably uniform in bore throughout their length and the majority are said to be about 1·1 mm. in bore.

Some tubes which I received from Col. Harvey and others which I obtained from the same source (Baird & Tatlock) do

not quite bear this out, but in this inquiry I have used the most uniform of them.

The tubes are about 90 mm. in length and when used, are marked at 70 mm. with indian ink to give a column of this length.

The anti-coagulant used is sodium citrate as in the Westergren technique - one part of 3·8% citrate to four of blood.

The technique is as follows.

A tested pipette is used and with this, one drop of citrate is expelled into a watch glass and the remaining citrate expelled. Some blood is then taken into the pipette from a finger prick and four drops are allowed to fall into the watch glass. The contents are mixed and ta ken up into the tube to the 70 mm. mark. The tube is then tilted and the blood allowed to run up a little. Both ends of the tube are then passed through a layer of plasticine to seal them and the tube set up vertically.

The process is repeated with one's own blood as a control. The authors state that once a standard has been established this may be dispensed with, since in their own cases they find that the test, so far as their blood is concerned, has a reasonable day to day constancy.

Their study of this method was done, using their own blood. Considering this, it was thought that it mi gh t be

interesting to study the behaviour of these tubes as compared with the standard Westergren practice, by doing a series of cases comparing the two and using the varying material found in general practice.

This was done. The same specimen of citrated blood was used both for the Westergren tube and for the capillary tubes.

The blood was taken from a vein in the usual way and probably gave a somewhat more accurate measurement than the drop method.

The cases, adopting the Westergren standard, varied from normal to a pathological rate of 127 in one hour.

The results are set out in the following pages.

The cases from which the figures were obtained will be found in the appendix.

Note:- The reading of the fall in the capillary tubes is done with a rule graduated in mm.

Comparison of Westergren and Capillary Readings.

			Westergren Tube.		Capillary tube.	
			1 hr.	24 hrs.	1 hr.	24 hrs.
Case.	1.	17:12:41	21		13.	
"	"	13:3:42.	47.		24.	
"	"	15:6:42	70.	125.	31.	
"	2	19:12:41	2.		1·5.	
"	3	23:1:42.	8.		7·5.	
"	4	21:3:42.	24.	110.	18·5.	45.
"	5	23:3:42.	12.	80.	12.5	35.
"	6	25:3:42.	7.	70.	6.	35.
"	7	27:3:42.	5.		4·5.	
"	8	28:3:42.	86.	138.	45.	52.
"	9	2:4:42.	5.	67.	4·5	39.
"	10	3:4:42.	6·5	67.	5.	45.
"	11	6:4:42.	45.	124.	28.	43.
"	12	24:4:42.	18.	95.	13.	38.
"	13	9:4:42.	6·5	82.	5.	38.
"	14	28:4:42.	6.	90.	6.	42.
"	15	2:5:42.	32.	120.	26.	42.
"	16	4:5:42.	21.	113.	19.	45.
"	5	27:4:42.	6.	92.	5.	39.
"	17	5:5:42.	4·5.	73.	4.	36·5.
"	18	6:5:42.	4·5	90	4.	36.
"	19	11:5:42	10·5	94.	9·5.	38.

Comparison of Westergren and Capillary Readings. (Cntd).

		Westergren Tube.		Capillary Tube.	
		1 hr.	24 hrs.	1 hr.	24 hrs
Case 20.	13:5:42	37.	119.	21.	45.
" 21	13:5:42	5·5	87.	4·5	43
" 22.	26:5:42	9.	90.	9.	41.
" 23	29:5:42	8.	80	5·5.	35·5
" 15	1:6:42.	18.	95.	15.	39.
" 24	3:6:42	3·5		2·5	
" 8	5:6:42.	127.		48.	
" 16	8:6:42	18.	100.	10·5	41.
" 25	9:6:42.	9.	83.	5.	45.
" 26	13:6:42	8.	75.	5.	38.
" 27	19:6:42,	13.	115.	14.	
" 28	27:6:42.	23.		15.	
" 2.	29:6:42.	1·5		1·5	
" 8.	30:6:42	105.		45·5	
" 15.	30:6:42.	10·5		9·5.	
" 29.	2:7:42.	85.		44.	
" "	5:7:42	95.		42·5	
" 16	13:7:42.	22.		18.	
" 30	13:7:42	35.		19.	
" 8	27:7:42.	109.		32.	
" 4	15:8:42.	32.		24.	
" 16.	24:8:42.	22.		18.	
" 31	26:8:42.	3.		3.5	

over

Comparison of Westergren and Capillary tube Readings (Cntd).

	Westergren tube.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 hrs.
Case. 8. 27:8:42.	75.		36.	
" 15. 1:9:42.	6·5		4·5	
" 30. 3:9:42	12.		12.	
" 32. 7:9:42.	7.		6·5	
" 20. 14:9:42.	37.		39.	
" 33. 16:9:42.	6·5		5·5	
" 34. 18:9:42.	11.		9.	
" 35. 19:9:42.	7.		8.	
" 8. 27:9:42.	73.		40.	
" 29. 15:10:42.	86.		41.	
" 36. 22:10:42.	8.	90.	8.	140.
" 15. 31:10:42.	3.	51.	2.	28.

To shew more clearly the relationship between these readings, in the following pages I have arranged them in the ascending order of the Westergren readings.

The 24 hrs. readings I have omitted. These give an approach to constant volume and are interesting as shewing the approximation between this and the respective sedimentation rates.

Readings of Westergren and Capillary tubes in ascending order of Westergren readings.

Westergren.	Capillary.	Westergren.	Capillary.
1·5	1·5	9.	5.
2.	1·5	9.	9.
3.	3·5	10·5	9·5
3.	2.	10·5	9·5
3·5	2·5	11.	9.
4·5	4.	12.	12·5.
4·5.	4.	12.	12.
5.	4·5	13.	14.
5.	4·5.	16.	9.
5·5	4·5	18.	13.
6.	5.	18.	15.
6.	6.	18.	10·5.
6·5	5.	21.	13.
6·5	5	21.	19.
6.5	5·5	22.	18.
7.	6·5	23.	15.
7.	6.	24.	18·5
7.	8.	32.	26.
8.	5.	32	24
8.	5·5	35	19.
8.	7·5	37	21.
8.	8.	37.	39.

Readings of Westergren and Capillary tubes in ascending order of Westergren readings. (Cntd).

Westergren.	Capillary.	Westergren.	Capillary.
45.	28.	86.	41.
47.	24.	86.	45.
70.	31.	95.	42·5.
73.	40.	105.	45·5
75.	36.	109.	32.
85.	44.	127	48.

It will be observed that relatively to the height of the column in the Westergren tube (200 mm.) and the height in the capillary tub e (70 mm.) sedimentation is faster in the capillary tube.

A closer inspection of the figures however reveals certain discrepancies in the capillary readings as compared with the respective Westergren readings.

These are difficult to explain. The authors of the article state that it might be advisable to take double the quantity of blood and set up two tubes "for sometimes sedimentation is unduly delayed - it may be from a particle of dust in the tube".

This does not seem to be the only explanation, as some of the readings shew an increase in the rate.

Variations in the bore of the tubes were considered as a possible explanation; though the tubes on casual examination seem to be fairly uniform, yet closer inspection shews that there is a fair difference between some of them.

The following observations were made on this:-

Capillary tubes were picked so as to have as true a circular bore and as much freedom from taper as possible.

The outside diameter about the middle was measured with a metric micrometer. The difference in the thickness of the wall may be taken as negligible.

They were filled to the usual height of 70 mm. and set up in a stand. Citrated blood as used in the Westergren test was utilized from two cases with a normal E.S.R. and from one with an abnormal rate.

Results,

Case. 38.

12:11:42.

E.S.R. of 1 hr. Westergren. 3.

Diameter of Tube.	1 hr.
1·25. mm.	2·75
1·27. mm.	3.
1·3 "	3.
1·32 "	2·75.
1·33 "	2·75.
1·34. "	3.
1·36. "	2·75.

Results.

Case. 37.

4:11:42.

E.S.R, of 1 hr. Westergren. 4.

Diameter of Tube.	1 hr.
1· 20. mm.	4.
1.25. "	3.
1·27. "	3.
1·30. "	3·75.
1·32. "	4.
1·33. "	3·5.
1·34. "	2·5.
1.36. "	4.
1·45. "	4.
1·50. "	3·5.
1·55. "	3·5.

Case. 29.

15.10:42.

E.S.R. of 1 hr. Westergren. 86.

Diameter of Tube.	1 hr.
1·27. mm.	42.
1·30. "	42.
1·32. "	43·5
1·34. "	44.
1·36. "	44·5.

These three experiments, though they shew minor variations, do not account for the greater discrepancies remarked upon.

Bore does not seem to enter into the question.

Temperature is known to influence the sedimentation rate. It has to be remember that the wall of these capillary tubes is very thin (almost paper thin) as compared to the wall of the Westergren tube and that the heat insulating effect must be very different to the two tubes. The varying temperature of a consulting room might have an effect.

This was tested by using micro-bore tubes with a thickness of wall corresponding to that of the Westergren tube.

One of these tubes had a bore of 1·07 mm. and an outside diameter of 4·5 mm. The bore was uniform and corresponded fairly closely to the bore of the capillary tubes. I have referred to it in my cases as the Micro-bore Tube.

The other tube had a bore of 0·88 mm. and an outside diameter of 5·3. mm. It is referred to as Veridia. This is the trade name for the precision bore glass tubing made by the Rotameter Co. who made it and two Westergren tubes for me.

The height of column used in both tubes was 100 M.m. Every care was taken in assuring the cleanliness of these and the Westergren Tube. They were washed and dried each time on an Edwards exhaust pump.

In the following tables I have arranged the figures in ascending order. Cases will be found in the Appendix.

Westergren and Micro-bore tube comparisons.

Westergren.	Micro-bore.	Westergren.	Micro-bore.
1.	0·75	19.	18.
1.	1.	22·5.	26.
1·25.	1·25.	23.	18.
2·5.	1·5	24.	26.
3.	3.	27.	27.
3.	3.	30.	32.
3.	2·5.	33.	35.
5.	6.	33.	25.
5.	6.	37.	39.
5.	5.	46.	39.
5.	6·5.	56.	34.
5·5.	5·5	57.	36.
6.	3·5.	59.	36.
6.	4.	60.	45.
7·5.	7·5	69.	47.
9.	9·5.	75.	45.
12.	12:5	76.	46.
13.	14.	83.	40.
15.	15.	98.	57.
17.	17.	108.	51·5.
17.	24.	114.	61.

It is evident that in this series there are also anomalies. A further small series is given of comparisons between the Micro-tube and the veridia tube. The Westergren figures are omitted.

Micro-bore tube.	Veridia tube.
1·5	2.
2·5	3·25.
6·5.	6.
9·5	9·5
12·5	15.
← 18.	8·5
24.	26.
26.	32.
32.	31.
39.	40.
45.	46·5
51·5	52.
57.	57.
61.	61.

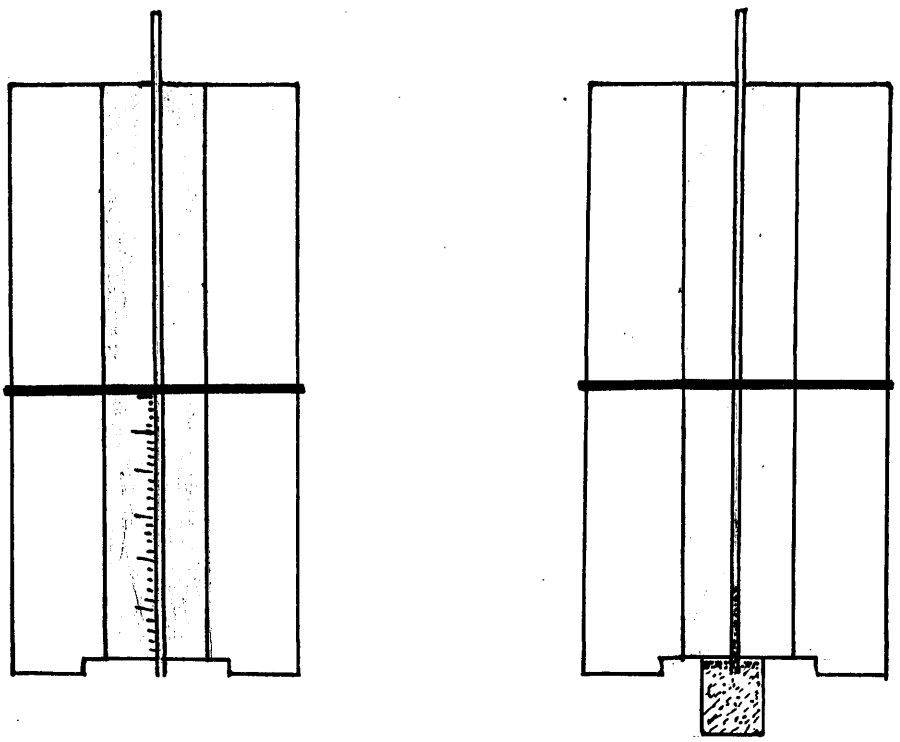
confirmed.

Even in this small series of comparisons between micro-bore tubes there are anomalies in the readings.

Temperature as an influence has, in these observations I think, been to a great extent eliminated.

The presence of foreign material in the tubes may also be claimed to have been dealt with fairly well.

The question of the influence of capillarity on various bloods, in causing these anomalies was considered and was tried in the following way.



A. B.

Full Size.

The simple device shewn above was used. It is made of card - board with a piece about 2 mm. in depth cut out of the foot. On it are pasted two other pieces, leaving a space between them which just takes one of the capillary lymph tubes. One of these pieces has a millimetre scale on it. To use it the tube is placed in the groove and held in place by a thin rubber band. It is then held vertically on the desk and the tube brought

down to desk level.

The small glass container shewn in B. is filled with the citrated blood to within about a mm. from the top and the card held vertically on the top of it as shewn. In a minute or two when the blood ceases to rise in the tube the height is read.

The average rise is about 8 to 9 mm. Observations were made with bloods of varying sedimentation rates but the variations were so slight that no definite conclusion could be drawn.

The one thing obv ious is that these tubes exercise a well marked capillary effect.

Capillarity in its ultimate analysis is a subject which I am unable to deal with, neither can I put forward any explanation of the anomalous readings described, but from my observations I think certain conclusions may be drawn.

The capillary vaccine lymph tubes may be usefully employed in rough experimental work where results can be checked by using more accurate apparatus. They require small quantities of material and so lend themselves to repetitive work. They are inexpensive and the trouble of cleaning apparatus is eliminated by discarding them.

In view of the inexplicable anomalies found with them and with the other micro-bore type of tubes it appears to me that the micro-bore type of tube is unsuitable for keeping

serious records or where different observers are recording their results.

The Westergren tube has stood the test of many years and is probably the best compromise between the smallest quantity of blood required and the reduction of the effects of capillarity.

VISCOSITY

Viscosity.

It seems natural in an inquiry such as this, to consider whether the viscosity of the plasma has any effect on the sedimentation rate.

The membrane and cytoplasm of the erythrocyte have their own viscosities, but this is not considered here.

The viscosity of colloidsolutions is a difficult subject which does not even yet appear to be thoroughly understaod.

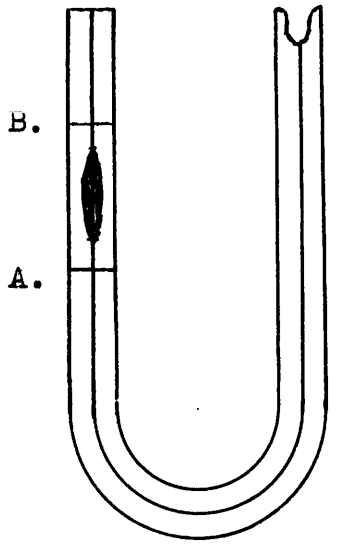
There is an interesting note in "Advances in Enzymology, Henry B. Bull." 1941. It is stated "Viscosity seems to depend to some extent on the asymmetry of the protein molecules."

This may have a bearing on fibrinogen and will be discussed later.

The relative viscosities of water and plasma are given as 1 to 1·7 to 2. (Thorpe.) Plasma here I take to be actual undiluted plasma.

In my experiments the plasma was that obtained from citrated blood; one of citrate to four of blood. The dilution of the plasma by the citrate will presumably reduce its viscosity.

Not having facilities for the proper scientific estimation of viscosity, I fell back on one of the dimple ones used for estimating the viscosity of whole blood.



Viscosimeter of Denning and Watson.

Above is a representation of the viscosimeter tried. It consists of a U shaped tube with a hair like bore. At the top of one end is a cup shaped opening for receiving the blood ; in the other limb there is a dilated portion of the bore with a mark above and below it.

The time taken by the blood to pass from the lower to the upper mark is taken as a measure of the viscosity of the blood as compared with that of water.

My tube was marked 3·5 secs. ; the time taken by normal blood. The relative viscosity of plasma and whole blood is given as 1·7 to 2 and 3·6 to 5·4. say roughly one to three.

The citrated plasma may be taken as somewhat less.

This meant that the citrated took about a second to pass between the marks. What between getting the plasma into the tube with hand and using a stop watch with the other the rapidity of the flow precluded any accurate observation.

It was then thought that if particles could be got, such as would sediment cleanly and in a reasonable time, these might be used to compare their sedimenting time in different plasmas and so might be used as a measure of the relative viscosities.

Several substances were examined.

Mag. carb.levis sedimented well in water and in a fairly reasonable time but the particles were difficult to colour.

Red precipited Ferric oxide and calcined Ferric oxide were both tried but no amount of levigation would give a particle with the desired qualities.

Kaolin was next dried. Even with a considerable amount of levigation there was still some cloudiness in the upper layer. It had the advantage that it coloured well with methylene blue, the negative charge on its particles absorbing the positive ones of the Methylene blue. This presumably rendering the particles neutral.

Some experiments were done with this and though not very conclusive, are detailed later.

It was then considered if it might be possible to use red cells themselves, preserved in some way, say with mercuric perchloride. A variation of this suggested itself through the knowledge that the red cells are agglutinable by the action of Tannic acid and the following procedure was adopted.

To about 12 cc. of a suspension of red cells in normal saline were added about ten drops of a freshly prepared 5% solution in saline of Tannic acid. The Tube was shaken vigorously to break up the larger clumps and allowed to a sediment. The supernatant fluid was pipetted off and the cells washed free of the Tannic acid by centrifuging gently several times in saline.

A saturated solution of Mercuric perchloride was then added and the cells shaken up in this. After about half an hour the cells were washed in several changes of saline and then kept in this. They keep well for a long time.

In use, when a suspension of them is centrifuged, they form a firm deposit at the bottom of the tube and the supernatant fluid can be poured off.

These preserved red cells give a colourable imitation of the clumps seen in high sedimentation rates.

Yeast cells being about the same size as red cells and more easily obtainable were considered as a substitute but it was/

was found that they were difficult to colour and curiously enough they did not agglutinate even with strong concentrations of Tannic acid. The membrane of the yeast cells evidently differs in its make up from that of the red cell. That yeast cells can agglutinate, is shewn by the fact that in brewing, after a certain stage, the yeast cells agglutinate and fall to the bottom. The ultimate cause of this agglutination is, like that of red cells, still imperfectly known.

A standard was thought of with which to compare the results obtained with various plasmas and two colloid solutions were tried.

1% solution of gelatine was unsatisfactory. ½% was the same. The preserved cells hung in the solution and did not sediment.

5% solution of gum arabic was unsatisfactory for the same reason.

Instead, pooled plasma was used.

Experiment. 1.

Case 48. E.S.R. 1 hr. Westergren. 15

Two capillary tubes were put up with A.B.Plasma and Kaolin suspension; two of plasma to one of Kaolin suspension.

Two others similarly but with patient's plasma. Sedimentation of the heavier particles took about two hours to attain a clear line of demarcation. Upper haze took about 24 hours to clear.

Result inconclusive. So far as could be judged the rate of fall seemed to be the same in each case.

One difficulty and possible source of error was, that in mixing plasma and suspension in the watch glasses, even in the short interval between mixing and filling the tubes, there was a certain amount of sedimentation of the larger particles.

Experiment. 2.

Case. 8. E.S.R. 1 hr. Westergren. 56.

Equal parts of patient's plasma and Kaolin suspension.
Equal parts of B. plasma "
Capillary tubes used.
Patient's put up 6.45 P.M. read 10.25 P.M. = 4 mm.
B. plasma " 6.46 " 10.39 " = 3·5 mm.

In both tubes there was a good deal of haziness above the solid deposit.

One or two experiments of a similar nature were tried and with somewhat similar results. As far as could be judged there was no difference but the results were considered as being unsatisfactory.

A point of interest was that after the tubes had stood for about a week, the upper part of the deposit became white. This, when treated with Hydrogen peroxide, regained its blue colour. Effect of light or vitamin C ?

Experiment 3.

Case. 58. E.S.R. 1 hr. Westergren. 91.

10 cc. of preserved cell suspension were divided into two equal parts and centrifuged. This left about 0·75 cc. of cells in each tube.

To one were added 2 cc. of the patient's plasma, to the other, 2 cc. Group B. pooled plasma.

Westergren tubes, 200 mm. column used.

Result.

Sedimentation.	1 hr.	2 hrs.	6 hrs.
Patient	6.	20.	100
Pooled plasma	5.	20.	100.

The only difference is that the patient's tube sediments a little more cleanly.

Experiment 4.

Case. 59. E.S.R. 1 hr. Westergren. 78.

About 0·3 cc. of cells was used this time and 2 cc. each of the plasma. A.B. plasma was used,

Westergren tubes. 200 mm. column.

Result

	1 hr.
Patient.	10.
A.B.plasma	12.

Both hazy; no clear line of demarcation.

	4 hrs.
Patient.	162.
A.B. Plasma	See note.

In the patient's tube there was a fairly well marked line of demarcation. In the A.B. tube there was haze, shading from top to bottom. No line of demarcation.

Experiment 5.

Case. 54. E.S.R. 1 hr. Westergren. 75.

Same procedure but A. pooled plasma used. 2 cc. of plasma to about 0·5 cc. of cells.

Result.

	1 hr.	1½ hour.	2 hrs.
Patient	72.	86.	93.
A. plasma	50.	66.	78.

The A. plasma tube was hazy and the readings were an estimate.

At 1½ hours. the patient's tube shewed a clear line.

Experiment. 6.

Case. 29. E.S.R. 1 hr. Westergren. 22·5.

In this case only 0·9 cc. of the patient's plasma was available and with about 0·25 cc of cells was used.

Pooled A.B. plasma was used.

The Westergren tubes were filled to a column of 170 mm.

Result.

Sedimentation.	1 hr.	1½ hours.	2 hrs.
Patient	64.	75.	85.
A.B.Plasma.	69.	87.	95.

These readings are the actual readings on the tubes.

The result of these various experiments are, I think, inconclusive.

There are one or two points to be considered.

I am not aware to what extent the sedimentation of particles in a colloid solution may be a measure of its viscosity. The preserved red cells mentioned, sediment faster in water than in plasma, in a ratio much more than the figures given as their relative viscosities.

In comparing different plasmas this might not matter but there are other consideration. For instance what is the charge on the red cells?

In/

In the Kaolin experiments, the negative charge on the Kaolin particles may be presumed to be neutralised by the positive charge on the methylene blue but there is the shape of these particles to be taken into account as well.

Microscopical examination shews them to be rather jagged crystals and this might well have a certain effect.

FIBRINOGEN.

Fibrinogen.

Of all the constituents of the plasma, the one which is regarded as having the most influence on the variation of the sedimentation rate, is fibrinogen, though as yet its mode of action is unknown.

Fibrinogen is one of the long molecule proteins and has some interesting relationships.

"When we consider that the fibrous proteins of the epidermis, the keratinous tissues, the chief muscle protein, myosin, and now the fibrinogen of the blood, all spring from the same peculiar shape of molecule and are therefore probably all adaptations of a single root idea, we seem to glimpse one of the great co-ordinating facts in the lineage of biological molecules".

(Astbury et alt. "Nature", June 26th. 1943.)

I take two other statements.

"Tissue injury and inflammation stimulate fibrinogen production".

(Thorpe)

"The main factor producing an increased sedimentation rate -------- is the extensive destruction of body cells which occurs in infections and toxic conditions".

(Whitby and Britten)

These may be linked together by the statement, that in most of these conditions there is wasting of muscles. The break -

down of muscle cells into their elements must lead to the liberation of the basic molecules referred to by Astbusy and it is an interesting speculation as to whether these molecules are utilised in the formation of the extra fibrinogen which is found in the plasma of these cases.

The latest work which I can find bearing on the action of fibrinogen as affecting the sedimentation rate is a paper by C.M.Gordon and J.R.Wardly, entitled "The Effects of the Plasma Proteins upon the Sedimentation of Human Blood".

(The Biochemical Journal. Sept. 1943.)

This is a very thorough and ingenious investigation and must have meant great labour.

I quote.

"From the work of Fahraeus (1929), Tiffeneau and Gysin (1937) and Fraser and Rennie (1941), it is known that the rate of sedimentation is determined by the protein constituents of the plasma, but no clear correlation has been yet found between the observed rate and the quantities of the various fractions."

"In this paper the problem has been tackled by building up pathological plasma with protein fractions isolated from normal plasma and observing the behaviour of normal cells suspended in them".

"The experiments were done in Westergren type tubes of 200 mm. column and 2·5 to 3 mm. bore."

Summary.

"1. The proteins from normal plasma were separated into 9 fractions, and the rate of fall of a 20% vol. of erythrocytes in 3% solutions of the proteins ~~during~~ was found to range from 100 mm. for fibrinogen to 1.5 for total albumin.

"2. The fractions were combined in equal amounts using 39 pairs. The action of the faster fractions, fibrinogen and euglobulin, is inhibited by the slow nucleo-proteid and globoglycoid.

"3. Artificial pathological plasma built from these fractions gave sedimentation rates similar to those of comparable material."

Discussion.

"From this series of experiments we conclude that the power of sedimentation is not controlled by the absolute concentration of either the total plasma proteins or the protein fractions but by the inhibition of one protein by another".

Which so far as the question of the ultimate mechanism of sedimentation goes, seems to me to leave the matter where it was.

Some remarks may be made on this paper.

With regard to the separation of the plasma protein fractions and their building up again into other plasmas, this may not be quite so simple a matter as it looks.

"It is highly probable that in the living animal there is only one plasma protein which is a labile complex unit dissociated into commonly isolated proteins by physico-chemical treatment such as salting out".

"Whatever the true nature of the proteins of serum, recent work leaves no doubt that we must consider them as a liable system in a delicately balanced equilibrium and not as several distinct and stable chemical individuals mixed in solution".

(Thorpe.)

In view of this statement it is difficult to comprehend Para. 3. of the above summary. Plasma built up from fractions can hardly be the same as actual plasma found in vivo, and yet they are said to give sedimentation rates similar to those of comparable natural plasmas.

One would have liked if the Authors had discussed certain other matters; for instance, the effect of the presence or absence of agglutinins, specific or otherwise, in the built up plasmas.

A more important omission to my mind is the absence of any discussion as to the part played by the red cells.

It is merely stated that washed cells were used. There is no mention as to how often they were washed or to their physical condition after. Were they crenated? It is known that other less obvious changes occur. For instance agglutinogens may be partially removed or their action impaired.

An indication as to whether aggregation of the cells took place during the more rapid rates would have been of interest.

Apart from these remarks, what struck me was the fact of the rapid sedimentation which took place in the fibrinogen fraction solution though the strength of this was ten times that of normal plasma - 3% as against about 0·3% for normal plasma.

There has been some difference of opinion as to the correlation between the amount of fibrinogen present in the plasma and the sedimentation rate.

"Gilligan and Ernstone (1934) and Oakley (1938) have observed a close relation between plasma fibrinogen and the rate of red cell sedimentation; this is the commonly accepted explanation of increased sedimentation though Brown and Munro 1934 found no relation at all between plasma fibrinogen plasma content and sedimentation rate."

(Whitby and Britten.)

To satisfy myself on this point I made a series of estimations of fibrinogen content and the corresponding sedimentation rates.

I give particulars of the method adopted and the results.

My first estimations were made by the biuret method, using the Lovibond Comparator. Here the fibrinogen is obtained from the plasma by a method which will be described later, and dissolved in Sodium hydroxide solution and then a solution of copper sulphate added.

The resulting precipitate is brought down in the centrifuge and the supernatant coloured fluid compared with the colour discs in the Comparator.

This method I found unsatisfactory; the biuret colour seemed to vary and the final shade was so light that comparison was difficult. My results were obviously not right.

The Tintometer people say themselves in their description of the method "Though clearly it cannot give quite the same degree of precision as the use of a good quality plunger type colorimeter ------ ".

A colorimeter was next used. The instrument is a Klett-Bio colorimeter using 5 cc. cups. A substage illuminator which provides neutral light was used in all estimations.

This instrument is illustrated and described in Cole's Practical Physiological Chemistry. 9th. edition. 1941.

The method adopted was that of Wu as described in Cole 7th. edition. 1926,

The standard used is Tyrosine, This is dissolved in N/10 Hydrochloric acid and contains 0·2 mg. tyrosine in 1 cc. The plasma is obtained by centrifuging the citrated blood and

pipetting off.

1 cc. of plasma is added to 28 cc. of normal saline and mixed. 1 cc. of 2·5% solution of Calcium chloride is added, mixed and allowed to stand for twenty-minutes. (In the Biuret method it is advised to keep 37°C. for half an hour.)

In my estimations it was kept in the incubator at 37°C. for one hour in all cases.

This was done in a large boiling tube. After standing for the hour, the tube is shaken slightly and a fine glass rod is introduced. A gentle whirling motion is imparted to this and the fibrin adheres to it. Any which does not is easily picked up. It is allowed to drain for a little and is then slipped off on a folded filter paper.

The fibrin is transferred to a 15 cc. centrifuge tube containing 4cc. of Sodium hydroxide solution (1%). The tube is placed in a beaker of boiling water and the contents stirred until the fibrin is completely dissolved. Water to 10 cc. is added and mixed. If solution is not clear it is centrifuged.

The supernatant fluid is transferred to a 25 cc. volumetric flask labelled F. and cooled under the tap. 1 cc. of 5% Sulphuric acid is added.

To another 25 cc. flask labelled S. is measured 1 cc. of the standard tyrosine solution and 15 cc. of water.

To each flask is added 0·5 cc. of Wu's reagent and 3 cc. of saturated solution of Sodium carbonate. (In my estimations

I used Folin and Ciocalteu's reagent which is somewhat similar to Wu's but keeps better.)

The flasks are shaken and made up to the 25 cc. mark with water. They are allowed to stand for fifteen minutes or until the colour develops. In my case they were allowed to stand for about an hour.

The solutions are placed in the colorimeter cups and the tyrosine standard is set at 20 mm.

Calculation.

1 mgm. tyrosine = 16·4 mgm. of Fibrin.

If standard is at 20 and the fibrin reads at F mm. then an amount of fibrin in 1 cc. = $\frac{20}{F}$ x 0·2 x 16·4. mgm.

So gms. in 100 cc. = $\frac{6·56}{F}$

In all my estimations I have taken the average of ten readings.

The following are the results.

Note. The correcting factor of $\frac{5}{4}$ had of course to be used.

1 hr. Westergren Readings and Fibrinogen Content.

		1 hr. Westergren.	Fibrinogen.
Case. 4.	30:12:42.	23.	0·47%.
" 8.	27: 8:42.	75.	0·7%.
" ".	27: 9:42.	73.	0·52%
" ".	18:11:42.	59.	0·5%
" ".	25: 1:43.	57.	0·48%
" ".	6: 5:43.	69.	0·6%
" ".	23: 6:43	56.	0·51%
" 15.	11: 6:43.	6.	0·37%
" 16.	28: 8:42.	16.	0·31%
" 20.	17: 5:42.	33.	0·66%
" "	14: 9:42.	37.	0·66%
" "	30:11:42.	33.	0·4%
" "	4:10:43.	17	0·41%
" 29.	15:11:42.	76.	0·49%
" "	1: 8:43.	22·5.	0·36%
" 31.	26: 8:42	3.	0·32%
" 34.	18: 9: 42.	11.	0·56%
" 35.	19: 9: 42.	7.	0·5%
" 39.	5:12:42 .	1·25	0·31%
" "	28: 4:43.	1.	0·31%
" "	2: 2:43.	1·75.	0·27%
" 41.	20.: 4:43.	5.	0·28%

over

1 hr. Westergren Readings and Fibrinogen Content. (cntd).

		1 hr. Westergren.	Fibrinogen.
Case. 42.	22: 4:43.	9.	0·37%
" 43.	27: 4:43.	1.	0·26%
" 44.	30: 4:43.	1·25.	0·31%
" 46.	9: 5:43.	19.	0·45%
" 47.	11:5: 43.	5.	0·27%
" 48.	15: 5:43.	5.	0·35%
" 49.	18: 5:43.	13.	0·37%
" "	29: 9:43.	12.	0·33%
" 50.	25: 5:43.	5·5.	0·32%
" 51.	12: 6:43.	3.	0·28%
" 52.	16: 6:43.	17.	0·33%
" 53.	13: 7:43.	15.	0·3%
" "	18:12:43.	5.	0·27%
" 54.	11: 8:43.	75.	0·41%
" "	6: 9: 43.	30.	0·36%
" 55.	18: 8:43.	3.	0·25%
" 56.	5: 9:43.	108.	0·71%
" "	25: 9:43.	114.	0·68%
" " "	26:11:43.	83.	0·67%
" 57.	21: 9:43.	24.	0·37%.
" 60.	16:12:43.	2·5.	0·32%
" 61.	18: 1:44.	23.	0·45%

On the following page is given a comparison of these 1 hr. Westergren readings and the respective fibrinogen contents in the ascending order of the Westergren readings.

Westergren.	Fibrinogen.	Westergren.	Fibrinogen.
1.	0·31%	17.	0·33%
1.	0·26%	17.	0·41%
1·25.	0·31%	19.	0·45%
1·25.	0·31%	22·5.	0·36%
1·75.	0·27%	23.	0·45%
2·5.	0·32%	23.	0·47%
3.	0·32%	24.	0·37%
3.	0·28%	30.	0·36%
3.	0·25%	33.	0·4%
5.	0·28%	33.	0·66%
5.	0·27%	37.	0·66%
5.	0·35%	56.	0·51%
5.	0·27%	57.	0·48%
5·5.	0·32%	59.	0·5%
6.	0·37%	69.	0·6%
7.	0·5%	73.	0·52%.
9.	0·37%	75	0·41%
11.	0·56%	75.	0·7%
12.	0·33%	76.	0·49%
13.	0·37%	83.	0.67%
15.	0·3%	108	0·71%
16.	0·31%	114.	0·68%

Though this is not a large series, I think it is sufficient to show fairly clearly that there is no direct correlation between the sedimentation rate and the amount of fibrinogen present in the plasma.

The higher rates are accompanied by an increased fibrinogen content, sometimes to almost double the normal amount, but this in itself of course can not be accepted as a proof that the fibrinogen, per se, is the cause of the higher rates.

The lack of a clear correlation between the two would also point to a somewhat similar conclusion.

The average fibrinogen content of normal blood is given between various limits.

Thorpe gives the figures 0·2 to 0·4%.

Taking up to six as the upper normal E.S.R. average limit in men and women then the average of my figures, up to this, works out at 3%.

In the course of my investigation I have had the privilege of being supplied with citrated plasma by Professor D.F. Cappell of University College, Dundee. The proportion of citrate in this has been the same as in the Westergren technique.

I give the results of the estimation of the fibrinogen contents of these plasmas.

A.B. plasma from two donors.	25:11:42.
A. and O. pooled plasmas	25: 5:43.
B. pooled plasmas	15: 6:43.

Results.

Colorimeter readings.

A.	B.	A.B.	O.
33·7.	25·7.	33·7.	23·8.
33·4	25·2.	33.	24·2.
33·5.	25·3.	32·3.	24·2.
33·3.	25·7.	32.7.	24·3.
33.	25·5.	32·-	24·2.
33·1.	25·2.	31·5.	24·3
33·3.	25·5.	32·-	24·4.
33·2.	26·6.	32·1	24·1.
33.	25·1.	31·8	24.
33·2	25·2.	32·1	24.
332·7.	255·0.	323·2.	241.5.

Averages.

33·3.	25.	32·3.	24.

Fibrinogen. Corrected.

0·25%,	0·31%.	0·25%	0·33%

Average of the four plasmas.

0·28%

From the above figures and my own cases it would appear that the average fibrinogen content of plasma (normal) is about 0·3%.

In the following are details of a few experiments done on the influence of fibrinogen in sedimentation.

Experiment 1.

Case. 29.

19:7:42.

Case of tuberculous pleurisy.

Blood was taken in citrate from one arm for the Westergren test. From the other arm was taken actual blood. (4 cc.)

This was whipped with a pipe cleaner and the fibrin removed.

Result.

E.S.R.	1 hr.
Citrated Blood.	
Westergren tube.	96.
Capillary tube. 1·4 mm. diam.	42.
Defibrinated blood.	
Westergren.	43.
Capillary tube. 1·3 mm. diam.	4·5.

Something seems to have gone wrong with the capillary tube.

Comparison of the two bloods put up in capillary tubes of opsonic index type (70 mm. column.) and kept in incub ator for one hour at 37°C.

Result.

E.S.R.	1 hr.
Citrated blood	47.
Defibrinated blood.	32.

The defibrinated blood shewed a little lysis due to

too prolonged whipping.

The opsonic index pipettes shew the influence of temperature.

I can not make out from my notes whether I made up the defibrinated blood with citrate to a comparable proportion or not,but in any case the results of the Westergren tests seem too greatly different for this to have made much difference.

It would seem from this experiment that the presence or absence of fibrinogen has made a difference.

Case. 29.

5:7:42.

E.S.R. 1 hr. Westergren. 95.

Pleural fluid was aspirated from this case. It was light amber in colour and clear. Part was retained in its original condition,part was treated with 3·8% citrate - one of citrate to four of fluid.

Original fluid. p/H. 7·6.

Citrated = p/H. 7·6.

The red cells were retained,washed in saline and used in the following experiment. They were put with various fluids,about one of cells to three of the particular fluid.

Capillary lymph tubes were used. 70 mm. column.

Results.

E.S.R.	1 hr.
A. Original citrated blood.	42·5.
B. Normal saline.	2.
C. Original pleural fluid less clot.	1.
D. Citrated pleural fluid.	15.
E. Plasma heated for 1 hr. at 60°C.	49.

C. This tube shews indication of some agglutination graduating from top to bottom.

D. Top 1 mm. clear, next 14 mm. a little hazy.

E. Appearance of slight agglutination in upper part of tube.

Microscope examination after 6 hours.

A. Cells irregular in shape, some crenated. Good deal of pseudo-agglutination.

B. Cells discrete, globular crenated.

C. Some small deformed rouleaux. Some discrete cells with clear outline, no bi-concavity on top view, a little on side view, Some pseudo-agglutination.

D. Some cells round clear outline, others globular crenated. No, bi-concavity. Some pseudo-agglutination.

It would appear that the fall, 15 mm. with the citrated fluid as compared to that of the fluid less clot, 1 mm. might be due to the presence of fibrinogen.

The case of the heated plasma is a little difficult to understand. Fibrinogen is coagulated at 56° C. one would

have expected a reverse effect.

A point of interest is the effect of the pleural fluid on the red cells. As with the hydrocoele fluid previously mentioned, the crenated globular cells are restored to shape and even the formation of roleaux takes place.

In another case of simple pleural effusion my experiment went astray.

I collected some of the fluid in a Heparinised tube to prevent the separation of the fibrinogen, but it had no effect. The fibrin clotted just the same.

I wrote B.D.H. about this and they replied:-

"We would now inform you that we understand the mechanism of coagulation of pleuritic fluid differs materially from that for the coagulation of blood, and that although Heparin is effective in inhibiting the coagulation of blood it is almost useless for inhibiting coagulation of pleuritic fluid."

An interesting point in connection with fibrinogen is the fact that it can be absorbed from plasma by Kaolin.

It is said that 6 to 10 g. of Kaolin absorbs practically all fibrinogen from 100 cc. of plasma.

What happens here is not exactly known. Is it that the fibrinogen molecule carries a positive charge? Or is it due to the physical character of the long chain molecule of the fibrinogen as opposed to the round molecules of the other

Proteins? Or is there an enzyme system attached to the fibrinogen?

Some time before I was aware of this fact I had done the following experiment.

Experiment. 3.

Adsorbment experiment with B,, plasma.

One part of colloidal kaolin was mixed with two parts of B. plasma (by volume) and allowed to stand at room temperature for two hours. the mixture was then centrifuged and the clear plasma pipetted off.

Capillary lymph tubes 1·36 mm. outside diameter were used. These were marked at 70 mm. and the average of two tubes was taken in each experiment.

0·5 cc. of 3·8: citrate solution was mixed with 0·2 cc. of my own blood and 0·2 cc. of the Kaolin treated plasma, in a watch glass and two tubes put up.

Sume procedure but with untreated plasma.

Result.

	1 hr.
Untreated plasma	34.
Kaolin plasma.	40.

Same procedure but B. plasma was treated with activated charcoal.

Result.

	1 hr.
Untreated plasma	41·5.
Charcoal plasma.	42·5.

Same procedure but B . plasma treated with Alumina.

Result.

	1 hr.
Untreated plasma	38·5.
Alumina plasma	38.

My own blood group is A. and agglutination was obvious in the mixtures in the watch glasses in all cases.

Agglutinin is evidently not adsorbed by any of these agents. These capillary tubes give somewhat uneven results but allowing for this it is interesting to note that in the case of the Kaolin treated plasma, the rate was somewhat similar to the untreated plasmas though a good deal of the fibrinogen must have been removed.

Experiment. 4.

30:9:43.

In this and the following experiment preserved red cells (see Section on Viscosity) were used.

B. group plasma (pooled) was treated with Kaolin for fifteen minutes and the mixture then centrifuged. The clear Plasma was pipetted off.

5 cc. of suspension of preserved cells were placed in each of two centrifuge tubes and centrifuged and the supernatant fluid poured off.

To the deposit in one was added untreated plasma and to the other an equal amount of defibrinated plasma.

The proportion of cells to plasma-was about one to five. The tubes used here were 1·3 mm. bore with a column of 200 mm.

Result.

	1 hr.
Untreated plasma.	5.
Defibrinated plasma.	65.

Experiment. 5.

10:10:43.

Same procedure with group B. plasma and preserved cells but using Veridia tubes of 2·4 mm. bore and column of 200 mm.

Result.

	1 hr.
Untreated plasma.	5.
Defibrinated plasma.	86.

Experiment. 6.

9:10:43.

B. group plasma untreated and defibrinated, was used with my own cells which were washed three times in saline.

The proportion was about one of cells to five of Plasma.

Tubes used were Veridia, 2·4 mm. bore, 200 mm. column.

Result.

	1 hr.	2 hrs.	6 hrs.	24 hrs.
Untreated plasma	4.	12.	49.	90.
Defibrinated plasma.	1.	3·5.	13.	40.

My group is A. and so a much faster rate might have been anticipated. Contrast the rate in experiment 3.

Is this due to agglutinogen being removed or its action weakened by washing the cells?

Experiment. 7.

24:2:44.

10 cc. of my own washed cells in saline were divided into two portions of 5 cc. each and centrifuged in separate tubes. After pipetting off the supernatant fluid this gave 0·6 cc. of cells in each tube.

Defibrinated A.B.plasma and untreated A.B. plasma were used. Equal parts of each - 3·2 cc. - were added to each of the tubes containing the cells and thoroughly mixed.

The mixtures were put up in the testing tubes described in the Section on Enzyme action and with a column of 75 mm.

Result.

	1 hr.	4 hrs.
Untreated plasma	1.	4.
Defibrinated plasma	2.	6.

It is a question as to how much the red cell is altered by repeated washing. With a few washings it may become crenated. Even if it retains its shape there may be changes in its constitution, unrecognisable by ordinary methods.

In the following experiment cells taken direct from citrated blood were used.

Experiment. 8.

14:3:44.

1 cc. of 3·8% citrate to 4 cc. of my own blood was used. This was divided into two portions of 2·5 cc. each. which were placed in two centrifuge tubes and centrifuged. The supernatant plasma was pipetted off, leaving in each tube 1·2 cc. of red cells.

To one tube were added 2·8 cc. of untreated A.B. plasma. To the other 2·8 cc. of defibrinated A.B.plasma.

Part of these mixtures were tested in the testing tubes described in the Enzyme Section and part in tubes of 1·3 mm.

The column in the testing tubes was 70 mm. and in the others 200 mm.

Result.(Testing tubes.)

	1 hr.
Untreated plasma.	1.
Defibrinated plasma.	1.

over

Result. (1·3 mm. bore tubes.)

	1 hr.	2 hrs.	3 hrs.	4 hrs.	15hrs.
Untreated plasma	1.	1.	1·5.	2.	6.
Defibrinated plasma	1.	3.	4.	6.	23.

These last three experiments with washed and unwashed cells are in fairly close agreement and shew ,that if anything, the presence of fibrinogen exercises a retarding effect on sedimentation.

More strikingly is this effect shewn in experiments4. and 5. where preserved red cells were used.

The results in experiments 7. and 8. are in rather striking contrast to those of Gordon and Wardly previously mentioned but I am rather more inclined to reply on my own.

Their results were obtained with artificial build ups, mine with natural plasma; even the defibrinated plasma is probably more natural.

Recalling the note mentioned in the previous section,

"Viscosity seems to depend to some extent on the asymmetry of some of the protein molecules."

(Advances in Enzymology).

This would seem to fit in with the results given above, Presumably the long fibre molecule of fibrinogen will shew some degree of asymmetry as compared with the round or globular molecules of the other proteins of the plasma.

Considering this last fact, the results of experiments 7. and 8. and the lack of correlation between the figures given by me of the comparison between sedimentation rates and fibrinogen content, I am left with the feeling that too much has been attributed to the effect of fibrinogen and that the increased content in the plasma of bloods shewing increased sedimentation rates is merely incidental.

THE ERYTHROCYTE.

It will be noticed that in some of the cases reported, I have typed the blood group of some of the patients. This was done in case I might want to make use of the plasma or the corpuscles and because of a lesson I learned from one of my earlier experiments.

This arose out of my interest in case. 8. in whose blood there was present a high degree of haem-agglutination.

I thought it might be interesting to see the effect of inter-mixing the cells and plasma of this case with the cells and plasma of two other cases.

The cases were,

Case. 8. E.S.R. 1 hr. Westergren. 116.
Case 10. E.S.R. " 6·5.
" 11. E.S.R. " 45.

Experiment 1.

One part of washed cells to three of plasma was used and put up in capillary tubes - 70 mm. column.

Result.

				1 hr.
Case. 10.	Cells with	Case. 8.	plasma	0·5.
11.	"	" 8.	"	46.

This result was a little puzzling until it was remembered about blood groups. These were determined.

Case. 8. Group. O.
" 10. " O.
" 11. " A.

Later, on reading Fahraeus' original work, "The Suspension Stability of the Blood" 1918, I found that I had erred in good company though more inexcusably.

I noticed that he had a similar experiment in which he intermingled the cells and plasma of three men and three women and from the absence of any reference to blood groups it is evident that at that time, Fahraeus was unaware of that factor.

A perusal of this work gives one an idea of the intricacy of the mechanism involved in sedimentation rates.

The erythrocyte is a living entity, Its life has now been extended to about one hundred days. It is a very complicated piece of mechanism, if one may be allowed to apply that term to a vital structure.

Its membrane is an involved structure and the cytoplasm is an elaborate affair holding the haemoglobin and possessing various enzyme systems.

Given fairplay in the matter of isotonicity and excluding anything deleterious in the medium in which it is placed, I have been struck with the fact of how, in spite of rough usage, it retains its integrity.

The fact of its vitality, as in other forms of living orgamisms, introduces a certain element of indeterminancy into any investigation regarding its behaviour.

The cell wall enters into the problem of sedimentation, though it is probable that the cytoplasm also may play a part in altering the condition of the cell membrane.

The structure of the cell membrane is still not yet fully understood.

I quote.

"Research in this field consisted in forming monolayers at interfaces composed of substances which exist in natural membranes and injecting into the underlying solution substances which are known to react with these membranes.

"It was quickly seen in seeking for biological analogies between the monolayer reactions and biological reactions that reactions taking place at cell surfaces were good examples, such as the cytolysis of red cells and unicellular animals.

"All compounds examined that penetrate cholesterol monolayers or disperse protein films haemolyse, and all compounds that only adsorbed on to protein films without dispersing them, agglutinate red cells.

"From results obtained it could be supposed that, since substances which penetrate cholesterol films and protein films are haemolytic and the substances which only adsorb on to protein films agglutinate red cells, there must be cholesterol and protein available on the cell surface. It could thus be imagined therefore that the cell surface was a mized protein cholesterol film"

(Cytology and Cell Physiology).

Astbury believes that "--- the protein of the red cell membrane may also belong to the collagen group of fiber proteins".

(Advances in Enzymology. Henry Bull.)

These are long fiber molecules which may be akin to, if not actually fibrinogen.

Astbury states "The appearance of long straight fibres radiating from corpuscles in the blood clot strongly suggests that they are formed primarily by end to end accretion of fibrinogen units"

(Nature, June 26, 1943)

This would suggest the presence of fibrinogen molecules in the cell membrane on to which the fibres accreted.

In addition to glutathione, part of whose function is known there is also present in the red cells another substance -ergothioneine - which contains the sulphur molecule (14% S) and about whose function I can find no mention.

(Cole, Practical Physiological Chemistry)

It is an interesting speculation as to whether some of the sulphur molecules are utilised to form cross linkages with the long fibrinogen fibres, such as occurs in the vulcanisation of rubber, thus giving additional strength and elasticity to the cell membrane.

There are two points which have struck me about the condition of the red cell in relation to the question of varying sedimentation.

Firstly, there is the suspension stability of mixtures of red cells and isotonic fluids or plasma or serum.

Secondly, there is the contrasting effect of agglutination in upsetting this stability and causing sedimentation.

Though the suspension stability of the mixtures mentioned above is fairly well known, it may be of interest if I give a few examples which I have done.

Where capillary tubes are used, these have a column of 70 mm, and the time interval is one hour.

The blood was collected as for the usual Westergren test and the cells washed three times in normal saline. The cells were used in the proportion of, roughly one of cells to three of the fluid.

Experiment. 2.

Case. 16. E.S.R. 1 hr. Westergren. 22.

In saline in capillary tubes of different diameters.

1·4 mm. diameter.	1.
1·45. "	1.
1·6 "	1.
1·7 "	1.

Experiment. 3.

Case. 16. E.S.R. 1 hr. Westergren. 22.

Cells in different strength saline.

0·45% Saline.	1.
0·6 "	1.
0·75 "	1.
Modified Ringer Soln.	1.

Experiment. 4.

Case. 15. E.S.R. 1 hr. Westergren. 32.

Cells in normal saline in capillary tub e.	0.
" Hydrocoele fluid "	1.

Experiment. 5.

Case. 8. E.S.R. 1 hr. Westergren. 86.

In saline in capillary tube	1·25.
In Hydrocoele fluid "	2,

Experiment. 6.

Case. 29. E.S.R. 1 hr. Westergren. 85.

In saline in capillary tubes	2·5.
In Hydrocoele fluid "	1.
In Pleural fluid from this case less some separated fibrin.	1.

Note. The Hydrocoele fluid was collected in a sterile flask and some of it was preserv ed in ampoules which I made for the purpose.

Before use I tested it for the presence of agglutin and found it free.

An effect of this fluid on the red cells will be discussed later.

In the following experiments A.B. plasma was used. This plasma I received from Professor D.F.Cappell,University College, Dundee. It was in the proportion of one of citrate to four of blood. With it, of course,could be used the corpuscles of any group.

In these experiments a Westergren tube and a micro-bore tube of 1·67 mm. bore and a column of 100 mm. were used.

Experiment. 7.

Case. 20. E.S.R. 1 hr. Westergren. 33.

Cells in A.B. Plasma.

Westergren tube.	0·25.
Micro-bore tube	0·25.

Experiment. 8.

Case. 8. E.S.R. 1 hr. Westergren. 57.

Cells in A.B.plasma.

Westergren tube.	0·25.
Micro-bore tube.	0.25.

Experiment. 9.

Case. 4. E.S.R. 1 hr. Westergren. 13.

Cells in A.B. plasma

Westergren tube	0·25.
Micro-bore tube	0·25.

Experiment. 10.

Case. 41. E.S.R. 1 hr. Westergren. 5.

Cells in A.B. plasma.

Westergren tube	0·25.
Micro-bore tube.	0·25.

Experiment. 11.

Case. 42. E.S.R., 1 hr. Westergren. 8.

Cells in A.B. plasma.

Westergren tube	0·25.
Micro-bore tube	0·25.

Experiment. 12.

Case. 43. E.S.R. 1 hr. Westergren. 1.

Some cells in A.B. Plasna, some in anti-diphtheria serum. Both in Westergren tubes.

With A.B. plasma.	Nil,
With anti-dip. serum .	Nil.

The "anti-dip." serum is interesting as being composed to a great extent of globulin.

Experiment. 13.

Some instances of cells in saline put up in capillary tubes and taken from cases of varying rates.

	Hb.	1 hr. Westergren.	Capillary tube.
Case. 10.	80%	6·5	0·5
" 11.	70%	45.	2.
" 15.	75%	10·5	0·75.
" 8.	60%	86.	2·5.
" ".	70%	105.	1·25.
" 2.	100%	1·5	0·5.
" 29.	92%	95.	2.

Hb. estimations are on Haldane scale.

This experiment will be referred to later.

In spite of the fact that the E.S.R. of the bloods used varies considerably, it will be noticed that the suspension stability of the mixtures is of a high order.

In the hydrocoele fluid experiments a few very small rouleaux may be found but in the others, the point to be noticed, is that the cells are in the discrete condition.

In citrated blood used in the Westergren test there are rouleaux present but even with these a high suspension stability may be present. The size of the rouleaux does not in itself seem to be a cause for sedimentation; it seems necessary for these to accrete in clumps.

In contrast to the high suspension stability of the mixtures of cells and the various fluids described in the foregoing experiments, is the effect of agglutination.

A. If a one in twenty dilution of red cell suspension is mixed with the serum of an imcompatible blood and the mixture is quickly put up in one of the capillary tubes, one gets a very good picture of the ordinary typing reaction.

There is the usual formation of macro-agglutinated clum ps and rapid sedimentation.

B. If citrated plasma instead of serum, is mixed with cells of an incompatible blood and put up in a Westergren tube, clumping is not quite so obvious but there follows fairly quick sedimentation.

This varies according to the groups involved.

C. Take a centrifuge tube or a small test tube filled with a suspension of red cells in saline - say one part of cells to about ten of saline. Add a few drops of a 5% fresh solution of Tannic acid in saline and invert and reinvert the tube. There is an immediate reaction as shewn by the contents of the tube assuming a curdled appearance. This is due to agglutination and on the wall of the tube at the top, where the contents have been in contact, there will be noticed clumps of agglutinated cells adhering to the wall.

Rapid sedimentation follows:-

D. If to a suspension similar to that used above, in a small test tube, a few mgs. of reduced glutathione are added, in a few minutes, varying with the quantity of glutathione used, there occurs agglutination as is evidenced by the appearance of clumps of cells adhering to the lower part of the tube.

There is also rapid sedimentation.

E. In high sedimentation rate bloods, if a close observation is kept on the upper part of the Westergren tube during the first fifteen or twenty minutes, it will be noticed that the cells in their fall are forming small clumps. This can be better seen if a hand lens is used - say one of X8.

Sedimentation is also going on fairly rapidly.

Comparing the results of the experiments described previously with what happens in the experiments described above, it is difficult to escape the conclusion that agglutination, in some shape or form, must play a considerable part in the phenomenon of sedimentation.

And here it is necessary to consider the question of agglutination and presently, what happens in the examples given above.

Agglutination - more properly iso-agglutination - is the term applied to a special phenomenon which is not confined to red cells.

Taking red cells, in iso-agglutination, there is almost what one might call a certain fierceness of action. The cells become fused together, individual cell outline disappears and cell identity is lost. Though the membranes in the cell mass become confused, yet the outside cell membranes must coalesce on the outside of the mass since there is no lysis.

The action is irreversible.

The agglutination brought about by Tannic acid would seem to be due to physico-chemical action. Cell outline in the agglutinated masses is retained and the action is to a certain extent reversible.

The causes of the agglutination produced by the action of reduced glutathione I am not clear on. This is discussed in section V.

In abnormal sedimentation rates, agglutination seems to be due to two factors; agglutinability of the red cells due to pathological causes and the action of so called non-specific cold agglutinins. The action is reversible.

In the experiments described above there is no question as to agglutination, using the term in its derivate meaning of adhesiveness.

As mentioned in connection with Tannic acid, if the

tube is inverted and reinverted, on the wall of the tube where the suspension has been in contact, there are many clumps left adhering. The suspension if now left to stand, sediments without any clumps adhering to the side of the tube,

There may be a little lysis but the reaction is to some extent reversible; if the tube is shaken vigorously some of the cells can be separated from the clumps.

With glutathione the action takes a little time to develop, but as sedimentation proceeds, clumps beging to form and are seen adhering to the lower part of the tube. The clumps here are evidently more adhesive. If the tube is inverted and reinverted the suspension reverts to its original state and if allowed to stand, the same reaction occurs again.

The reaction is reversible.

In both the above instances, microscopical examination shews that the clumps are irregular in size and shape; the cells adhere by any part of their surface. Cell outline is not lost, Any increased apparent density of colour is due to overlapping of the cells in the clumps.

The small clumps referred to above as seen forming in the upper part of the Westergren tube are another example of pseudo-agglutination but here there is clumping of rouleaux as well.

That the cells accrete as well, seems to me to follow a priori, since both they and the cells of the rouleaux are exposed to the same conditions which give rise to adhesiveness.

If after performing the sedimentation test for an hour and the citrated blood is agitated and allowed to sediment again, it sediments at about the same rate again.

The action is reversible.

There is a point I would mention here. Most authorities state that it is the accretion of large rouleaux which is the cause of rapid sedimentation.

Many observations by myself of the conditions of citrated blood seem to shew that this is not invariably true.

Conditions of about equal parts of comparatively small rouleaux and discrete cells to complete rouleaux formation are found and it has not always happened that the complete rouleaux formation bloods have had the higher sedimentation rate.

Agglutination involves certain fundamental problems, such as change or electrical charge at the interface of cell membrane and medium with which I am not competent to deal.

Though the scale is greatly different, it is tempting to apply to agglutination the analogy of coacervates.

The following quotation is from "Cytology and Cell Physiology". (Bourne.)

"The term coacervate ---- refers to the structures formed during the slow separation of a colloid from a solution. Coacervates are formed of a large number of small droplets which lump together. In a typical colloidal solution each

particle is surrounded by concentric sheaths of water. The inner layer is very firmly bound to the particles and the succesive water layers are less and less strongly bound until there is a gradual gradation into the free water of the sol. One can conceive each colloid particle,therefore,as being surrounded by an envelope of water. Change of the electrical charge on the colloid particles,or the addition of dehydrating agents,causes the removal of the envelope of loosely bound water,leaving only the strongly bound water attached to the particle. In this way the particles come in contact with one another,and the various shells join up.

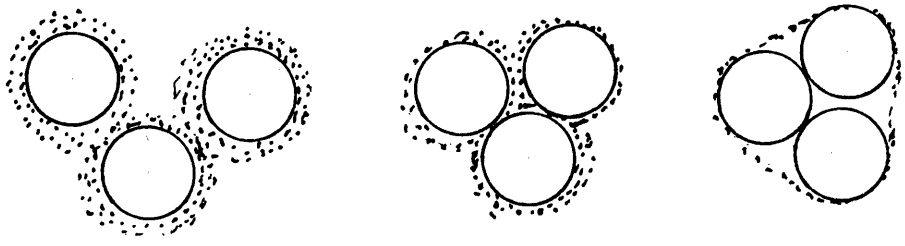


Diagram. (Cytology and Cell Physiology.)

It has been mentioned previously,that substances which adsorb on to the protein element of the cell membrane,cause agglutination of red cells. Such a substance is Tannic acid and this has been dealt with.

It was considered whether it might be possible to act on the cholesterol element,short of causing lysis,so that an agglutinating effect might be produced on the red cells.

The following experiments were done.

The bile salt Sodium Tauroglycocholate was used. This was dissolved in normal saline. Washed red cells from two cases were used. These were suspended in various dilutions of the bile salt and put up in capillary tubes.

It was thought that an agglutination might manifest itself as a difference in the sedimentation rate.

Experiment 14.

Cells from Case. 27.

Result.

	1 hr.	24 hrs.
Normal Saline.	1·5	41.
0·000015% Bile Salt	1·75	46.
0·0003. "	3.	48.
0·0005. "	1·25.	42.
0·00075. "	1·5	47.
0·0015. "	1·5	46·5
0·003 "	1·25	45·5
0·006 "	1·5	43.
0·01. "	1.	41·5.
1. "	0.	0.

Microscopical examination of the sediment after 24 hrs.

0·003% tube. Cells all discrete.

1% tube. Contents are a structureless gel.

Experiment 15.

Cells from Case. 28.

Result.

	1 hr.
0·01% Bile Salt.	0·5.
0·05. "	0·5.
0·1. "	0·5.
0·4. "	0.
0·6. "	0.
1. "	0.

After 22 hrs. the first three tubes shew lysis. The other three are to all appearance unchanged.

Microscopical examination of contents of tubes.

0·01% tube. Cells laked; discrete and round; streaming freely; no rouleaux.

0·05% tube. Cells laked; in single layer are in islets cells touching and hexagonal in shape; in channels. cells still discreteand streaming but shape altering.

0·1% tube. Structureless red. gel.

0·4% tube Structureless red gel.

The critical point for gel formation seems to be between 0·05% and 0·1%.

There is no evidence that agglutination or clumping occurs at any stage.

Experiment. 16.

Case. 29. Cells and plasma used.

The citrated plasma from this case was heated for one hour at 60°C. Capillary tubes were used.

The heated plasma at the end if the hour was slightly turbid. A certain amount if denaturation had evidently occurred Fibrinogen clots at 56°C. but the appearance of the tube seemed to indicate that not all the fibrinogen was affected.

Result.

		1 hr.
A.	Ordinary E.S.R.	44.
B.	One of cells to three of treated plasma	11.
C.	" " saline	2.5.
D.	" " hydrocoele fluid	1.

Microscopical examination of tube contents after 3 hours.

A. Cells discrete; closely packed.

B. Upper 11 mm. of tube clear. The lower 12 mm. is densely uniform. Intermediate part has the appearance of agglutination. Contents of lower part are almost entirely composed of very long rouleaux. The very few discrete cells are normal in appearance.

C. Cells all discrete; crenated globular.

D. Cells discrete; normal in shape; a very few small rouleaux.

The appearance of the coarse agglutination in the middle part of the tube using the heated plasma is difficult to understand.

as is also the appearance of the rouleaux and the normal shape of the cells.

This can be paralleled by the action of the hydrocoele fluid which I was using.

When red cells are washed in saline a few times they tend to become crenated and globular in shape.

During the experiments on suspension stability, when hydrocoele was being used, it was noticed, on examining the contents of the tubes, that the cells had been restored to their pristine condition. Their size and bi-concave shape became normal and there was even the tendency to rouleaux formation.

Experiment. 17.

An experiment is detailed, comparing the action of A.B. plasma and hydrocoele fluid.

My own cells were washed in saline until they were markedly crenated.

The hydrocoele fluid was over a year old and had been preserved in one of my ampoules. The A.B. plasma was about nine months old but in perfect condition.

Opsonic index type tubes were used, one with the hydrocoele fluid and cells and the other with the plasma and cells.

They were kept in the incubator for two hours at 37° C.

Result.

In the hydrocoele fluid tube there was a small percentage of crenated cells but the bulk were normal in size; bi-concave

in side and full view.

In the A.B. plasma tube there was no change in the crenated condition of the cells.

This lack of effect with A.B. plasma had been noted before.

Experiment. 18.

An earlier experiment done when the hydrocoele fluid was fresh is described. The time allowed for sedimentation was one hour. Cells from different cases with different rates were used. Put up in capillary tubes.

	Westergren rate.	Saline.	Hydrocoele fluid.
No. 1.	1·5.	0·5.	3·5.
" 2.	9·5.	1.	1.
" 3.	45·5.	1·25.	2.
" 4.	44.	2.5	1.

Microscopical examination of hydrocoele fluid tube.

No. 1. Cells discrete; normal in size and shape; very small rouleaux present; no agglutination.

No. 2. Cells discrete; Beautifully typical bi-concave cells; a few rouleaux; no agglutination.

No. 3. Cells discrete; bi-concavity not so clear as in 2; some mis-shapen cells; some rouleaux; no agglutination.

No. 4. Cells discrete; normal shape; very few small rouleaux; no agglutination.

Here again though the cells were discrete and somewhat crenated yet they are restored in size and shape and there is the tendency to rouleaux formation.

I noticed the following in "Cytology and Cell Physoilogy", " ------- it is interesting to find that Ponder and Purchgott have found that maintenance of the discoid form of the mammalian erythrocyte is connected with the adsorption of a crystallizable fraction of the serum albumin upon the erythrocyte. Reversible disk-sphere changes occur, according to whether the albumin is, or is not, present in the fluid bathing the erythrocyte ". (Page. 78.)

This may be a possible explanation of the action of the hydrocoele fluid, though it is difficult to understand why the plasma does not act in a similar way.

The osmotic balance between cell and medium also also be restored.

Still more interesting is the power to form rouleaux.

If, as I take it, the authors above, mean by adsorption upon the erythrocyte, adsorption upon the membrane, then the observation above that the protein of the cell membrane belongs to the collagen group would seem to require qualification - Serum albumin belongs to the globular or round molecule type of proteins.

It is stated that the sedimentation rate is influenced by the red cell count and that in anaemia the rate is increased.

I have not made any observations comparing haemoglobin content or red cell counts.

"A correction may be applied in terms of haemglobin content (Gram,1929) by the volume of packed cells (Fig.55) or by the red cell count. The various methods for applying correction for anaemia are reviewed by Gibson (1939) and Schuster (1938). No method of correction is entirely reliable".

(Whitby and Britten, Page 558.)

With the last sentence I am entirely in agreement.

To talk of corrections for haemglobin content or red cell counts seem to me to be due to a misunderstanding of the problem of the mechanism of sedimentation, which to me appears to be one of pseudo-agglutination and of what causes this.

The only correction for haemglobin which I can think of, would be for the gravitational effect of the varying amounts of haemglobin present in the cells of different cases.

I don't think that this would amount to much. A glance at the figures in Experiment. 13. in this section will shew what I mean.

Instead of red cell counts I have made a few comparisons of the red cell volume of the blood and the Westergren sedimentation rates.

The red cell volume estimations were done in a Wintrobe Haematocrit tube.

They were centrifuged for an average of forty minutes, this being the time that constant volume was attained by my centrifuge.

Instead of the oxalated blood normally used with the Wintrobe tube, I used the Westergren mixture of one of citrate to four of blood.

This of course required the correction factor X $\frac{5}{4}$.

In the following pages I give the results.
The first page gives these according to cases.

The second gives them in ascending order of the Westergren rates.

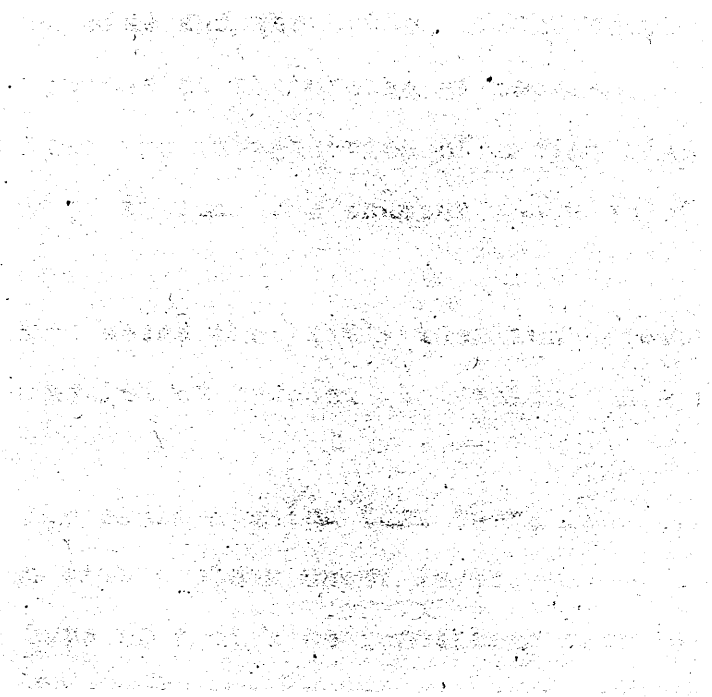
	Westergren. 1 hr.	Constant Volume.
Case. 1.	70.	36%.
" 8.	116.	35%.
" "	69.	37%.
" "	60.	35%.
" 20.	33.	32·5%
" 44.	1·25.	48%.
" 45.	6.	40%.
" 46.	19.	33·7%.
" 47.	5.	35%.
" 48.	5.	39%.
" 49.	13.	25%.
" ".	12.	25%.
" 50.	5·5	36%.
" 51.	3.	40%.
" 52.	17.	39%.
" 54.	30.	35%
" 56.	108.	35%.
" "	98.	33%.
" "	114.	33·6%.
" "	72.	40%.
" 57.	24.	27·5%.
" 20.	17.	32%.
" 4.	9.	36%.
" 60.	2·5.	46%.
" 61.	23.	41%.

On the following page these figures are arranged in ascending order of Westergren.

Westergren. 1 hr.	Constant Volume.
1·25.	48%.
2·5.	46%.
3.	40%.
5.	35%.
5·5.	39%.
5·5	36%.
6.	40%.
9.	36%.
12.	25%.
13.	25%.
17.	32%.
17.	39%.
19.	33·7%.
23.	41%.
24.	27·5%.
30.	35%.
33.	32·5%.
60.	35%.
69.	37%.
70.	36%.
72.	40%.
98.	33%.
108.	35%.
114.	33·6%.
116.	35%.

Though the above series is not a large one, it seems to me to shew that there is no definite correlation between the red cell volume of the blood and sedmentation rate.

It seems to shew that it is not the number of cells so much as what happens to them in pathological states.



On the following page is shewn a piece of apparatus which I made to study what happens during sedimentation.

In the Westergren tube it is difficult to see what is happening in the body of the blood.

The idea was to get a chamber of such a depth that the blood would sediment and yet that the film of blood would be thin enough to see what was happening in it.

This was done by cementing a piece of brass 1 mm. thick between two pieces of perspex. This gave a chamber 1 mm. in depth.

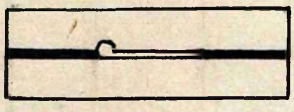
Along one side and the bottom of the chamber a groove was milled in one of the pieces of perspex.

This allows the introduction of a fine glass pipette for the purpose of filling the chamber and also of washing it out after.

The figure shews a slightly idealised picture of what happens during the action of reduced glutathione on a suspension of red cells.

During the sedimentation test there is a clear line of demarcation with a clear upper layer.

In the case of a high sedimentation rate blood the clumping which takes place in the body of the blood is well shewn.



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ELECTRICAL

It is stated that the sedimentation rate is affected by electrical causes. In terms of modern molecular physics this may be accepted as true but it appears to me that some authorities use the expression in a some what cruder way. For instance,

"The red cells carry a negative charge and anything which increases the positive charge in the plasma tends to cause an abnormal aggregation of cells and hence an increased sedimentation rate."

This statement is from Whitby and Britton. 1939.

It seemed to me that a simple experiment,using cataphoresis might throw some light on this and the following experiments were carried out.

Experiment. 1.

The apparatus shewn was used with my own blood; one of citrate to four of blood.

Microscopical examination of this mixture shewed no free cells and complete rouleaux formation.

After a preliminary trial the U tube was filled with the citrated blood so that it stood at a height of 100 mm. in each limb.

At the same time two Westergren tubes were set up; one filled to a height of 100 mm. and the other to a height of 200 mm.

A current of 6 volts was passed through the U tube for one hour.

Result.

E.S.R.	1 hr.	2 hrs.
Westergren. 100 mm.	12.	24.
" 200 mm.	12.	33.

U tube result.

In limb connected to positive pole.	20.	-
" negative "	14.	-
One hour after current cut off.		
Positive limb	-	36.
Negative limb	-	25.

24 hours after.

Red cell level was equal in both limbs. The clear super - natant fluid was pipetted off both limbs and then the cells were examined microscopically.

Positive limb. Cells all discrete; half crenated, half normal in shape, though bi-concavity not well marked.

Negative limb. Rouleaux formation, four to about fifteen cells in each. Mostly discrete cells. Bi-concavity well marked.

After 36 hours.

The fluid level in each limb was now 52 mm. but the conditions of sedimentation were reversed.

Further sedimentation has now occurred with the following result.

Positive limb.	3.
Negative limb.	15.

A result rather difficult to explain. The presence of more normal cells and rouleaux in the negative limb might be a possible explanation.

Experiment. 2.

In this experiment the citrated blood from case 53, was used. E.S.R. 1 hr. Westergren. 7·5.

Column in each limb = 100 mm.

Result.

	Positive limb,	Negative limb.
E.S.R. 1 hr.	14.	11.
" 2 hrs.	27.	22.
" 4 "	39.	35.
" 15. "	45.	45.

The current was cut off after one hour.

After 21 hrs.

The clear supernatant fluid was pipetted off and the cells in each limb examined.

Positive limb.

Cells all discrete. No rouleaux. Cells about half and half crenated and normal in outline. Bi-concavity practically absent.

Negative limb.

Many perfect rouleaux. Many discrete cells, some of which are crenated and others normal in outline, though there is no bi-concavity.

Experiment. 3.

In the two previous experiments, the only material I had at hand for electrodes was silver solder. This contains silver, copper and zinc and to obviate any side effects from these metals, in this and the following experiments, electrodes of platinum wire have been used.

My own blood - one of citrate to four of blood used.

Result.

Current passed for one hour. Column in each limb was 94 mm.

E.S.R.	1 hr.
Positive limb.	17.
Negative "	13.

The clear supernatant fluid was pipetted off and tested for its p/H value.

B.D.H. Capillator with Phenol indicator was used.

Positive limb.

H ion concentration is greater than p/H. 6·8.

Negative limb.

H ion concentration is less than p/H, 8·4.

Experiment. 4.

My own blood was used. One of citrate to four of blood. Column of 100 mm. in each limb. Current passed for one hour.

Result.

E.S.R.	1 hr.
Positive limb.	17.
Negative limb.	20.

Current cut off. Both electrodes not exposed in clear upper layer.

Positive electrode shews red cells adhering to it. The negative electrode is clear.

The clear supernatant fluid was now pipetted off and the contents of each limb examined microscopically,

Positive limb. Rouleaux formation largely clumped. No free cells.

Negative limb. Rouleaux formation, clumped also. A few free cells are present.

Height of column in each limb now 90 mm.

Sedimentation after 6 hours.

Positive limb.	30.
Negative limb.	32.

After 27 hours.

over.

After 27 hours.

Positive limb. 40.

Negative limb 37.

Microscopical examination of limb contents.

Positive limb.

Rouleaux formation, compacted.

Negative limb.

Rouleaux formation, compacted, but some crenated cells present.

In experiment 1. it looks as if the electrolyte produced by the silver solder electrodes had interfered with the reaction. Apart from this and the fact that passing a current of 6 volts is a rather heavy handed procedure one would have expected theoretically, in experiment 4. a larger fall in the positive limb, considering the difference in the p/H values in the two limbs as shewn in experiment 3.

In some of the cases recorded I have made a comparison of sedimentation rates and the plasma p/H.

For this purpose I used a B.D.H. Capillator. This consists of a series of capillary tubes filled with buffer solutions containing an indicator, The indicator used here was Phenol Red. This indicates from p/H 6·8 to p/H 8·4 in stages of 0·2.

A capillary tube containing plasma and Phenol Red indicator is compared with the standard tubes. This gives a fair approximation to actual values,

		1 hr. Westergren.	Plasma p/H.
Case.	2. 29:6:42	1·5.	Between 7·6 and 7·8.
"	8. 27:7:42.	109.	" 7·6 " 7·8.
"	" 6:5:43.	69.	" 7·6 " 7·8.
"	" 23:6:43.	56.	" 7·6 " 7·8.
"	" 19:9:43.	60.	7·4.
"	15 30:6:42.	10·5	7·6.
"	16. 13:7:42.	22.	Between 7·6 amd 7·8.
"	20. 17:5:42.	33.	7· 6
"	" 14:9:42.	37.	7·6.
"	29. 2:7:42	85.	Betwee n 7·6 and 7·8.
"	" 1:8:43	22·5.	7·6
"	30. 13:7:42	35.	Between 7·6 and 7·8.
"	39. 28:4:43	1.	7·4.
"	43. 27:4:43	1.	7·4
"	46. 9/5:43	19.	Between 7·6 and 7·8.
"	47 11:5:43.	5.	" 7·6 and 7·8.
"	48. 15:5:43	5.	7·4.
"	49. 18:5/42	13.	Between 7·6 and 7·8.
"	" 24:9:43.	12.	7·4
"	50 25:5:43.	5·5	7·4.
"	51. 12:6:43.	3.	7·6.
"	52 16:6:43	17.	7·6
"	53. 17/8:43	7·5	7·4.
"	" 18:12:43	5.	7·4.

over

Westergren and p/H. Comparisons. (cntd).

		1 hr. Westergren.	Plasma p/H.
Case. 54.	24:8:43.	46.	Betwee n 7·6 and 7·8.
" ".	6:9:43	30.	" 7·6 and 7·8
" 55.	18:8:43.	3.	7·6
" 56.	5/9:43.	108	7·6
" "	25:9:43	114.	7·6
" "	26:11:43.	83	7·4
" 60.	16:12:43	2·5.	between 7·2 and 7·4.

There is no necessity to rearrange these figures. A casual glance seems to shew that there is no correlation between the electrical condition of the plasma and the sedimentation rate.

Even this rather crude method of ascertaining the p/H. value of the citrated plasma shews that there may be a large variation in this.

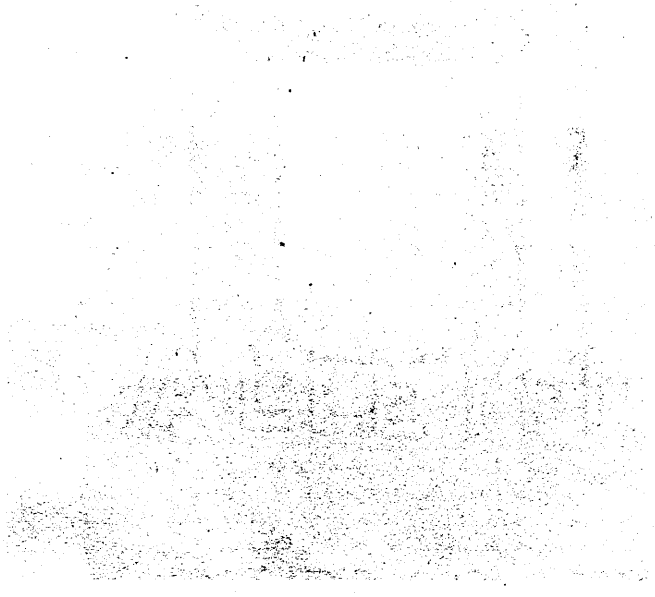
It has to be remembered that a difference of 0·2 or 0·3 in the p/H value means a considerable difference in the Hydrogen ion content.

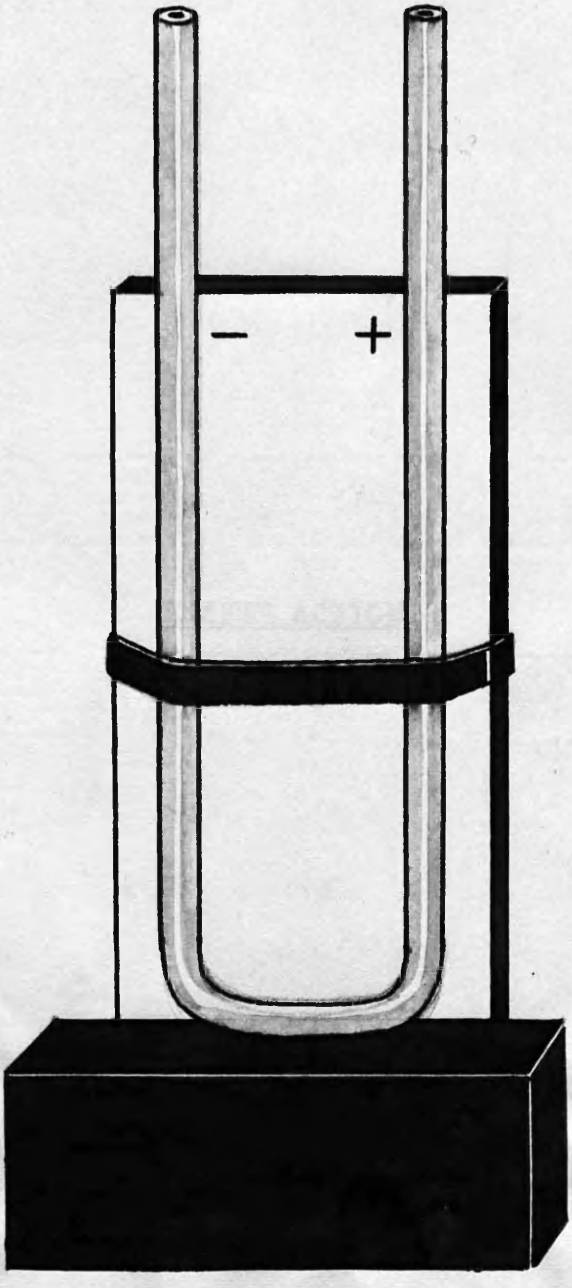
On the following page is shewn a piece of apparatus used for experiments in this section.

It is made from a plain ungraduated standard Westergren tube which I bent into a U shape.

The tube is the standard bore of 2 5 mm.

It is held vertically on the stand by the rubber band shewn.





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ENZYME ACTION.

In certain diseases with much wasting there is usually an increased sedimentation rate. Tissue waste is accompanied by cell destruction and this again is ultimately caused by the action of certain intra-cellular ferments.

It seemed pertinent to inquire if there might be any connection linking increased sedimentation rates and enzyme action.

The enzymes taking part in cell destruction are proteolytic in nature and seem to be present in nearly all tissues.

In what way could the erythrocyte be affected? In certain pathological conditions would its own condition be altered in such a way that its own intra-cellular proteinases might be called into action?

Could an excess of the products of protein breakdown, such as creatinine or creatin have any effect? Could the presence of active proteinase in the plasma act on them? Or could it be that the intra-cellular proteinase might be activated by substances present in the plasma?

An answer was sought to these questions.

Experiment 1.

Case. 32. E.S.R. 1 hr. Westergren. 7.

To 3·5 cc. of the citrated blood of this case were added 5 drops of a saturated solution of creatinine in 3·8% citrate. Shaken and put up in a Westergren tube.

The resultant fall in one hour was 5.

Experiment 2.

Case. 33.

E.S.R. 1 hr. Westergren. 6·5.

To 3·5 cc. of the citrated blood of this case were added 5 drops of a saturated solution of creatin in 3·8% citrate. Shaken and put up in a Westergren tube.

Resultant fall in one hour. 5·5.

Experiment. 3.

Case. 2.

E.S.R. 1 hr. Westergren. 1·5.

As a source of active proteinase, fresh pus seemed to be suitable. To 3·5cc. of the citrated blood was added 0·4 cc. of pus from a case of osteomyelitis and the mixture well shaken. Westergren tube used.

Resultant fall in one hour. 3.

Evidently in neither of these cases was there any practical difference.

"Recent work indicates the almost universal presence of Kathepsin, an enzyme that acts on native proteins, together with a mixture of pepsidases. Kathepsin is peculiar in that it is activated by HCN and HS, thus resembling of being identical with papain, the protease found in certain plants. Both in animal and plant cells there exist other activators of Kathepsin. Amongst these the most important seem to be reduced glutathione and/

and cysteine."

(Practical Physiological Chemistry. S.W.Cole. 1941.)

Intra-cellular Proteolytic Enzymes.

"There seem to be several of these and attempts have been made to classify them by means of their action on various substrates, natural and synthetic.

"The fact that the specificity of an intra-cellular proteinase is not rigidly determined but may be altered in various ways is of obvious significance for the general problem of protein metabolism.

"While glutathione has been accepted as a possible naturally occurring activator, it is clear that other substances (cysteine, ascorbic acid etc.) shown to occur in living tissues are also potential activators and by combining with an intra-cellular proteinase may modify its specificity.

"There appears to be one intra-cellular proteinase acting at a weakly acid p/H (named Cathepsin by Willstatter.) The proteinase is activated by HS. "

(M.Bergman and J.S.Fruton. in Advances in Enzymology. 1941.)

Glutathione.

It was decided to try the effect of reduced glutathione on the erythrocyte.

Glutathione is almost universally present in animal tissues. It is present in the erythrocyte - 25 to 50 mg. in 100 cc. Blood.

Hopkins has shewn that glutathione is concerned with respiration and forms a system independent of thermolabile enzymes.

Glutathione also plays the part of a co-enzyme in the conversion of glucose to lactic acid in the erythrocyte.

It contains the SH group and in the reduced form is an activator of proteinase.

Though the erythrocyte contains a small quantity of glutathione it was decided to try the effect on the erythrocytes of adding a small quantity of reduced glutathione to a suspension of red cells in normal saline.

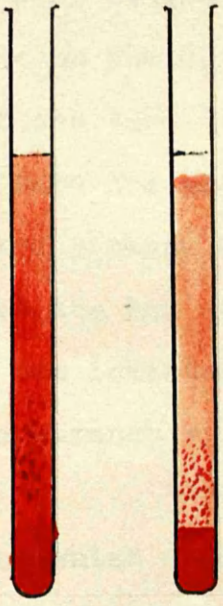
This revealed the interesting fact that the addition of the glutathione caused agglutination and fairly rapid sedimentation. The rapidity of the reaction varied according to the quantity used.

Experiment No. 4.

My own cells were used. They were collected in 3;8% citrate solution in a centrifuge tube - one of citrate to four of blood. Normal saline was added to about 10 cc. mixed and centrifuged. The supernatant fluid pipetted off, repeated and lastly make up with saline to about 10 cc.

The cells are now in the discrete state - there are no rouleaux present.

It has to be remembered that the cells are now in an abnormal environment and removed from their metabolites.



A. B.

Some of the cell suspension was put in one of the tubes of the apparatus illustrated at the beginning of this section.

A few mgs. of glutathione were added and the tube inverted and reinverted a few times.(Glutathione is very soluble.)

There is a gradually developing reaction and in about five minutes the appearance is as in A. above.

There is obvious sedimentation going on and clumps of cells are seen adhering to the side of the tube - agglutination.

In about an hour the appearance is as in B. Sedimentation is now far advanced and the clumps are well seen on the side of the tube.There is a coloured layer above the sedimented cells and above this a clear layer shewing that so far there is no

no lysys.

At this stage if the tube is inverted and reinverted the clumps disappear and the same action takes place again.

Some hours later, depending on the quantity of the glutathione and more especially if the tube is kept in the incubator at 37 C. the contents of the tube has altered. The colour is becoming darker, the sedimented portion is becoming looser like and the colour has diffused to the top of the tube. Lysis has taken place. If the tube is now inverted and reinverted the contents shew a flocculent appearance with semi-transparent floccules apparent.

Later the colour becomes brownish - the haemoglobin has become changed - and the floccules disappear. There has been evidently complete dissolution of the cell content.

Microscopic examination confirms all the changes described above.

In the early stages the cells are found to be forming simple clumps. There are no rouleaux and no appearance of iso-agglutination. Any darkening of the centre of the clumps is due to super-imposition of cells.

The appearance is very similar to that found with the action of Tannic Acid but with the glutathione the clumps are very easily dispersed.

About the midstage the cells are seen to be slightly swollen, globular and lysed.

In the later stages the cells in the clumps are becoming smaller, their outline becoming more indistinct and finally they disappear. The changes have the appearance of the digestion of the cell elements.

The permeability of the cell membrane in connection with this action will be discussed later.

This experiment took place in a medium of about p/H. 6. It was asked if the same action would happen in citrated blood with a p/H of about 7·4. and with the cells in the presence of their metabolites.

This was a little difficulty on account of the greater density of the citrated blood but the following experiment was done.

Experiment 5.

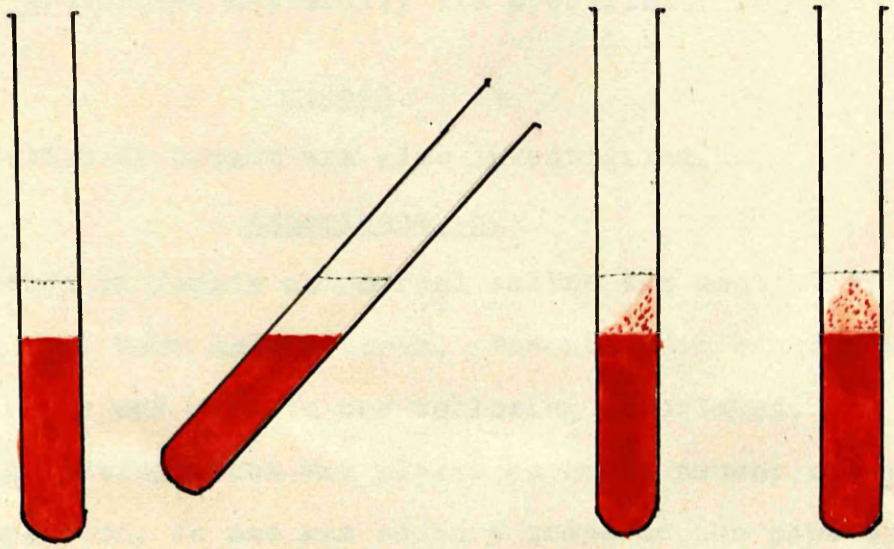
In this experiment a somewhat larger tube was used. My own blood was used - one part of citrate to four of blood.

The tube was first allowed to stand for an hour and the appearance was then as shewn at A. in the following diagram.

On tilting the tube gently and returning to the vertical there was a thin film of blood left on the side but this gradually subsided into the body of the cells.

There was no sign of macro-agglutination on the side of the tube.

The citrated blood was then agitated until it was mixed again. A few mgs. of glutathione were then added and the tube



A. B. C. D.

agitated and allowed to stand for one hour. At the end of that time the appearance was again similar to A.

On tilting the tube as at B. and returning to the vertical the appearance was similar to that at C. Full on the appearance was as in D.

Clumps of macro-agglutination were evident, adhering to the side of the tube as in the last experiment.

Evidently glutathione can act in a mildly alkaline medium also.

If this is an enzyme action is the proteinase the Cathepsin of Willstatter ? I quote again.

" While glutathione has been accepted as a possible natur - ally occurring activator, it is clear that other substances (cysteine, ascorbic acid etc.) shown to occur in living tissues

are also potential activators and by combining with an intracellular proteinase may modify its specificity".

Papain.

The action of papain was also investigated.

Experiment. 6.

A mixture of papain and normal saline was kept at 37°C. for about half an hour and filtered. The filtrate was light brown in colour and was used in the following experiment.

In the testing tubes was placed an equal amount of washed cell suspension. To one was added 5 drops of the papain filtrate and 5 drops of Acid Hydrocyan. Dil. To the other 10 drops of saline.

The tubes were then kept at 37°C. for one hour. At the end of that time the appearance of both tubes was much the same. A slight amount of sedimentation had occurred in both.

The tubes were then kept at room temperature for another four and a half hours.

The appearance was again much the same in both. Height of column 75 mm.

About 18.mm. clear layer at top, then coloured layer to near the bottom where there was a small dark deposit.

Evidently the papain filtrate had had no effect. Possibly a solution of a different p/H might have extracted any enzyme present.

Experiment. 7.

At the end of the first hour in the previous experiment, papain itself was added to the remainder of the cell suspension in the centrifuge tube. To this was added about 20 drops of Acid Hydrocyan. Dil. and the tube then kept in the incubator for four hours when the appearance was as shewn.

There were four well defined layers with a fine frothy layer at the top. These layers were pretty much as shewn.

On tilting the tube as described in exper. 5. a fine granular clumping was left adhering for a short time to the side of the tube.

This was a somewhat similar appearance to that produced by the glutathione but the clumps were very much smaller and not so adhesive. There seemed to be a slight agglutination but the association of the cells was not so strong as with the glutathione.

Under the microscope a slide from the bottom layer shewed the cells to be pretty compacted but a little pressure on the coverslip caused dispersal into discrete cells. These were well coloured and bi-concave.

Agglutination if it had occurred must have been mild and easily dissociated.

It is possible that in certain circumstances, the technique of tilting the tube and watching for the appearance of clumps on the side may be a more delicate test than microscopical examination.

Note:-
B.D.H. from whom I got the papain, could make no definite statement as to the proteolytic or diastatic content of it.

The question now was, are the changes produced by the glutathione due to the activation or enzyme action.

It was remembered that in collecting blood samples for the estimation of sugar, if this is not to be done at the time, it is customary to add Sodium fluoride to prevent the glucose from being transformed to lactic acid. This action of course is due to enzyme activity and the sodium fluoride is an enzyme poison.

The following experiment was tried:-

Experiment 7.

1·5 cc. of my own red cells, collected in 3·8% Sod. citrate solution and washed as described previously, were suspended in 12 cc. of normal saline.

Equal parts of this were put in the test tubes.

To one tube was added 0·5 cc. of 1% solution of sodium fluoride in normal saline and to the other 0·5 cc. of normal saline.

Then to each was added 0·5 cc. of a solution of glutathione

in normal saline. The amount of glutathione in this would be of the nature of a few mgs.

In half an hour there was evident agglutination and active sedimentation in the control tube.

In the Sod. fluoride tube there was no change.

In one hour there was almost complete sedimentation in the control tube with the familiar appearance of agglutinated clumps adhering to the lower part of the tube.

In the Sod. fluoride tube there was practically no change, with the exception of a very small deposit at the bottom of the tube and no evidence of agglutination.

The above experiment would seem favourable to the idea that the glutathione was the activator of some enzyme action and that the enzyme action was stopped by the Sod. fluoride.

Two hours later the addition to the fluoride tube of three drops of a 5% solution of Tannic acid in normal saline caused the usual immediate agglutination and rapid sedimentation.

This action would seem to shew that the condition of the cell membrane remains unchanged both by the glutathione and by the Sod. fluoride.

One aspect of the glutathione phenomenon may be remarked upon. Though the bulk of the cells are affected and sediment, there is a residue which remains in suspension. In all living organisms there is a gradation through youthful vigour to

maturity and senescence and it seems allowable to think that the young erythrocyte may be more resistant.

The changes produced in the erythrocyte by the action of glutathione may, I think, be divided into three stages.

a. Agglutination.

b. Haemolysis

c. Lysis.

Each of these involves fundamental problems to which there is at present no clear answer, but recognising this fact one may perhaps be allowed to ask some questions.

Presuming that this is an enzyme action and assuming Wilstatter's theory that enzymes can be absorbed on protein molecules, is it possible that proteinase may be present in the cell membrane and that in this way the membrane is first affected, causing changes which lead to agglutination?

Or assuming the "sieve" theory of membranes ~~and set~~ does the glutathione molecule penetrate the membrane and set up changes in the cytoplasm which react on the membrane and produce the conditions favourable to agglutination?

That the cell membrane is affected is evidenced by the next stage of haemolysis. Examination at this stage, of the cells under the dark ground condenser, shews them to be globular somewhat swollen and bereft of haemoglobin. The cell membrane stand out clear cut and apparently unchanged but the cell is now dead. The changes in the cell are not due to any change in the tonicity of the medium but to the fact that in the dead

cell there is complete permeability and diffusion has occurred into the cell.

"No one can fail to be impressed with the great difference in properties of living and dead cells. The dead are completely permeable to diffusible substances ----------"

(The Permeability of Natural Membranes. Davson and Danielli.)

The third stage is lysis and complete dissolution of the cell probably due to proteolytic changes since one cannot envisage the glutathione as being a solvent of protein.

It was considered that in certain pathological states the erythrocyte, though to all appearance unchanged, might be altered in some way and that that difference, if it existed, might be revealed by the action of reduced glutathione.

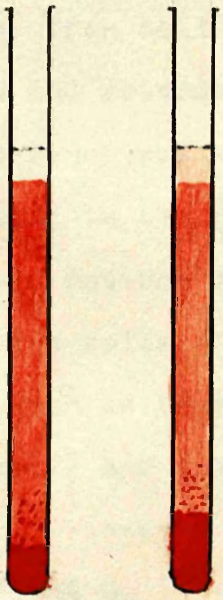
To this end the following experiments were done.

The tubes are lettered C and P.

C. Here my own cells are used.

P. Patient's cells.

Experiment. 8.



Case. 49. E.S.R. 12. (24:9:43). Nephritis.

17:9:43.

Citrated blood from this case was used and my own citrated blood used as a control.

The cells were washed in saline and a suspension of them in saline put in the test tubes. Colour was matched as evenly as possible.

A few mgs. of glutathione were added to each and the tubes placed in the incubator. 37 C.

In ten minutes the cells in the patient's tube were almost completely sedimented. The rapidity of this was rather striking.

Microscopically there were now large clumps of

agglutinated cells and a few discrete cells.

In fifteen minutes my own cells shewed a little sediment - ation. Microscopically, many free cells and some small clumps.

The tubes were now shaken and returned to the incubator.

In forty minutes the appearance was as shewn in the figure.

Lysis is evidently beginning in the patient's tube.

In two and a half hours the patient's tube shewed lysis and complete dissolution of the cells.

My own cells were pretty much as they were at the end of the fifteen minutes. Free cells and small clumps.

Experiment 9.

Case. 8. E.S.R. 60.

18:9:43. Cells from this case and my own were used and the same procedure followed.

8. P. M. Tubes placed in incubator.

8.5 P.M. Active sedimentation going on. Clumps of macro-agglutination are adhering to the sides of both tubes.

8.10.P.M. Sedimentation practically complete in both tubes. The supernatant fluid in patient's tube is a little clearer. Microscopically there are free cells and small clumps in both tubes. Tubes shaken up and returned to incubator.

10.10.P.M. There is complete lysis in both tubes. There are a few ghosts in my tube but otherwise and in patient's

tube there is complete dissolution of the cells.

Note. I had just turned on the incubator and did not notice that at first the temperature was 40 C. This may have disturbed the experiment.

Experiment. 10.

Case. 54. E.S.K. 30. (6:9:43).

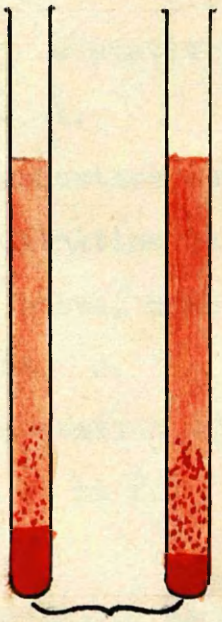
20:9:43.

Cells from this case and my own used as before. A minute quantity of glutathione added and tubes placed in incubator. In ten minutes no change. The small amount of glutathione was tried to slow down the action but more is evidently required. A further quantity was added.

In a minute or two macro-agglutination was occurring in both tubes. In five minutes sedimentation was complete in patient's tube and the colour was becoming brownish. In my own tube sedimentation was about half completed and the colour was red.

Tubes were shaken and returned to the incubator. In another ten minutes patient's tube shewed flocculated clumps and the colour was now definitely brown. In my own there was flocculation but the colour was still good. In another five minutes in patient's tube there was a clear brown fluid and microscopically complete dissolution of cells. In my tube colour was changing a little and micro-scopically, agglutinated clumps of "ghost" cells undergoing dissolution.

Experiment. 11.



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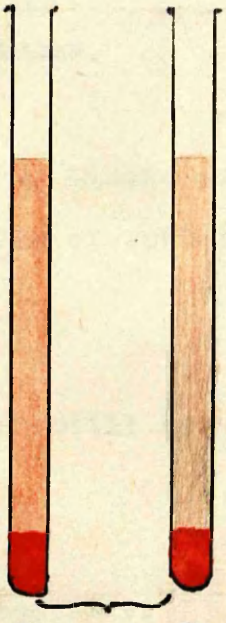

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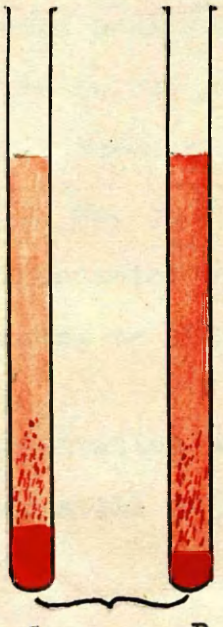

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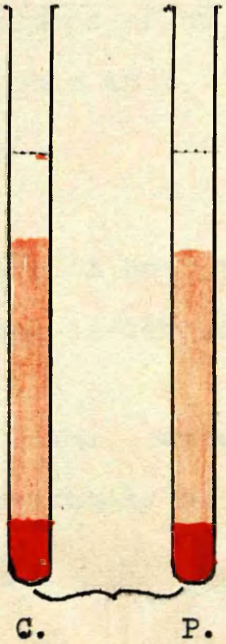


4.

Experiment. 11.

Case. 57. E.S.K. 24.

21:9:43. My own and patient's cells used as before.

4.50 P.M.
Glutathione added to both tubes.

5.P.M. No. 1.

Sedimentation advanced more so in C. Clumps or macro-agglutination ad hering to the sides of both tubes. Hazy above, more so in P.

5.10 P.M. No. 2.

Sedimentation equal in both tubes. Still hazy above. more so in P.

5.15.P.M.

Tubes shaken and examined microscopically.

C. There are clumps, more of a pavement appearance and cells seem a little swollen.

P. Cells mostly discrete. Small clumps of up to about five cells. Cells look better than in C.

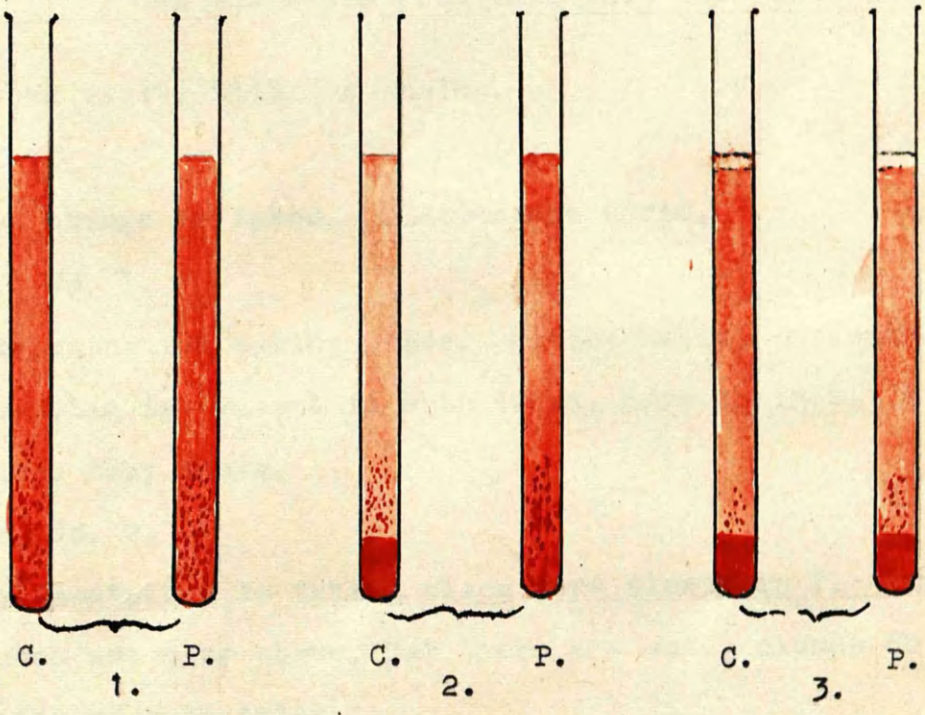
5. 20. P.M. Tubes placed in incubator.

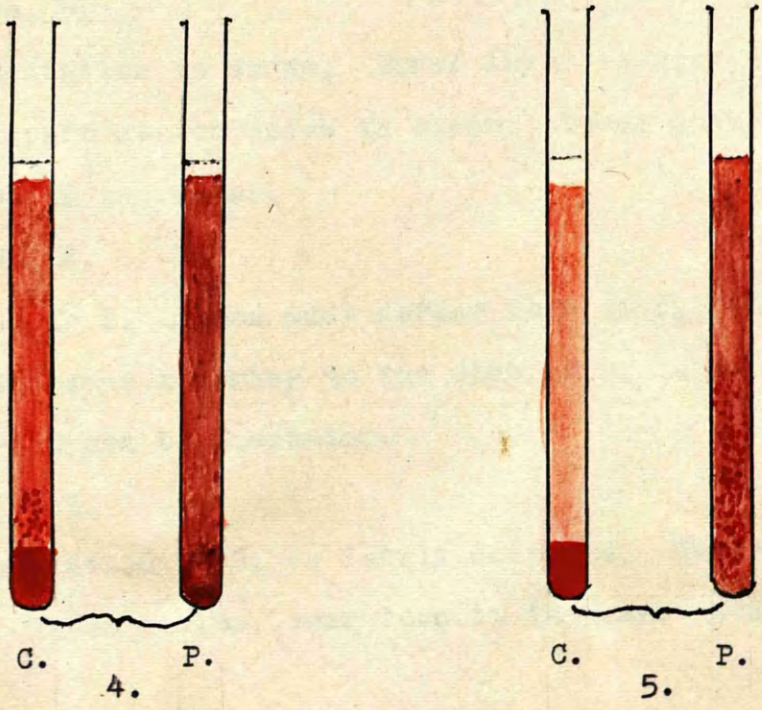
5. 25. P.M. No. 3.

Sedimentation as shewn. Hazy above in both tubes. Clumps adhering to sides of tubes, somewhat larger and streaky.

7. 5. P.M.
Sedimentation as shewn. Top layer clear. Intermediate layer still hazy but clearing. Evidently no Lysis

Experiment. 12.





Experiment 12.

Case. 56. E.S.R. 114.

25:9:43.

My own and cells from this case were used as before.

4.35.P.M.
Tubes filled with suspension.

4.45.P.M.

No. change in tubes. Glutathione added.

5. P.M. No. 1.

Agglutination taking place. Sedimentation advancing. Clumping is evident in both tubes, more so in C. Both tubes hazy above.

5.5. P.M. No. 2.

Sedimentation is taking place more slowly in P. Both tubes are hazy above, but there are small clumps on the sides of both tubes.

5.25 P.M. No. 3.

Sedimentation as shewn. Upper layer is hazy except a small part at top which is clear. Tubes shaken and placed in incubator.

5.50.P.M. No. 4.

Colour in P. is now much darker than in C. There are small clumps adhering to the side of C. tube. Shaken and returned to incubator.

6.35.P.M. No. 5.

Sedimentation in C. is fairly complete. There is a small clear layer at top, lower down it is hazy. Colour good.

Experiment. 12. (Contd).

In P. the whole tube is much darker, almost of a brownish tinge. The appearance is that of Lysis. The deposit is flocculent in appearance.

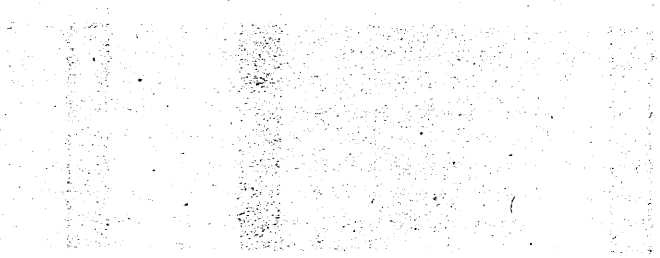
Tubes shaken and returned to incubator.

12. P.M.

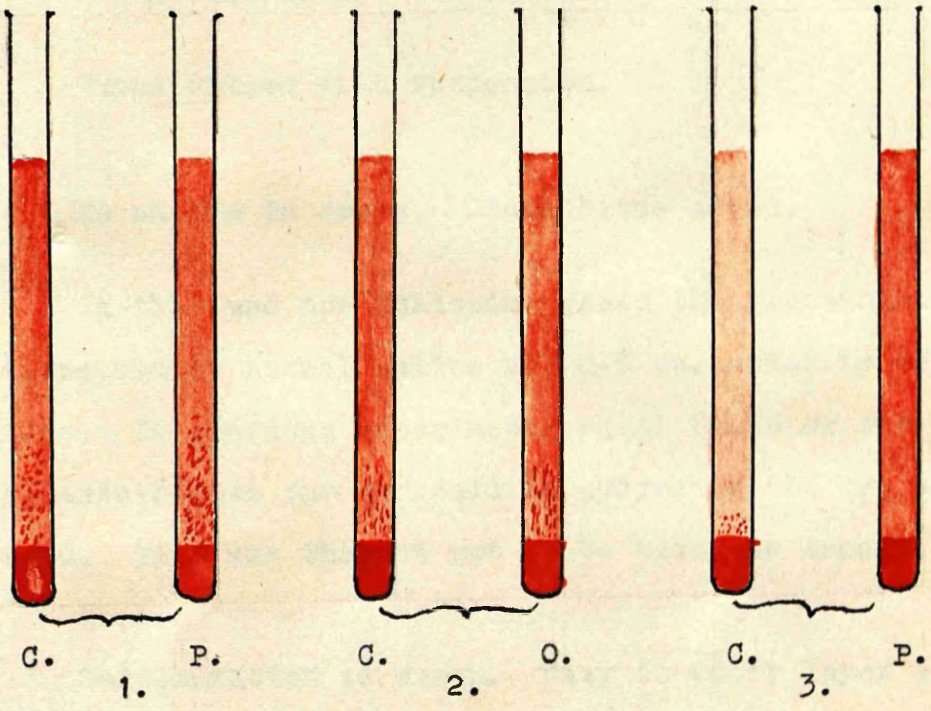
Tubes shaken and contents examined microscopically.

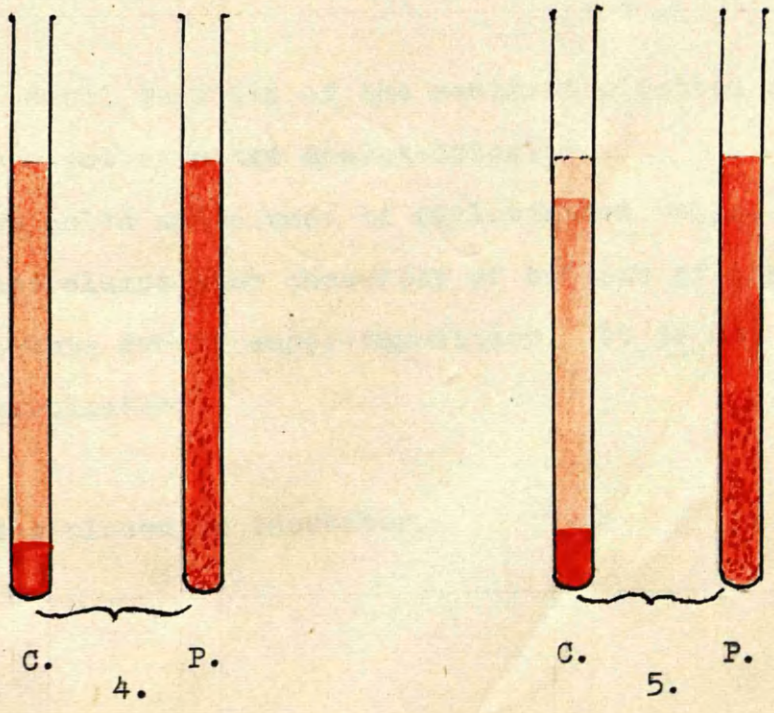
In C. the shells are mostly discrete with clear outlines. There are clumps here and there of two or three cells. Some ghost cells whose membrane is clearly out lined. The cells seem to have retained their colour fairly well.

In P. there are masses of agglutinated ghost cells the outline of some of which has disappeared. The appearance is that of dissolution.



Experiment. 13.





Experiment. 13.

Case. 49. E.S.R. 12.

24:9:43. My own and patient's cells used.

4.40. P.M.

Tubes filled with suspension.

4.50. P.M.

No change in tubes. Glutathione added.

Note.

In this and the following cases the glutathione was dissolved in normal saline and 0·5 cc. added to each tube. In previous experiments equal parts of the actual glutathione so far as could be judged by the eye were used. This was thought not to be accurate enough.

5. P.M. No.1.

Sedimentation as shewn. Hazy in upper layer but clumps are adhering to the sides of both tubes.

5.15.P.M.

Small quantity of the sediment pipetted off both tubes and examined microscopically.

Free cells and clumps of agglutinated cells. Some of these clumps shew obscurity of outline of the cells but this due to super-imposition. It is not iso-agglutination.

5.20. P.M.

Tubes placed in incubator.

Experiment. 13. (Contd).

5.25.P.M. No. 2.

Sedimentation as shown. Clumps adhering to sides of tubes especially in C. are larger and more of a streaky nature. Supernatant fluid hazy.

7. P.M. No. 3.

In C. the upper fluid is clear and practically colourless. Just above the sediment there are clumps which are of good colour.

In P. the upper fluid is clear but darker crimson in colour, especially a small layer just above the sediment.

8.15.P.M.

Tubes shaken and put back in incubator.

8.45.P.M. No. 4.

In C. sedimentation is as shewn. The upper layer is hazy but is clearing at the top.

In P. the colour deepens gradually from top to bottom. The clumps now seem lighter in Colour and have a flocculent appearance.

10.15.P.M.No. 5.

In C. sedimentation is as shewn. The intermediate layer is hazy but the top layer is clear. Lysis is very slight.

In P. the sediment is of a loose flocculent appearance. The upper layer is clear and dark crimson in colour. Lysis has evidently taken place.

Experiment. 13. (Contd.

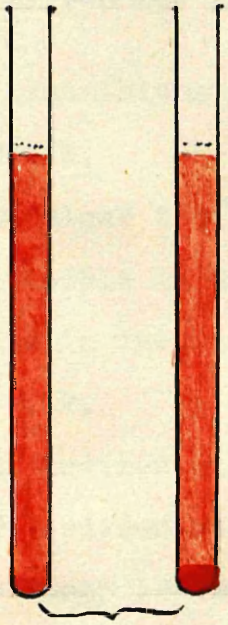
11. P.M.

Tubes shaken and the contents examined microscopically. In C. the cells are mostly discrete but her and there are small clumps of a few cells.

The cells shew a good outline and appear to retain their colour.

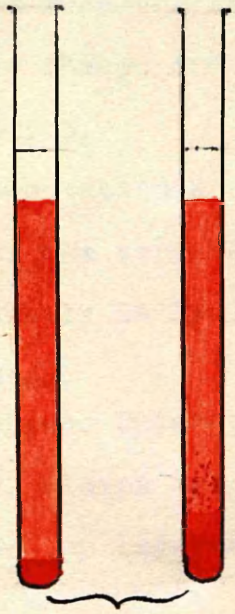
In P. the cells are in process of dissolution. There are some clumps of ghost cells in which the cell membrane can still be seen faintly.

Experiment. 14.



1.

2.



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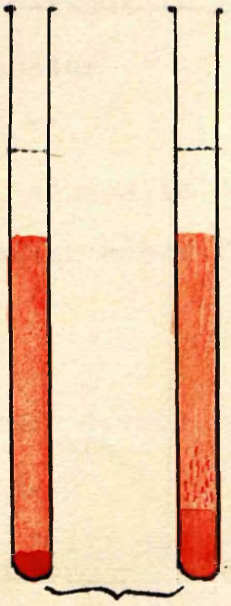


4.

Experiment 14.

Case. 20. E.S.R. 17.

4:10:43.

My own and patient's cells used.

4.25 P.M.

Glutathione added.

4.55.P.M. No. 1.

Small clear top layer in both tubes. Sedimentation not yet visible in C. In P. sedimentation is proceeding. Placed in incubator.

5.15.P.M. No. 2.

In C. sedimentation is much slower. No clumps

In P. sedimentation is advanced. Small clumps on side of tube upper layer lighter in colour than in C.

Clear layer in both at top. About 4 m.m.

6. 50.P.M.

Tubes much the same. Top layer now about 10 mm.

Tubes shaken and returned to incubator

8.35.P.M. nO. 3.

Sedimentation again as shewn. No. clumps in C. Clumps in P. now very small. Top layer clear about 10 mm.

Evidently no Lysis.

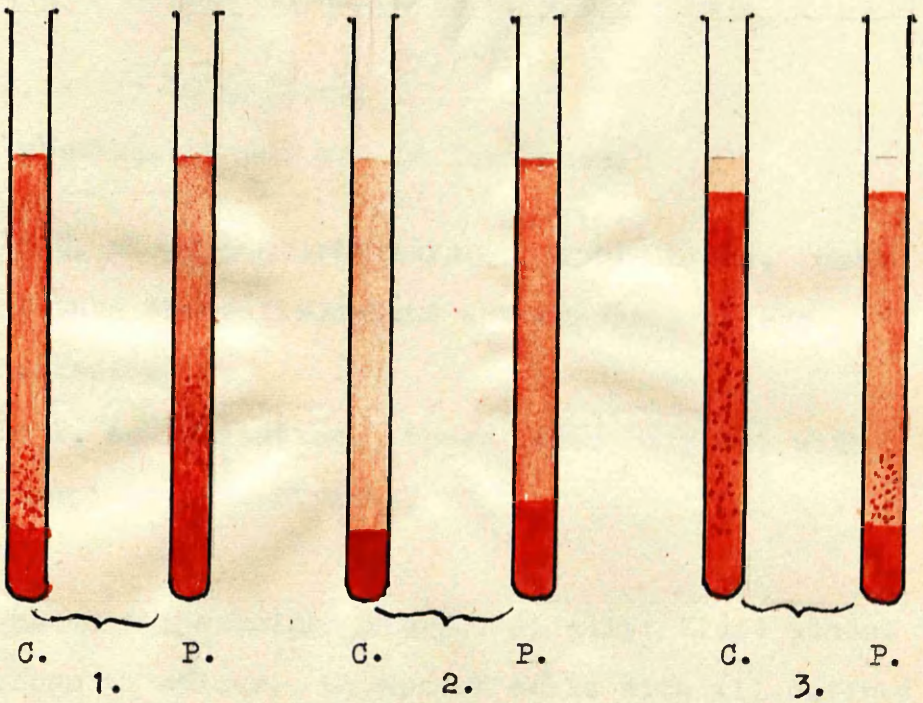
11.P.M. No.4.

As shewn. Upper layer in C. slightly darker. No clumps.

In P. clumps are small and streaky.

Clear top layer now about 20 mm. Still no Lysis

Experiment. 15.



Case. 58. E.S.R. 1 hr. Westergren. 91.

25:10:43.

Patient's blood collected at 9.A.M.

My own at 9.30. A.M.

Cell suspensions prepared.

5.3. P.M. Glutathione added.

5.8. P.M. Active agglutination in both tubes, more marked in C.

5.15. P.M. No. 1.

Well marked clumping on side of tube C. in lower part. Clumping in P. also.

Over.

Experiment. 15. Contd).

5.30. P.M. No. 2.

Complete sedimentation in C. clear above.

P. still hazy above.

Tubes shaken and put in Incubator.

5.45. P.M.

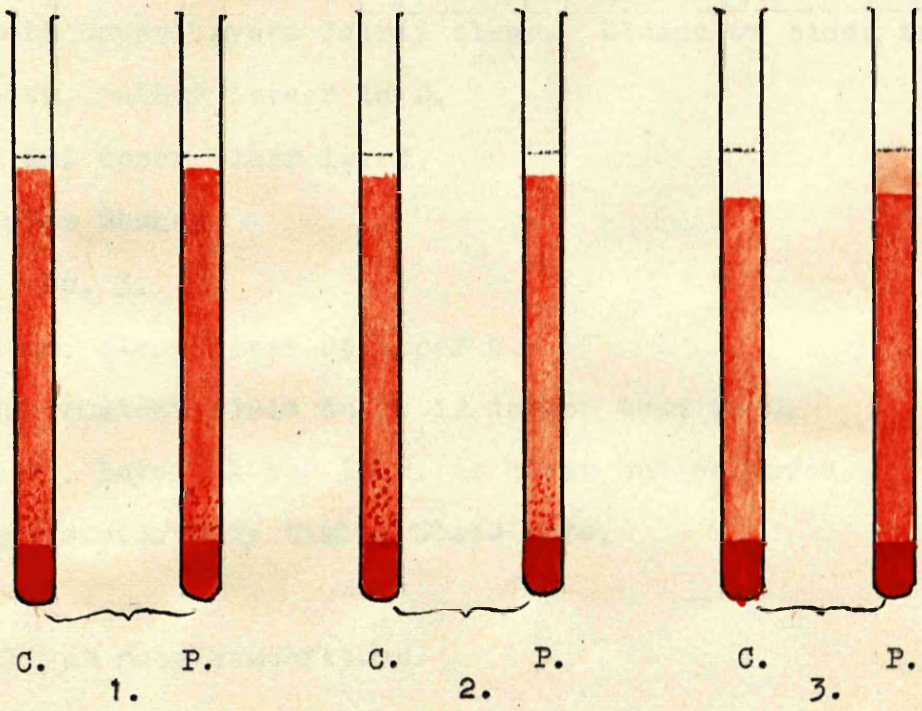
In C. clear coloured layer at top. Lysis. Rest of tube shews flocculent appearance. Colour darkening.

In P. sedimentation. Upper layer hazy but with clumps on side of tube.

7. P.M.

Shaken and examined microscopically. Fluid almost brown in colour. Clumps of cells with ill defined membrances. Dissolution taken place.

Experiment. 16.



Case. 56. E.S.R. 93.

26:10:43.

Patient's blood and my own taken at 4.50.P.M.

7.45.P.M.

Glutathione added.

7.55.P.M.No.1.

Both upper layers about the same. Clumps on sides in both tubes. Not very large.

About 1 mm. clear layer at top.

Experiment. 16. (Contd).

8.25.P.M. No. 2.

Both upper layers fairly clear. Clumps on sides in both, rather larger in C.

3 mm. upper clear layer.

Tubes Shaken.

10. P.M. No. 3.

5 mm. clear layer at topof C.

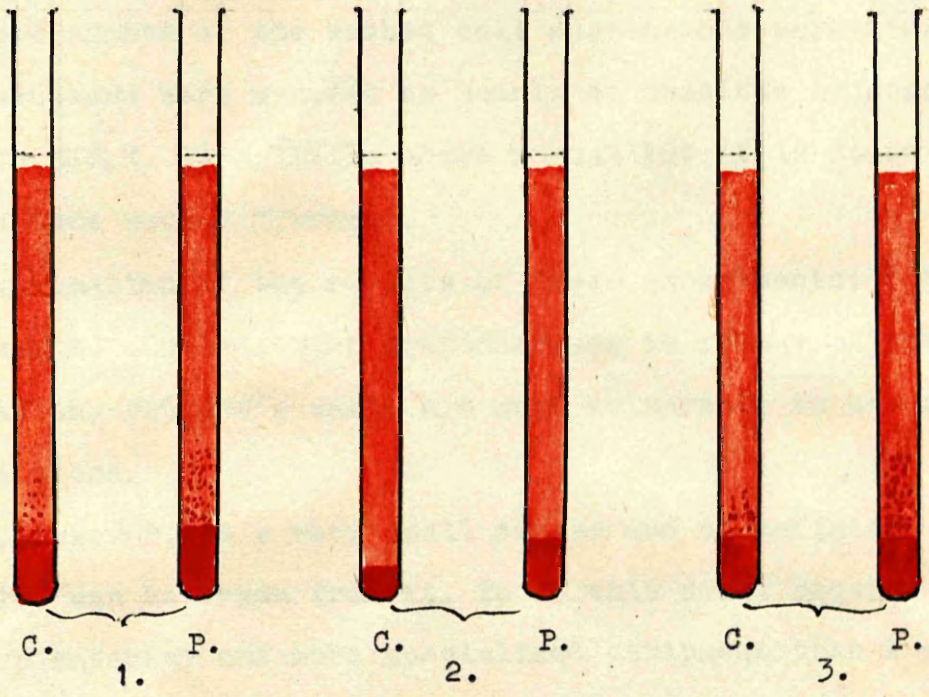
Supernatant fluid in P. is darker than in C.

5 mm. layer at top in P. is clear but coloured.

Lysis evidently taking place here.

All at room temperature.

Experiment. 17.



Case. 59. E.S.R. 78.

16:11:43. Patient's blood collected at 9.A.M. My own at 9.30.

4.42.P.M. Glutathione added.

5.5.P.M. No.1.

Slightly more sedimentation in P. Adhering clumps are small. More in P.

8.P.M. Tubes shaken.

8.45.P.M. NO.2.

No clumps.Sedimentation as shewn.Clear top layer. No Lysis. Colour good.Shaken and put in incubator

9.45.P.M. No.3.

As shewn. No lysis. Colour good.

In some of these cases where the blood was taken at the bedside, I collected some of my own at the same time - in both cases, four of blood to one of citrate.

No cell counts of the washed cell suspensions were done, but the suspensions were matched as nearly as possible by colour.

My own E.S.R. is a little above normal but it is doubtful if this has made much difference.

An examination of the results of these experiments; 8 to 17; would seem to shew a slight preponderance in favour of the idea that the patient's cells are more vulnerable to the action of glutathione.

This, however, is a very small series and no definite conclusion can be drawn from it. To do this would require more time, more material and more specialised equipment than I can command.

It has been previously quoted from "Advances in Enzymology" "--- it is clear that other substances (cysteine, ascorbic acid etc., shown to occur in living tissues are also potential activators ---".

The effect of pure ascorbic acid on similar red cell suspensions was tried.

This was found to have an effect somewhat similar to that of glutathione; agglutination, then haemolysis and finally lysis.

As observed in the testing tubes the action seemed to differ.

The clumps formed did not appear to be so adhesive and the flocculent appearance observed with glutathione before final cell dissolution was not noticed.

Like glutathione, its action was inhibited by Sodium fluoride. Though sedimentation did not occur, the contents of the tube seemed somewhat denser and darker in colour.

On the addition of Tannic Acid solution to the fluoride treated tube the reaction again differed. Instead of the rapid agglutination, sedimentation and retention of colour in the glutathione experiment, there was agglutination with practically no sedimentation, and the colour changed to a chocolate shade.

No further experimenting was done with the ascorbic acid.

Ascorbic acid is present in plasma but it may be questioned whether it has any effect on sedimentation rates. In some of the conditions where there is a very high rate, such as rheumatic fever and acute infections, there is a deficiency of ascorbic acid in the plasma.

Glutathione is not present in plasma so that the investigation of its effect on red cell suspensions can have no direct connection with the clumping which takes place in abnormal sedimentation rates.

The point of interest is that it can act as an agglutinin. Justification of its use in investigating what I have termed the vulnerability of the red cell in various conditions might

be found in another aspect of agglutination.

In the Thomsen phenomenon agglutination can be caused by certain types of contaminating bacteria which have gained access to stored blood.

"Agglutination then occurs because the cells themselves have become agglutinable by practically any normal human or animal serum".

"The Determination of Blood Groups." M.R.C. Memorand) 9

The point here is that the cells themselves have become altered and presumably the amount of alteration will be proportionate to the strength of the toxin which causes it.

If so, then it is a natural assumption, that in pathological conditions and in the variableness of these conditions, the agglutinability of the red cells will also vary.

That this is so, I believe, is shewn by the varying sedimentations rates.

The other question is whether the agglutinating action of Glutathione is similar or comparable to the action of naturally occurring agglutinins.

A possible answer to this may be found in the following experiments.

Experiment. 18.

24:10;43.

My own blood was used.

4 cc. of a mixture of 1 cc. of 3·8% Sod. citrate and 3 cc. of blood, were divided into two parts.

To one part was added 0·2 cc. of a 1% of Sod. Fluoride in normal saline.

To the other was added 0·2 cc. of normal saline.

Put up in Westergren tubes.

Result.

E.S.R.	1 hr.	2 hrs.	24 hrs.
Control tube	14.	36.	92.
Fluoride.	15.	37.	62.

Experiment. 19.

Case. 56.

26:10;43.

Citrated blood - 1 cc. of citrate to four of blood.

Collected at 4.45.P.M.

At 5.20.P.M. was divided into two parts.

To one part were added a few mgs. of Sod. fluoride.

Put up in Westergren tubes.

Result.

E.S.R.	1 hr.
Control	93.
Fluoride.	37.

During the first fifteen or twenty minutes there were obvious aggregations of cells in the top layer of the untreated tube.

This was not nearly so marked in the fluoride tube.

At the end of the hour the top layer of the fluoride tube was hazy with no clear line of demarcation.

The untreated tube shewed a clear line of demarcation with the upper layer clear.

Experiment. 20.

Case. 56.

31:1:44. One half of the citrated blood was treated with Sod. fluoride. A very small quantity on the point of a scalpel.

Westergren tubes were used.

Result.

E.S.R.	1 hr.	2 hrs.	4 hrs.
Untreated tube.	72.	108.	120.
Fluoride tube	67.	95.	110.

Experiment. 21.

Case. 56.

24:3:44. Same procedure followed.

Result.

E.S.R.	1 hr.
Untreated tube.	93.
Fluoride tube.	90.

In the upper part of the fluoride tube there was definite clumping.

In each of these experiments the quantity of fluoride used was much in excess of that evidently required to inhibit the enzyme forming lactic acid. It may have caused some physical change in the plasma itself.

The results of experiments 19. and 21. are difficult to reconcile but I am inclined to accept the result in 21.

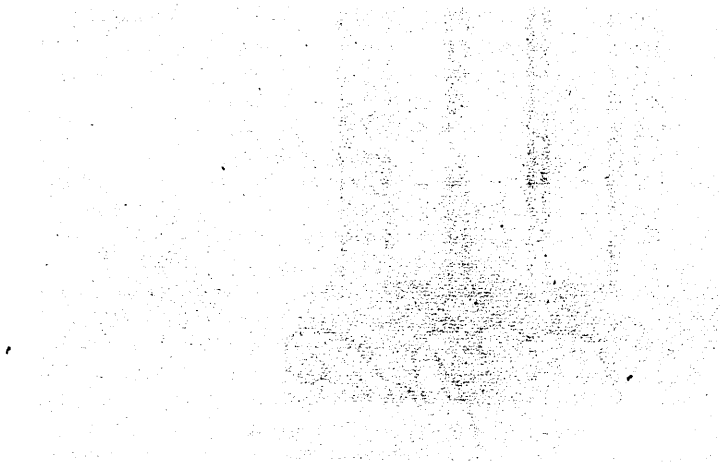
It has been shewn that the action of glutathione is inhibited by sod. fluoride. Evidently this does not apply to the substance or agglutinin which causes clumping in sedimentation.

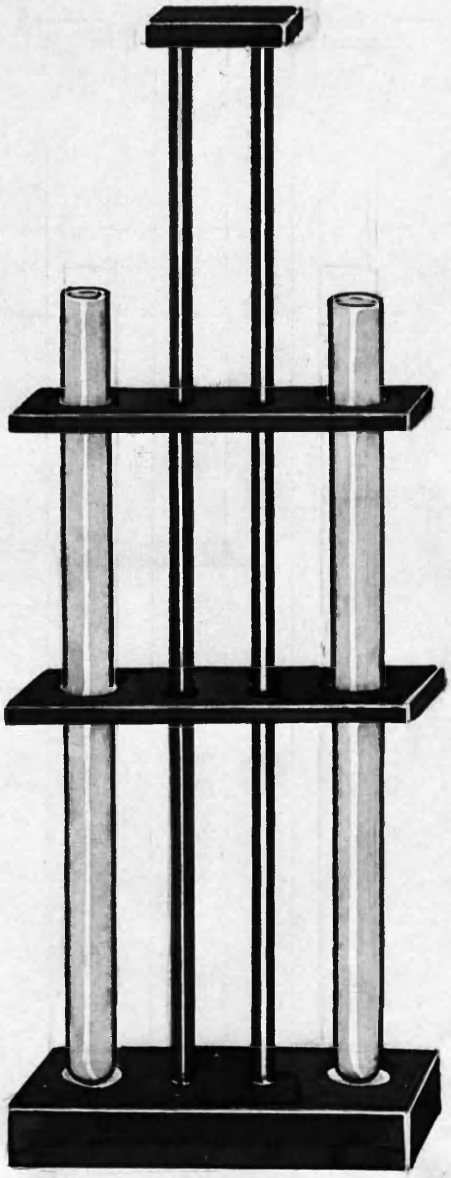
On the following page is shewn a piece of apparatus which I made to study the effects produced in the action of reduced glutathione on red cell suspensions.

The original tubes were slightly different in bore and were replaced by two Haldane haemoglobinometer testing tubes of practically identical bore.

The bottom is solid brass so that it can be immersed in the water in the chamber of the incubator.

It has two plasticene inserts for the tubes to rest on.





Full Size.

CONCLUSION.

I have instanced the clumping and sedimentation which takes place when cells and serum of incompatible bloods are put up in the capillary lymph tubes.

This in a medium free of fibrinogen.

The tannic acid reaction, though more rapid, follows the same pattern.

This in a medium devoid of protein.

In abnormal sedimentation rates there is again clumping followed by sedimentation, though more slowly.

This in a medium containing fibrinogen.

It would seem that the second essential part of the phenomenon of sedimentation is clumping of cells or of rouleaux. This accomplished, the third part would seem to be a simple gravitational action the rapidity of which would very according to the size of the clumps.

It has to be remembered that in the sedimentation test, the fall of the cells is taking place in a colloid solution.

According to most authorities the variation of the rate of sedimentation is caused by variation in the constitution of the plasma. It is stated that disturbance of the balance of the proteins in the plasma causes an increased rate.

I cannot speak directly as to this but in two or my cases (20 and 49) with heavy albuminuria and in which one would naturally expect a considerable disturbance in the protein balance, the sedimentation rate was only moderate.

Another explanation of the variation in the sedimentation rate is offered (Gordon and Wardley) - "the inhibition of one protein by another".

A happier expression to my mind would have been Variation in the lubricity of the plasma.

Neither of these statements of course is a strictly scientific explanation.

Increased fibrinogen content of the plasma has also been assigned as a cause of increased rates. As I have stated previously this, as an explanation, has seemed to me to be doubtful.

There is one aspect of the clumping which takes place, especially in high rates, which is worthy of note and that is the loose association of the cells and rouleaux which form the clumps.

The action is easily reversible and one would naturally expect this to be so. In vivo, the turbulence of the blood stream is probably sufficient to prevent agglutination.

So far, agglutination and the sedimentation subsequent upon this, have been discussed. These are phenomena which lend themselves to direct observation.

The first essential part of the phenomenon of sedimentation is largely of a speculative nature.

What causes the red cells to become agglutinable?

In the case of infectious diseases is it caused by the action of toxins?

What happens in chronic kidney and heart disease? Is the cause here nutritional?

In simple fractures with cell destruction and absorption due to enzyme action, has this action any effect?

Granting the presence of an agglutinogen - using this term in a generic sense - is the agglutinogen specific or non-specific?

Again, what activates the agglutinogen? Assuming - again in a generic sense - that it is an agglutinin, is this specific or non-specific? Is it present in normal blood or is it produced by the same causes which produce the agglutinogen?

The answers, so far as I can gather, to the most of these questions has still to be found.

I have only one observation bearing on one of them. In speaking of the Thomsen phenomenon it was stated that the cells became agglutinable to normal serum.

In section II, I have shewn some experiments where the cells from cases with varying sedimentation rates were treated with normal A.B.plasma. In them nothing occurred. There was no agglutination. I am not prepared to found on this because I don't know in what way the cells might be changed by washing.

Otherwise it might have been deduced that the particular agglutinin was not present in normal plasma.

Enzymology to-day is impinging more and more on medical science and the possibility of enzyme action entering into the causation of abnormal sedimentation rates was considered.

Tentative experiments to find a connection with enzyme action by the use of reduced glutathione have been described.

Though the results from this were somewhat analogous to natural sedimentation yet one can not found an analogy alone.

Whether the effect of the reduced glutathione is a bio-chemical or simply a physico-chemical one I am unable to say. The inhibition of its action by sodium fluoride is suggestive but not, I think, conclusive.

The acid test, the trial of sodium fluoride direct on citrated blood with abnormal rates, would seem to indicate, if this can be taken as a criterion, that the question of enzyme action can be excluded.

To myself and I expect to many others, the question of the causation of the first stage in abnormal sedimentation rates remains obscure.

CASES.

Case. O.

Mrs. I----.W----. Age. 28 Yrs.

1:11:41. Has had T.B. spine for about three years. Still wearing spinal support.

Complaining of urinary symptoms for about a year. Painful and frequent micturition. Pain and tenderness in right loin. General condition is good.

In the centrifuged deposit of one of the specimens of her urine I got a clump of T.B.

E.S.R. Westergren. 1 hr. 4.

A few weeks later the kidney was removed.

Case mentioned because it is one of the few occasions on which I have got T.B. in a urine, and also on account of the normal sedimentation rate in the presence of fairly extensive tubercular disease.

Case 1.

A----.H----. Age. 32 Yrs. Vanman

Nov. 1940 Complaining of cough, breathlessness and weakness. Examination shewed the usual thin, anaemic and listless subject. Foci present in both lungs.

E.S.R. Westergren. 1 hr. 57.

Notified and entered a Sanatorium.

17:12:41/

Case 1. (continued)

17:12:41.

Discharged from Sanatorium for disciplinary reasons. General and chest condition very much improved, but some cough still present.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	21.	13.

Refused to re-enter Sanatorium and started work.

13:3:42.

General and chest condition deteriorating. Still refused to enter Sanatorium.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	47.	24.

15:6:42.

Condition still deteriorating. Consented to enter Sanatorium.

	Westergren.		Capillary.tube.
	1 hr.	24 hrs.	1 hr.
E.S.R.	70.	125.	31.

Constant Volume. Wintrobe tube. 29%. Corrected. = 36%.

Case. 2.

H----.B----. Age 40 Yrs. Painter.

19:12:41.

Complaining of intense headache, associated sometimes with vomiting. Some acidity also complained of too. Though somewhat neurotic and easily excitable the headache is not typically migrainous. Knee jerks active. Otherwise healthy. No definite signs of lead poisoning. Blood picture normal.

	Westergren.	Capillary tube.
E.S.R.	2.	1·5.

29:6/42.

Not much change in symptoms or general condition.

	Westergren.	Capillary tube.
E.S.R.	1·5.	1·5.

Hb. 98 %. (Carbon monoxide - Haldane).

Citrated plasma p/H. between 7·6 and 7·8.

Blood group. B.

Case 3.

Mrs. A----. Age. 43. Yrs. Housewife.

23:1:42.

Menopausal; Flushing; periods of mental depression and intense lassitude. Weight increasing. Some arthritis in both feet.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	8.	7·5.

Case. 4.

Miss E----.H----. Age. 46 Yrs. Weaver.

10:5:41.

Subacute rheumatoid arthritis. Knees, wrists, meta-carpo-phalangeal and finger joints affected. Any of these would swell up separately at times and in a day or two subside again. One or other knee would fill up and in a few days be down again. Finger joints spindle shaped. General condition poor, never been off work.

Hb. 62%.

E.S.R. 1 hr. Westergren. 50.

Iron internally and Myocrisin injections.

After four injections the skin of the forearms shewed/

Case. 4. (continued)

shewed signs of irritation and the Myocrisin was stopped.

28:6:41.

General condition better but joints no better. Warren Crowe vaccine started.

3:10:41.

Joint condition seems a little better.

Hb. 68%.

E.S.R. 1 hr. Westergren. 12.

1:11:41.

General condition better. Joints not much more difference.

Hb. 72%.

21:3:42.

Stationary. Vaccine stopped.

	Westergren.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 hrs.
E.S.R.	24.	110.	14.	54.

29:8:42.

General condition and joints much the same. Myocrisin injections started again.

30:12:42.

General condition better but joints still troublesome/

Case. 4. (continued).

troublesome. No sign of skin com plications with the Myocrisin.

	Westergren. 1 hr.	24 hrs.	Capillary tube. 1 hr.	24 hrs.
E.S.R.	23.	110	14.	54.

Plasma Fibrinogen.

Colorimeter readings.

17·6
16·3
17·
17·1
18.
17.
17·2
17·4
17·5
17·8
172·9

Average. 17·3

Fibrinogen. (corrected). = 0·47%

16:10:43.

Finished another course of Myocrisin three weeks ago. General condition and joints much improved.

E.S.R.	1 hr.
Westergren.	9.
Veridia. 100 mm.	9·5
Micro-bore. 100 mm.	9·5
Wintrobe.	9·5

Constant/

Case. 4. (continued).

Constant volume. (Wintrobe). 29%. Corrected. 36%.

Plasma Fibrinogen.

Colorimeter readings.

22.
21.4
22.
21.8
21.7
22.
22.
22.1
21.8
22.
218.8.

Average. 21.9

Fibrinogen. (corrected). = 0.37%

Case. 5.

Miss B----.B----. Age. 57 yrs. Weaver

23:3:42.

Only complaint is of tiredness. No physical signs. General examination negative. Anaemic and debility.

	Westergren. 1 hr.	24 hrs.	Capillary tube. 1 hr.	24 hrs.
E.S.R.	12.	80.	12.5	35.

27:4:42.

After treatment and rest.

E.S.R./

Case. 5. (continued)

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube. 1 hr.	Capillary tube. 24 hrs.
E.S.R.	6.	92.	5.	39.

Case. 6.

Mrs. A----.H----. Age. 38 yrs. Housewife.

25.:3:42.

Pain and tenderness in left loin. No urinary symptoms. No albumin. Centrifuged urinary deposit shews a few pus cells and a varied microbial flora. Examination in Infirmary of kidney and urinary tract shewed no abnormality.

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube. 1 hr.	Capillary tube. 24 hrs.
E.S.R.	7.	70.	6.	35.

Case. 7.

Mrs. M----. Age 54 yrs. Housewife.

27:3:42.

Arthritis of right knee joint. Some thickening round the joint. Small amount of fluid present in the joint. General condition is good.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	5.	4·5

Case. 8.

J----. S----. Age. 59 yrs. Farmer

9:2:42.

Complaining for the last few days of pain in the front of the chest and cough.

History of not being well for about two months before. Is under weight and in rather poor condition.

Extensive pleuritic friction over the lower two thirds of the chest in front, in the left side, extending into the axillary region.

Lungs normal. Heart normal. Temperature normal. Good deal of fluid in the left pleurs.

Seen a few times. Fluid increasing. Beyond cough, there is no respiratory distress. Temperature always normal.

21:2:42.

This day he was having a profuse haematuria. There was present in the urine a number of thin worm-like clots, taken to be ureteral. Temp. normal. Aspirated his chest and withdrew 80 ounces of clear pleuritic fluid, without any respiratory distress.

Next evening he developed very severe pain in the left loin and was sent into Dundee Infirmary.

25:3:42/

Case 8. (continued)

25:3:42.

Seen on return home. Report from Infirmary. - "Examination of urinary tract reveals no abnormality. "Remains of fluid in pleura lessening".

A few days later was complaining of pain in the lower cardiac and epigastric area. He thought this was worse after eating and also on bending forward.

Further examination in the Infirmary Report. - "X ray of chest shows a little fluid remaining at left "base, but most of what was there has been absorbed. "Barium meal shews a normal gastro-intestinal tract."

Some days later he developed a swelling of the group of glands at the angle of the right jaw. Swelling fairly hard, large and painful, Remained hard, no softening. No abscess formation, but broke down superficially in one or two places with oozing of pus, more like a granuloma. Film of pus, stained Gram was negative.

	Westergren.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 hrs.
E.S.R.	86.	138.	45.	52.

28:4:42.

Glands improving.

over

Case. 8. (continued)

Hb. 60%
R.B.C. 3·4 mill.
W.B.C. 8800

	Westergren.	Capillary tube.
E.S.R. at end of 10 mins.	55.	24.
" 20 "	92.	36.
" 30 "	100.	41.
" 40 "	103.	44.
" 50 "	113.	45.
" 60 "	116.	46.

Constant volume. Wintrobe. 28%. Corrected. 35%.

The Hb. was done with a Lovibond Comparator using their blood slide with a film of actual blood 0·0045 inches thick. Pseudo-agglutination occurred so quickly that it was difficult to get a reading. Even diluting half with normal saline, did not help much.

It was this which stimulated my interest in the mechanism of the sedimentation rate.

5:6:42.

		Westergren.	Capillary tube.
E.S,R.	1 hr.	127.	48.

30:6:42.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	105.	45·5.

Pseudo-agglutination present in the citrated blood.

27:7:42.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	109.	32.

Plasma p/H. between 7·6 and 7·8.

Hb/

Case. 8. (continued)

Hb. 78%

	Westergren.	Capillary tube.
E.S.R. 1 hr.	73.	40.

Fibrinogen.

Colorimeter readings.

	14·7.
	14·7.
	15·6.
	15·4.
	15.
	15·4.
	15·7.
	15·9.
	16.
	15·7.
	154·1.
Average.	15·4.

Fibrinogen. Corrected. = 0·52%

18:11:42.

E.S.R.	1 hr.	Westergren	59.
"	"	Micro-bore. 200 mm.	54.
"	"	Micro-bore. 100 mm.	36.

Fibrinogen.

Colorimeter readings.

16.
16.
15·7.
15·7.
16·4.
16·5.
16·2.
16·1.
16·4.
16·2.
161·2.

Average/

Case. 8. (continued).

Average. 16·1.

Fibrinogen. Corrected. = 0·5%.

25:1:43.

		Westergren.	Micro-bore.
E.S.R.	1 hr.	57.	31·5.

Fibrinogen.

Colorimeter readings.

16.
15·7.
16·8.
16·6
16·6
17·2.
16·9.
16·9.
17·2.
17·6.
167·5

Average. 16·7.

Fibrinogen. Corrected. = 0·48%.

6:5:43.

Plasma p/H. between 7·6 and 7·8.

E.S.R.	1 hr.	24 hrs.
Westergren.	69.	128.
Micro-bore. 100 mm.	47.	63.
Wintrobe.	43.	-.

Constant volume. (Wintrobe) 30%. Corrected. 37%.

Fibrinogen/

Case. 8. (continued)

Fibrinogen.

Colorimeter readings.

13·5.
13·4.
13·3.
13·4.
13·8.
13·6.
13·3.
13·7.1
13·7.
13·4.
135·1.

Average. 13·5.

Fibrinogen. Corrected. = 0.6%.

Plasma p/H. between 7·6 and 7·8.

		Westergren.	Micro-bore.
E.S.R.	1 hr.	56.	34.

Fibrinogen.

Colorimeter readings.

16.
16.
15·8.
16.
16·3.
16·6.
15·9.
15·5.
16·2
15·7.
160·0.

Average. 16.

Fibrinogen. Corrected. = 0.51%

18:9:43./

Case. 8. (continued).

19:9:43.

E.S.R.	1 hr.	24 hrs.
Westergren	60.	124.
Veridia. 100 mm.	45·5.	63.
Micro-bore. 100 mm.	45·5.	63·5

Constant volume. (Wintrobe). Corrected. 35%.

Plasma p/H. 7·4.

Citrated blood shews a few discrete cells. Rouleaux formation. A few clumped rouleaux.

Fibrinogen.

Colorimeter readings.

18·5.
18·6.
19·2.
19·1
19·6.
19·6.
19·3.
19·3.
19·5.
19·6.
192·3.

Average. 19·2.

Fibrinogen. Corrected. = 0·42%.

Case. 9.

W----.L----. Age. 21 Yrs. Joiner.

2:4:42.

Persistent furunculosis and abscesses in axillae. Otherwise quite a good specimen.

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube. 1 hr.	Capillary tube. 24 hrs.
E.S.R.	5.	67.	4·5.	39.

Case. 10.

W.....R----. Age. 47 yrs. Factory Worker.

3:4:42.

Chronic bronchitis. For several years has had persistent attacks lasting several weeks at a time. Otherwise general condition is fairly good.

Blood group. O.

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube. 1 hr.	Capillary tube. 24 hrs.
E.S.R.	6·5.	67.	5.	45.

Case. 11.

Miss G----.B----. Age 57 Yrs. At home.

6:4:42.

Seen in April of last year. Complaining of being/

Case. 11. (continued).

being tired and a little breathless. No definite chest signs but there is a bad family history of T.B.

Hb. 70%.

E.S.R. 1 hr. Westergren. 50.

Improved and not seen again till now. Same Symptoms but breathlessness worse. Asthmatic type rhonchi with a little suspicious background. Some cough and a little sputum which is not purulent. No Lung signs. Suspicious of T.B.

Hb. 75%.

Blood group. A.

	Westergren.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 hrs.
E.S.R.	45.	124.	28.	43.

Note. A year later developed a rapidly increasing abdominal swelling, evidently a cystic ovarian condition. Operation shewed this to be a malignant ovarian cyst.

Case. 12.

Miss J----.D----. Age. 63 Yrs. Weaver.

24:4:42.

For a year or two has had attacks which shew râles and rhonchi appearing in different parts of the chest at different times. Practically no cough and no sputum obtainable. X ray examination negative. Suggested diagnosis of a condition of allergy.

	Westergren.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 hrs.
E.S.R.	18.	95.	13.	38.

Case. 13.

Mrs. D----. Age 76 Yrs. Housewife.

9:4:42.

Swelling of abdomen and discomfort. Some loss of weight. Loculated cystic tumour present in lower abdomen. Specialist uncertain whether simple or malignant. Hb. and normal E.S.R. seemed to indicate a simple condition, and this, at operation, it turned out to be.

Hb. 86%.

	Westergren.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 hrs.
E.S.R.	6·5.	82.	5.	38.

"The testis also of value in differentiating simple and malignant pelvic tumours".

"Whitby and Britton". 1939. p.115.

Case. 14.

H----.L----. Age. 36 Yrs. Nurse.

28:4:42.

Mild generalised arthritis-- not very typical. On holiday here for a week. Came to get an injection of Myocrisin, part of a course which she was getting elsewhere. General condition good.

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube. 1 hr.	Capillary tube. 24 hrs.
E.S.R.	6.	90.	2.	42.

Does not seem quite a suitable case for gold treatment.

Case. 15.

H----.R----. Age. 40 Yrs. Factory Worker.

2:5:42.

This case had been operated upon on 13:2:42 for tuberculous ascites. Been putting on weight and general condition fair.

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube. 1 hr.	Capillary tube. 24 hrs.
E.S.R.	32.	120.	26.	42.

1:6:42.

Hb. 80%.

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube. 1 hr.	Capillary tube. 24 hrs.
E.S.R.	18.	95.	15.	39.

30:6:42.

Hb. 96% Plasma p/H. 7·6.
Blood group. A.

E.S.R./

Case. 15. (continued).

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube. 1 hr.	Capillary tube. 24 hrs.
E.S.R.	10·5.	-.	9·5.	-.

28:7:42.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	8.	7.

1:9:42.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	6·5.	4·5.

31:10:42.

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary. 1 hr.	Capillary. 24 hrs.
E.S.R.	3.	51.	2.	28.

11:6:43.

E.S.R.	1 hr.	24 hrs.
Westergren	6.	80.
Micro-bore. 100 mm.	3·5.	39.

Fibrinogen.

Colorim eter readings.

22·7.
21·9.
21·6.
21·5.
22.
22·1.
22·1.
22.
22·1.
22·2.
220·2.

Average. 22.

Fibrinogen. Corrected. = 0·37%.

Case. 16.

T----. S----. Age. 54 Yrs. Farmer

4:5:42. Suffering from Lichen Planus of about nine months duration. General condition very good.

	Westergren.		Capillary tube.	
E.S.R.	1 hr.	24 hrs.	1 hr.	24 hrs.
	21.	113.	19.	45.

8:6:42.

	Westergren.		Capillary tube.	
E.S.R.	1 hr.	24 hrs.	1 hr.	24 hrs.
	18.	100.	10·5.	41.

13:7:42.

	Westergren.		Capillary tube.	
E.S.R.	1 hr.	24 hrs.	1 hr.	24 hrs.
	22.	-	18.	-

Bh. 92%. Plasma p/H. between 7.6 and 7·8.

Blood group. O.

28:8:42.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	16.	9.

Fibrinogen.

Colorimeter readings

25·6.
24·9.
25·0.
24·7.
25·6.
26.
25·6.
25·8.
26.
27.
257·1.

Average. 25·7.

Fibrinogen. Corrected. = 0.31%.

Case. 17.

C----.M----. Age. 45 Yrs. F. Shop-Assistant.

5:5:42.

Seen about six or seven weeks previously. Troublesome cough. Temp. running 102° - 103° for about a fortnight. No physical signs to be made out in the chest. Ten days after inception, test for T.A.B. and Brucella negative.

At the end of a fortnight the temp. fell to normal and a profuse purulent spit appeared. Moist rales now appeared at the bases, particularly at the right side. Sputum shewed an almost complete absence of microbial flora. T.B. negative. Complaining of weakness and profuse sweating at night.

	Westergren.		Capillary tube.	
E.S.R.	1 hr.	24 hrs.	1 hr.	24 hrs.
	4·5.	73.	4.	36·5.

T.B. taken to be absent.

Case. 18.

J----.M----. Age. 40 Yrs. Housekeeper.

6:5:42.

Small carcinoma of breast. No. glands. Excised later.

	Westergren.		Capillary.tube.	
E.S.R.	1 hr.	24 hrs.	1 hr.	24 hrs.
	4·5.	90.	4.	36.

Case. 19.

G----.G----. Age 21 Yrs. Housewife.

11:5:42.

Erythema Nodosum. Slightly anaemic.

Otherwise normal.

	Westergren.		Capillary tube.	
E.S.R.	1 hr.	24 hrs.	1 hr.	24 hrs.
	10·5.	94.	9·5.	38.

Case. 20.

W----.M----. Age. 65 Yrs. Farmer.

17:5:42.

Been under my care for the past four years. Hyper-tension and albuminuria. History of kidney trouble for the past fifteen years. His systolic pressure at first was running about 230 to 250 mm., though latterly it has been keeping round about 170. The albuminuria has always kept at a high level. There has never been any sign of oedema. He has a chronic anaemia which is iron resistent.

Hb. 65%
R.B.C. 4·3. Mill.
C.I. 0·76.
Plasma p/H 7·6.
Blood group. A.

E.S.R./

Case. 20. (continued)

E.S.R. 1 hr.

Westergren. 33.

Micro-tube. 100 mm. 35.

Wintrobe tube. 30.

Constant volume. (Wintrobe). Corrected. = 32·5%

Fibrinogen.

Colorimeter readings.

12.
12.
12·4.
12.
12·4.
12·4
12·7.
12·4.
12·1.
12·5.
122·9.

Average. 12·3.

Fibrinogen. Corrected. = 0·66%.

14:9:42.

Plasma p/H. 7·6.

E.S.R. 1 hr.

Westergren 37.

Micro-tube. 100 mm. 39.

Fibrinogen/

Case. 20. (continued).

Fibrinogen.

Colorimeter readings.

11·9.
12·4.
12·5.
13·4.
13·6.
12·5.
11·9.
12.
11·9.
11·8.
123·9.

Average. 12·4.

Fibrinogen. Corrected. = 0·66%.

30:11:42.

Hb. 70% .

E.S.R.	1 hr.
Westergren.	33.
Micro-tube. 100 mm.	25.

Fibrinogen.

Colorimeter readings.

19·7.
19·3.
20·5.
20·3.
20·5.
19·6.
19·5.
20·6,
19·9.
19·7.
199·6.

Average 19·9.

Case. 20. (continued)

Fibrinogen. Corrected. = 0·4%

4:10:43.

The urine to-day is turbid with hyaline and granular tube casts. These are the only abnormal constituents present. Albumin still present in large quantity.

Hb. 73%.

R.B.C. = 3·75. Mil.

Constant volume. (Wintrobe tube) 26.0 Corrected. = 32%

E.S.R.	1 hr.
Westergren	17.
Micro-tube. 100 mm.	24.
Veridia. 100 mm.	26.
Wintrobe.	24.

The citrated blood shews the bulk of the cells to be discrete. Rouleaux present, are small in size.

Fibrinogen.

Colorimeter readings

20.
19·9.
20.
20·1.
19·6.
19·8.
19·7.
20·1.
19·6.
20·2.
199·0

Average 20.

Fibrinogen. Corrected. = 0·41%.

Case. 21.

H----.J----. Age. 34. Housewife.

13:5:42.

Complaining of weakness and rather profuse vaginal discharge - leucorrheal, due to cervicitis.

Hb. 70%.

	Westergren.		Capillary tube.	
E.S.R.	1 hr.	24 hrs.	1 hr.	24 hrs.
	5·5.	87	4·5.	43.

Case. 22.

26:5:42.

Mrs. M ----. Age. 58 Yrs.

Seen two years ago. Rheumatic arthritis. Fingers of both hands and one wrist affected.

E.S.R. 1 hr. Westergren. 18.

Course of Myocrisin and seen about a year later.

E.S.R. 1 hr. Westergren. 12.

Another course of Myocrisin and seen to-day. Much improved.

	Westergren.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 hrs.
E.S.R.	9.	90.	9.	41.

Case. 23.

Mrs. D----. Age 49 Yrs.

29:5:42.

Severe dermatitis of scalp and face.

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube. 1 hr.	Capillary tube. 24 hrs.
E.S.R.	8.	80.	5·5.	35·5.

Case. 24.

3:6:42.

R~~~~~~~~-.S----. Age. 39 Yrs. Nurse.

Complaining of lassitude and loss of weight which have gone on for many months. Sent home from Hospital on long leave. Report from Hospital says nothing organically wrong discovered. Is now very thin and under weight.

Hb. 75%

		Westergren.	Capillary tube.
E.S.R.	1 hr.	3·5.	2·5.

Organic trouble would seem to be excluded.

Case. 25.

9:6:42.

Mrs. M----. Age. 59 Yrs.

Eighteen months ago had excision of breast for extensive carcinoma. Radical operation and thereafter deep X rays.

	Westergren.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 hrs.
E.S.R.	9.	83.	5.	45.

Case. 26.

13:6:42. J----.S----. Age. 65 Yrs. Retired Teacher.

Myocarditis. Slight glycosuria. Oedema of legs.

	Westergren.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 hrs.
E.S.R.	8.	75.	5.	38.

Case. 27.

19:6:42.

E----.B----. Age 37 Yrs. House-keeper.

Complaining of slight cough and great lassitude. Had been five years in a Sanatorium and discharged three years ago. Examination shews no physical signs in the chest.

Hb. 60%.

Plasma p/H. 7·4.

E.S.R./

Case. 27. (Continued).

	Westergren. 1 hr.	Westergren. 24 hrs.	Capillary tube, 1 hr.	Capillary tube, 24 hrs.
E.S.R.	13.	115.	1½.	-.

Case. 28.

27:6:42.

D----.N----. Age. 18 Yrs. Apprentice Engineer.

Extensive area of deep ulceration on posterior surface of left buccal membrane, extending on to the palate and lower gum. Sloughing and foul smelling. Temp. 99° - 99·5?

Examination of pus by dark ground condenser. Giemsa stain and Indian ink film shews fusiform bacilli, Vincent's spirochaetes and spirochaetae dentata.

Urine excretion test for Vitamin C. (dichlorphenol-indo phenol indicator) shews much deficiency. 3·65 gms. Ascorbic acid required to produce saturation.

Hb. 85%.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	23.	15.

Case. 29.

2:7:42.

C-----.P----. Age. 42 Yrs. Hotel-keeper.

Seen first three days ago. Complaining of pain over the lower part of the left side of the chest in front. No definite pleural friction, but fluid present at left base. Not much cough and no sputum. Some albumin in urine. Temp. runn ing 99·6 - 100·7.

Hb. 92%

W.B.C. = 8000

Plasma p/H between 7·6 and 7·8.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	85.	44.

5:7:42.

Fluid increasing. Temp. 100·8: Pulse 70.

Plasma p/H. between 7·6 and 7·8.

Blood group B.

10 cc. pleural fluid aspirated. Clear light amber.

p/H. 7·6.

Some mixed with 3·8% Sod. Citrate soln. and centrifuged. Stained deposit is mostly lymphocytes.

Cytology of fluid indicates tuberculous pleurisy.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	96.	42·5.

19:7:42./

Case. 29. (continued).

19:7:42.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	96.	42.

16:8:42.

General condition not so good. Has developed a fistula in ano.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	105.	42·5.

15:10:42.

General condition improving. Fluid absorbing well.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	86.	41.

15:11:42.

Fluid still absorbing well. General condition much better. Sent away for the winter.

E.S.R.	1 hr.
Westergren.	76.
Micro-bore. 200 mm.	69.
Micro-bore. 100 mm.	46.

Fibrinogen/

Case 29. (Continued).

Fibrinogen.

Colorimeter readings.

16·6.
16·4.
16.
16·6.
16·5.
16·6.
16·7.
16·9.
16·2.
16·4.
165·0.

Average 16·5.

Fibrinogen. Corrected. = 0·49%

1:8:43.

Hb. 80%

Plasma p/H. 7·6.

E.S.R.	1 hr.
Westergren.	22·5.
Veridia. 100 mm.	26.
Veridia. 200 mm.	40.

In the Veridia 200 mm. column there was a good deal of haze. Citrated blood shews only discrete and also rouleaux formation. These are not large - two or three to seven cells in each rouleaux. Groups of about 2 cells clumping with one or two similar groups.

Fibrinogen/

Case. 29 (continued)

Fibrinogen.

Colorimeter readings.

21.
20·8.
21.
21·4.
20·6.
21·2.
20·8.
21.
21.
21.
209·8.

Average. 21.

Fibrinogen. Corrected. = 0·37%.

Case. 30.

13:7:42.

M----.N----. 58. Yrs. Weaver.

Only complaint is of feeling very tired. Nothing organically wrong is to be discovered.

Hb. 70%

Plasma p/H between 7·6 and 7·8

Blood group O.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	35.	19.

3:9:42./

Case. 30. (continued)

3:9:42 .

		Westergren.	Capillary tube.
E.S.R.	1 hr.	12.	12.

Case. 31.

26:8:42.

M----.R----. Age 48 years. Weaver.

Complaining of feeling tired and of stomach acidity and abdominal tightness. Nothing organically wrong to be made out.

	Westergren.	Capillary tube.
E.S.R.	3.	3·5.

Fibrinogen.

Colorimeter readings.

27·4.
26·6.
25·7.
25·1.
25·6.
27·6.
26.
26·4.
26.
26·4.
262·8.

Average 26·3.

Fibrinogen. Corrected. ∓ 0·31%.

Case. 32.

7:9:42 .

P----. B----. Age. 20 Yrs. Farm Worker.

Large abscess of fore-arm, Otherwise a very healthy specimen.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	7.	6·5.

Case. 33.

A----.R----. Age. 25 Yrs. Clerkess.

16:9:42.

Com plaining of occasional pain and discomfort in lower abdomen. Some tenderness over appendix area. Tentative diagnosis of chronic appendix. Otherwise normal.

		Westergren.	Capillary tube.
E.S.R.	1 hr.	6·5.	5·5.

Note. Examined some months later by Gynaecologist who diagnosed retroversion of uterus.

Case. 34.

18:9:42.

L----.H----. Age. 34. Yrs. Weaver.

Complaining of intense lassitude and inability for work. Nothing organically wrong discovered either here or in the Infirmary.

Hb. 70%.

Westergren. Capillary tube.

E.S.R. 1 hr. 11. 9.

Fibrinogen.

Colorimeter readings.

14·4.
14·5.
14·6.
14·6.
14·4.
14·6.
13·9.
14·4.
15.
14·2.
144·6.

Average. 14·4.

Fibrinogen. Corrected. ∓ 0·56%

Case. 35.

19:9:42.

D-----.F-----. Age. 39 Yrs. M. Factory Worker.

Rheumatic arthritis of joints of both hands.

Otherwise nothing abnormal

		Westergren.	Capillary tube.
E.S.R.	1 hr.	7.	8.

Fibrinogen.

Colorimeter readings.

15·5.
15·8.
16·1.
15·8.
16·4.
16·5.
16·5.
16·8.
17.
163,3.

Average. 16·3.

Fibrinogen. Corrected. = 0·5%

Case. 36.

22:10:42.

L----. B----. Age. 39 Yrs. Weaver.

For several years has had regular spells of anaemia, each lasting for about two months. Somewhat resistant to iron; irresponsive to liver therapy.

	Westergren.		Capillary tube.	
	1 hr.	24 hrs.	1 hr.	24 Hrs.
E.S.R.	8.	90.	8.	40.

Case. 37.

4:11:42.

Mrs. T----. Age. 32. Yrs.

Anaemia, inter-costal myalgia (?) and subacute synovitis of knee joint.

	Westergren.	Capillary tube.
E.S.R.	4.	4.

Case. 38.

12:11:42.

Mrs. K----. Age. 42. Yrs.

Menopause, troublesome on account of excessive hemorrhage. Latterly had to have radium treatment. Recently much better.

E.S.R.	1 hr.
Westergren.	3.
Micro-bore. 200 mm.	3.
Micro-bore. 100 mm.	3.

Case. 39.

5:12:42.

J----.S----. Age. 32 Yrs. Clerk.

Seen first two years ago. Weakness, sweating and temp. (100·5). Cough and fairly profuse haemoptysis. I got T.B. in his sputum.

E.S.R. 1 hr. Westergren. 60.

Went/

Case. 39. (continued).

Went into Sanatorium for about eighteen months. Is now very much improved.

E.S.R. 1 hr.

Westergren. 1·25.

Micro-bore. 100 mm. 1.

Fibrinogen.

Colorimeter readings.

26.
26·2.
26·8.
26.
25·5.
25·4.
26·4.
26·8.
27.
26·6.
262·7.

Average. 26·3.

Fibrinogen. Corrected. = 0·31%

28:4:43.

Plasma p/H. 7·4.

E.S.R. 1 hr.

Westergren 1.

Micro-bore. 200 mm. 0·75.

Micro-bore. 100 mm. 0·75.

Fibrinogen./

Case. 39. (continued).

Fibrinogen.

Colorimeter readings.

26.
26·5.
26·5.
27.
26·6.
27.
26·2.
26·6.
27.
26·8
266·2.

Average. 26·6.

Fibrinogen. Corrected. = 0·31%

2:2:44.

E.S.R.	1 hr.
Westergren.	1·75.
Micro-bore. 100 mm.	2.
Veridia. 100 mm.	1·75.
Wintrobe. Tube.	1:25.

Constant volume. (Wintrobe). 34% Corrected. 42·5%

Citrated blood shews rouleaux formation and free cells. The rouleaux are comparatively small.

Fibrinogen/

Case. 39. (continued)

Fibrinogen.

Colorimeter readings.

29·6.
29.
30·3.
30·7.
30.
30·4.
29·9.
30·2.
30·7.
29·8.
300 ·6.

Average. 30.

Fibrinogen. Corrected . = 0·27%.

Case. 40.

22:1:43.

A----. C----. Age 43 Yrs. F.

Swelling of glands on both sides of neck. Been running a temp. (100° - 102°) for the past four weeks. Evidently T.B. Definitely milk infection. Operated on ultimately.

E.S.R.	1 hr.	24 Hrs.
Westergren.	27.	121·5.
Micro-bore 100 mm.	27.	56·5.

Case. 41.

20:4:43.

Mrs M----. Age. 29 Yrs.

Persistent cough of some months duration, and feeling of lassitude. Bad T.B. family history. Examination of chest negative.

Hb. 80%

E.S.R.	1 hr.
Westergren	5.
Micro-bore. 100 mm	6.

Fibrinogen.

Colorimeter readings.

27·4.
27·8.
27·1.
27·4.
27·4.
27·6.
27·6.
27·5.
27·4.
27·4.
274·6.

Average. 27·4.

Fibrinogen. Corrected. = 0·28%

Case. 42.

J-----. M-----. Age. 58 Yrs. M.

Complaining of persistent pain slightly above right loin. Has got much thinner during the last six months. Some resistance in epigastrium. X ray report, suspicion of appendix. Otherwise nothing much to be made out.

E.S.R.	1 hr.
Westergren.	9.
Micro-bore. 100 mm.	4·5.

Plasma is pretty yellow in colour though there is no jaundice.

Fibrinogen.

Colorimeter readings.

20·8.
21·1.
21·1.
21.
21·5.
21·5.
21·4.
21·6.
21·9.
21·8.
213·7.

Average. 21·4.

Fibrinogen. Corrected. = 0·37%

Note. Operation three days later disclosed malignant growth in liver.

Case. 43.

27:4:43.

R----. B----. Age. 35 Yrs. Clerkess.

Complaining of persistent pain over right shoulder and in right arm. Evidently some neuritis in the brachial plexus. No discoverable focus of infection.

Hb. 80%.

Plasma p/H. 7·4.

Plasma milky. Evidently due to presence of chyle.

E.S.R.	1 hr.
Westergren.	1.
Micro-bore. 100 mm.	1.

Fibrinogen.

Colorimeter readings.

30·3.
30·2.
31·1.
31·3.
30·7.
30·5
30·2.
30·5.
30·3.
30·2.
305·2.

Average. 30·5.

Fibrinogen. Corrected. = 0·26%

Case. 44.

30:4:43. W----. W----. Age. 31 Yrs. Mechanic.

Complaining of headache, abdominal swelling and discomfort. Vomiting immediately after meals. Bulimia. Nothing organically wrong to be made out.

E.S.R.	1 hr.
Westergren,	1·25.
Micro-bore. 200 mm.	1.
Micro-bore. 100 mm.	1·25.
Wintrobe tube	1·25.

Constant volume. (Wintrobe). 39%. Corrected..48%

Fibrinogen.

Colorimeter readings.

25·5.
26.
26.
25·2.
25·9.
25·2.
26.
25·7.
26.
26.
257·5.

Average. 25·7

Fibrinogen. Corrected. = 0·31%

Case. 45.

4:5:43.

Mrs. L----. Age. 50. Yrs. Housewife.

Convalscent after pleura-pneumonia.

Hb. 80%

E.S.R.	1 hr.
Westergren.	6.
Micro-bore. 200 mm.	3·75.
Micro-bore. 100 mm.	4.
Wintrobe tube.	4.

Constant volume. (Wintrobe) 32 %. Corrected. 40%.

Case. 46.

9:5:43.

W----. S----. Age. 65 Yrs. Blacksmith.

Been under treatment during the past four years for pernicious anaemia. Kept going on maintenance doses of Neo-Hepatex. For a little time past this has not been very satisfactory. (See Note).

Hb. 64%

R.B.C. 2·8. Mill.

C.I. 1·1.

Plasma p/H. between 7·6 and 7·8.

E.S.R./

Case. 46. (continued)

E.S.R.	1 hr.	24 hrs.
Westergren	19.	110.
Micro-bore 100 mm.	18.	60.
Wintrobe tube	14.	

Constant volume. (Wintrobe). 27%. Corrected. 33·7%.

Fibrinogen.

Colorimeter readings.

17·7.
17·6.
17·9.
18.
18·2.
17·8.
18.
17·8.
18.
17·8.
178·8.

Average. 18.

Fibrinogen. Corrected. = 0·45%.

Note.

A remark by Professor A. Patrick that he had been dissatisfied with Neo-Hepatex, led me to try Anahaemin in this case. Eight days after the administration of 4 cc., slides (prepared with Brilliant cresyl blue and stained later with Leishman) shewed a very marked reticulocytosis. The appearance as if the reticulum was/

Case. 46. (continued)

was being extruded from the corpuscles was striking.

Professor D.F.Cappell who saw one of my slides remarks:-

"When the substance is precipitated by "the dye there is no doubt that it can be extruded "from the cell. I have often watched this happen "under the dark ground illumination, but it has "first to be precipitated".

Case 47.
11:5:43.

Mrs. S----. Age. 38 Yrs. Housewife.

Anaemia, which seemed to be simple in type. Iron resistant for about a year. Latterly, responed to "Lextron" (Liver-stomach concentrate with ferrous iron and Vitamin B. complex.)

Hb. 80%

R.B.C. 3·35 mill.

Plasma p/H between 7.6 and 7·8.

Blood group. B.

Typed at first visit as group A. (with whole blood). One part of red corpuscles to three of group B. plasma/

Case. 47. (continued).

11:5:43.

plasma put up in a Westergren tube gave a 1 hr. fall of 5. This was suspicious. Typed at next visit with diluted blood (1 in 20) gave group B.

E.S.R.	1 hr.	24 hrs.
Westergren.	5.	85.
Micro-bore. 100 mm.	6.	49.
Wintrobe tubee	5.	-.

Constant volume. (Wintrobe). 28%. Corrected. 35%.

Fibrinogen.

Colorimeter readings.

28·8.
28·2.
28·2.
29.
28·9.
29.
28·7.
28·9.
29.
28·4.
287·1.

Average. 28·7.

Fibrinogen. Corrected. = 0·3%.

Case. 48.

15:5:43.

D----. C----. Age. 34 Yrs. Factory Worker.

Persistent large boils on forearms. Otherwise Normal.

Hb. 90%.

R.B.C. 4·5 mill.

Plasma p/H. 7·4.

Group O.

E.S.R.	1 hr.	24 hrs.
Westergren.	5.	79.
Micro-bore. 100 mm.	5.	46·5.
Wintrobe tube	5.	-.

Constant volume. (Wintrobe). 31·5. Corrected. 39%.

Fibrinogen.

Colorimeter readings.

22·7.
22·3.
22·4.
22·6.
22·4.
22·5.
22·5.
22·8.
22·2.
22·6.
225·0.

Average. 22·5.

Fibrinogen. Corrected. = 0·35%.

Case. 49.

18:5:43.

M-----.B-----. Age. 50 Yrs. Housekeeper.

Complaining of weakness and attacks of severe headache with vomiting. Anaemic, oedema of legs, hyperpiesis and fairly heavy albuminuria. B.P. Systolic 230.

Hb 65%

R.B.C. 3·2 mill.

Plasma p/H between 7·6 and 7·8.

Group. A.

Cells in citrated blood are all discrete.

E.S.R.	1 hr.	24 hrs.
Westergren	13.	130.
Micro-bore. 100 mm.	14.	66.
Wintrobe tube.	12·5.	-.
One part cells to three parts B. plasma in Westergren tube	116.	135.

Constant volume. (Wintrobe). 20% Corrected. 25%

Fibrinogen.

Colorimeter readings.

22.3.
23.
22.
22·1.
22·2.
22·4.
22·5.
22.
22·5.
22·3.
223·3.

Average. 22·3.

Case. 49. (continued.

Fibrinogen. Corrected. = 0·37%

24:9:43.

Plasma p/H. 7·4.

E.S.R.	1 hr.
Westergren	12.
Veridia. 100 mm.	15.
Micro-bore. 100 mm.	12·5.
Wintrobe tube	12.

Constant volume. (Wintrobe). 20%. Corrected. 25%

Fibrinogen.

Colorimeter readings.

23·5.
23·7.
23·6.
23·5.
23·7.
24·2.
23·8.
24·3.
24·2.
24·4.
238·9.

Average. 23·9.

Fibrinogen. Corrected. = 0·33%.

Case. 50.

24:5:43.

B----.B----. Age. 52 Yrs. Housewife.

Complaining of weakness and inability to do her work. No other symptoms and nothing organically wrong to be discovered.

Hb. 78%.

R.B.C. 3·8 mill.

Plasma p/H. 7·4.

Group A.

E.S.R.	1 hr.
Westergren.	5·5.
Micro-bore. 100 mm.	5·5.
Wintrobe.	5·5.

Constant volume. (Wintrobe). 29%. Corrected. 36%.

Fibrinogen.

Colorimeter readings.

25.
25·5.
25.
25.
24·7.
24·6.
24·6.
25·2.
24·9.
25.
249·5.

Average. 25.
Fibrinogen. Corrected. = 0·32%.

Case. 51.

12:6:43.

E-----.N-----. Age. 32. Yrs. Nurse.

Complaining of feeling tired. Has lost over a stone in weight during the past year and has been X rayed three times with negative results. Some tachycardia. Otherwise nothing organically wrong to be discovered.

Hb. 90%.

R.B.C. 4·3 mill.

Plasma p/H 7·6.

Group O.

Group was typed at first as A. Subsequently corrected. Patient's cells and Group O. plasma in capillary tubes shewed nil drop.

E.S.R.	1 hr.	24. hrs.
Westergren.	3.	60.
Micro-bore. 100 mm.	3.	35.
Wintrobe tube	3.	-.

Constant volume.(Wintrobe). 32%. Corrected. 40%

Fibrinogen/

Case. 51. (continued).

Fibrinogen.

Colorimeter readings.

28·8.
27·4.
28·2.
27·9.
28.
27·6.
28·2.
28·4.
28·1.
28·3.
280·9.

Average. 28·1.

Fibrinogen. Corrected. = 0·28%.

Case. 52 .

16:6:43. Mrs. F----. Age. 47 Yrs. Housewife.

Arthritis of right hip joint. Otherwise nothing abnormal. After X ray examination about two years ago, had a course of Myocrisin which improved the condition. Is now starting another course.

Hb. 90%.

R.B.C. 4·8 mill.

Plasma p/H 7·6.

Group A.

E.S.R./

Case. 52. (continued)

E.S.R. 1 hr.

Westergren 17.

Micro-bore 100 mm. 17.

Wintrobe tube 17.

Constant volume. (Wintrobe). 31%. Corrected. 39%.

Fibrinogen.

Colorimeter readings.

24·6.
24·7.
24·2.
24·3.
23·8.
24·4.
24·4.
24·3.
23·8.
23·8.
242·3.

Average. 24:2.

Fibrinogen. Corrected. = 0·33%.

Case. 53.

13:7:43.

J----.L----. Age. 48 Yrs. Weaver.

Complaining of feeling tired. Arthritis of finger joints of both hands. Swollen and painful. Evidently rheumatoid. Otherwise nothing abnormal to be made out.

Hb. 82%.

Citrated/

Case. 53. (continued.

Citrated blood shews rouleaux formation, but the cells are mostly discrete.

E.S.R.	1 hr.
Westergren.	15.
Micro-bore. 100 mm.	15.

Fibrinogen.

Colorimeter readings.

26·7.
27.
27·1.
26·7.
26·8.
26·6.
26·2.
27·1.
27.
27.
269·2.

Average. 26·9.

Fibrinogen. Corrected. = 0·3%.

17:8:43.

Plasma p/H 7·4.

Citrated blood shews rouleaux formation with occasional free cell.

E.S.R.	1 hr.	24 hrs.
Westergren.	7·5.	77.
Micro-bore. 100 mm.	7·5.	49.
Veridia. 100 mm.	7·5.	41.

18:12:43/

Case. 53. (continued).

18:12:43.

Plasma p/H. 7·4.

Citrated blood shews half short rouleaux, half discrete cells.

E.S.R.	1 hr.
Westergren.	5.
Micro-bore. 100 mm.	6.
Veridia. 100 mm.	6·5.
Wintrobe.	5.

Constant volume. (Wintrobe). 32·5%. Corrected. 40%.

Fibrinogen.

Colorimeter readings.

29·6.
29·7.
30.
29·7.
29·8.
30·4.
30·3.
30.
29·9.
30·3.
299·7.

Average. 30.

Fibrinogen. Corrected. = 0.27%.

Case. 54.

11:8:43.

Mrs. S----. Age. 52 Yrs. Housewife.

Subacute rheumatism, Fingers, wrists, elbows, knees and ankles affected. Heart untouched. Temp. 101·5°.

Citrated blood shews rouleaux formation. Rouleaux formation is not so large as in normal rouleaux. Very few discrete cells.

E.S.R.	1 hr.
Westergren.	75.
Micro-bore. 100 mm.	45.
Veridia. 100 mm.	44·5.

In about fifteen or twenty minutes the upper part of the tubes shew macro-clumping, very obvious with a hand lens. Cells examined after one hour's sedimentation shew more free cells and compacted rouleaux.

11:8:43.

Plasma p/H. between 7·6 and 7·8.

Rouleaux formation. Many of the rouleaux are short. Many free cells.

E.S.R.	1 hr.
Westergren.	46.
Micro-bore. 100 mm.	39.
Veridia. 100 mm.	40.

Fibrinogen/

Case. 54. (continued).

Fibrinogen.

Colorimeter readings

20·2.
20.
20·3.
19·9.
20.
20·3.
20·2.
20·1.
20.
20·4.
201·4.

Average. 20.1.

Fibrinogen. Corrected. = 0·41%.

6:9:43.

Hb. 72%

Plasma p/H between 7·6 and 7·8.

Citrated blood shews a few discrete cells. Rouleaux present - not large, and forming clumps here and there.

E.S.R.	1 hr.
Westergren	30.
Micro-bore. 100 mm.	32.
Veridia. 100 mm.	31.
Wintrobe tube.	24.

Constant volume. (Wintrobe). Corrected. 35%.

After/

Case. 54. (continued)

After about twenty minutes, clumping is well seen in the upper layers of the Veridia tube with an X 8 lens.

Fibrinogen.

Colorimeter readings

22·3.
22·5.
22·2.
22·5.
22·5.
22·7.
22·5.
22·8.
23.
22·7.
225·7.

Averages. 22·5.

Fibrinogen. Corrected. = 0·36%.

Case. 55.

R----. M----. Age. 33 Yrs. Plumber.

Complaining of much lassitude at his work and cough of several months duration. Sputum at times is blood streaked. Has been losing a bit of weight. In November last was marked Grade 3. by Medical Board.

Examination of chest does not disclose any special signs.

Hb. 95%

Plasma p/H 7·6.

Citrated/

Case. 55. (continued)

Citrated blood shews rouleaux present - not large and some of them loosely put together. Many free cells.

E.S.R.	1 hr.
Westergren	3.
Micro-bore. 100 mm.	2·5.
Veridia. 100 mm.	3·25.

Fibrinogen.

Colorimeter readings.

31·5.
31·3.
30·7.
32·2.
30·9.
31·7.
31·5.
31·4.
31·2.
31·4.
312·8.

Average. 31·3.

Fibrinogen. Corrected. = 0·25%.

Case. 56.
5:9:43.

Mrs. P----. Age. 61 Yrs. Housewife.

Intra-capsular fracture of right thigh about five months ago. Treated by Smith-Petersen pin. For several weeks past has been complaining of pain in the hip/

Case 56. (continued)

hip and in the groin. X ray taken three weeks ago shews a little bone absorption at seat of fracture and fairly extensive arthritic changes in the hip joint. Otherwise general condition is fairly good.

Hb. 72%

R.B.C. 4·2. mill.

Plasma p/H 7·6.

E.S.R.	1 hr.
Westergren.	108.
Micro-bore. 100 mm.	51·5.
Veridia. 100 mm.	52.
Wintrobe tube.	39.

Constant volume. (Wintrobe). Corrected. 35%.

Citrated blood shews very few discrete cells. There is rouleaux formation; the rouleaux forming large clum ps, not unlike true agglutination, but there is no condensation of haemoglobin though the cell outline in the centre of some clumps is indistinct.

In about twenty minutes, formation of large clumps visible to the naked eye and well shewn by an X 8. lens. were appearing in the upper layer of the Westergren tube.

In the Veridia tube the X 8. lens shewed very marked/

Case 56. (Continued)

marked clumping throughout the column of sedimenting corpuscles.

Fibrinogen.

Colorimeter readings.

11·5.
11·7.
11·5.
11·6.
11·7.
11·4.
11·5.
11·6.
11·5.
11·4.
115·4.

Average. 11·5.

Fibrinogen. Corrected. = 0·71%

9:9:43.

Hb. 72%

R.B.C. 3·45 mill.

W.B.C. 10600.

E.S.R.	1 hr.
Westergren.	98.
Micro-bore. 100 mm.	57.
Veridia. 100 mm.	57.
Wintrobe tube	39.

Constant volume. (Wintrobe.) 26·5. Corrected 33%.

Citrated/

Case. 56. (continued)

Citrated blood shews few discrete cells. Rouleaux formation. Rouleaux are gathered chiefly in large clumps. In some of these it is difficult to define the cell outlines. There is an appearance of real agglutination but no concentration of haemoglobin.

25:9:43.

Patient moving about quietly and to all appearance is in fairly good health.

Hb. about 75%.

Plasma p/H. 7·6.

E.S.R.	1 hr.
Westergren	114.
Micro-bore. 100 mm.	61.
Veridia. 100 mm.	61.
Wintrobe tube.	63.

Constant volume. (Wintrobe), 27%. Corrected. 33·6%.

The citrated blood shews free cells and rouleaux. There are present also clumps of rouleaux.

Fibrinogen/

Case. 56. (continued)

Fibrinogen.

Colorimeter readings

11·7.
11·8.
12.
11·6.
11·9.
11·7.
11·7.
11·8,
11·8.
11·7.
117·7.

Average. 11·8.

Fibrinogen. Corrected. = 0·68%.

26:11:43.

W.B.C. 14000.

Plasma p/H. 7·4.

E.S.R.	1 hr.
Westergren	83.
Micro-bore. 100 mm.	40.
Veridia. 100 mm.	44·5.
Wintrobe tube	45.

Constant volume.(Wintrobe). Corrected. 38·5%

Fibrinogen/

Case. 56 (continued).

Fibrinogen.

Colorimeter readings.

12.
12·2.
12.
12.
12·1.
12.
11·9.
12·3.
12·2.
12.
120·7.

Average. 12.

Fibrinogen. Corrected. = 0.67%.

31:1:44.

Hb. 72%.

W.B.C. 12600.

E.S.R. 1 hr.

Westergren. 72.

Micro-bore. 100 mm. 35·5.

Constant volume. (Wintrobe). 32% Corrected. 40%.

Citrated blood shews practically complete rouleaux formation - a very few scattered discrete cells.

The slide examined by transmitted light and with/

Case. 56. (continued)

with an X 8. hand lens shews the clumps well - appearance of pseudo-agglutination, but the cell outlines are clear and there is no appearance of condensation of haemoglobin.

Case. 57.

Mrs. B----. Age. 32. Yrs.

Six months pregnant. Complaining of pruritis of vulva. Examination of urine shews some glycosuria. 2 para.

E.S.R.	1 hr.	24 hrs.
Westergren	24.	130.
Micro-bore. 100 mm.	32.	67.
Veridia. 100 mm.	26.	67.

Constant volume. (Wintrobe). Correc ted 27·5%.

Citrated blood shews rouleaux formation and a good few discrete cells. No rouleaux clumps.

Fibrinogen.

Colorimeter readings.

21.
20·3.
21·2.
21·4.
21·2.
21·2.
21·3.
21·5.
21·5.
21·3.
211·9.

Average 21·2.

Fibrinogen. Corrected. = 0·37%.

Case. 58.

A----. H-----. Age. 14. Yrs.

Rheumatic Fever.

E.S.R.	1 hr.
Westergren	91.
Hellige Micro-tube	56.

Case. 59.

Mrs.L----.

Sub-acute Rheumatism and Mitral Stenosis.

E.S.R.	1 hr.
Westergren	78.

Both the above cases are in Dundee Royal Infirmary under the care of Professor Patrick by whose courtesy citrated blood from them was sent to me for experimental purposes.

Case. 60.

W----. F----. Age. 46. Shopkeeper.

16:12:43.

Complaining of muscular rheumatism of back and shoulders. X ray examination shews some arthritis of spine. Some hyperacidity. Otherwise normal.

E.S.R./

Case. 60. (continued)

E.S.R.	1 hr.	24 hrs.
Westergren	2·5.	63.
Micro-bore. 100 mm.	2.	40.
Veridia. 100 mm.	1·5.	38.
Wintrobe	2.	-.

Citrated blood shews mostly rouleaux formation. There are a few discrete cells.

Plasma p/H. between 7·2. and 7·4.

Constant volume. 37%. Corrected. 46%.

Fibrinogen.

Colorimeter readings.

24·6.
25·4.
25·2.
25·6.
25·6.
25·8.
25·4.
25·6.
25·9.
25·5
254·6.

Average. 25·4.

Fibrinogen. Corrected. = 0·32%.

Case. 61.

J----. D----. Age. 43 Yrs. Ploughman.

Developed acute rheumatism three weeks ago. Heart involvement about four days later.

Hb. 86%.

Plasma p/H. 7·4.

Citrated blood, largely discrete cells with some small rouleaux.

E.S.R.	1 hr.
Westergren	23.
Micro-bore. 100 mm.	18.
Veridia. 100 mm.	8·5.
Wintrobe tube.	17.

Constant volume. 33% Corrected. 41%

Fibrinogen.

Colorimeter readings.

18·2.
18·2.
18.
17·9.
18·3.
17·9.
17·8.
18·7.
18·4.
18.
181·4.

Average. 18·1.

Fibrinogen. Corrected. -- 0·45%.

The cases mentioned here are not meant to be full case histories.

They may be of interest in shewing the various conditions in which abnormal rates may be found.

But rather, they are to be regarded as the quarry from which have been extracted the materials for this inquiry.

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APPARATUS.

INCUBATOR.

This was designed and made by myself for consulting room use about 25 years ago.

It was made to replace a large square water jacketed one made locally to my design in 1906.

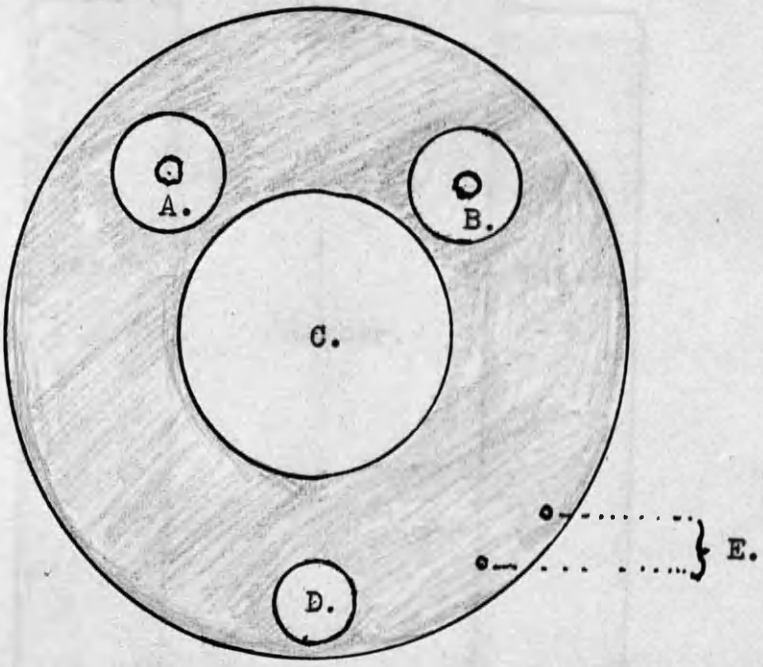
The small one it will be noticed ran on gas - using a Reichert mercury thermostat - but since the photograph was taken a few years ago I have changed it to electricity by fitting a ring heater to the bottom and using an electric thermostat.

The diagrams - elevation and plan - will explain the construction of it.

The chamber can hold one or two culture tubes. It can have a certain amount of water put in it if desirable - in some of my experiments it has been used.

A thermometer can be fixed in the covering of the chamber if necessary.

Incubator.



A. Opening for Thermostat.

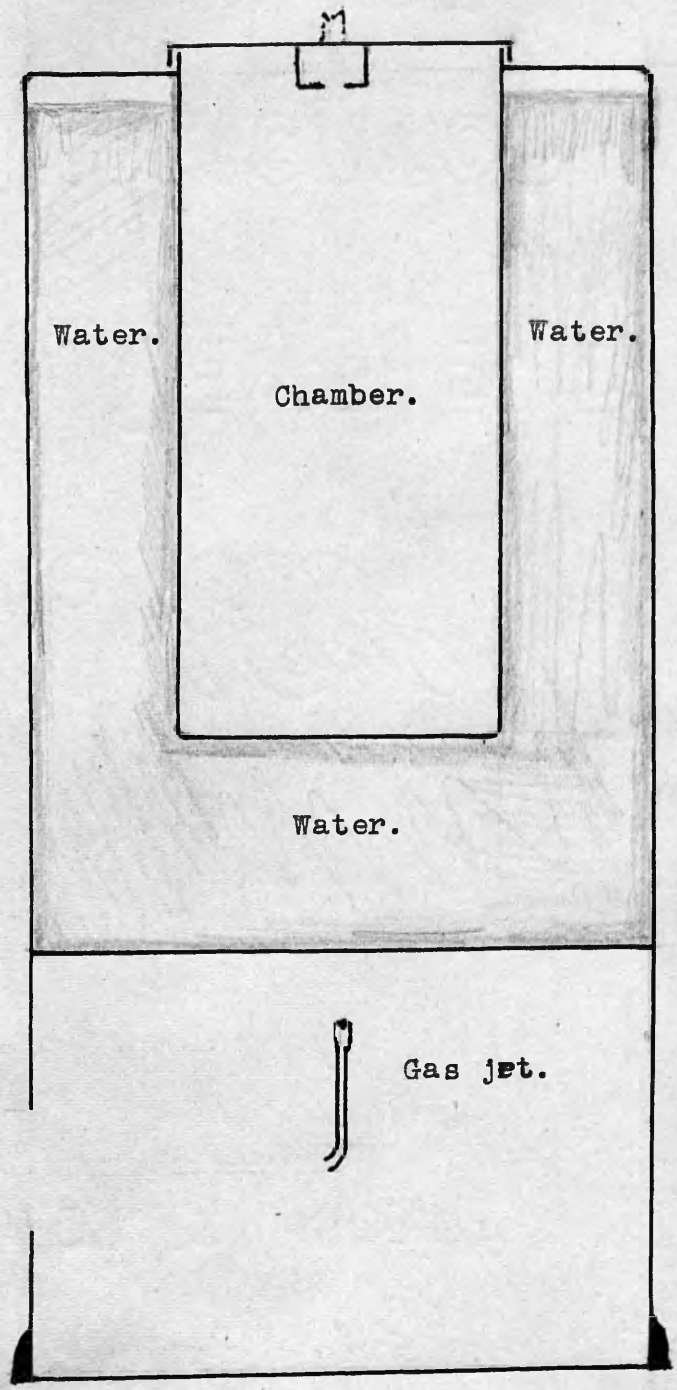
B. Opening for Thermometer.

C. Chamber.

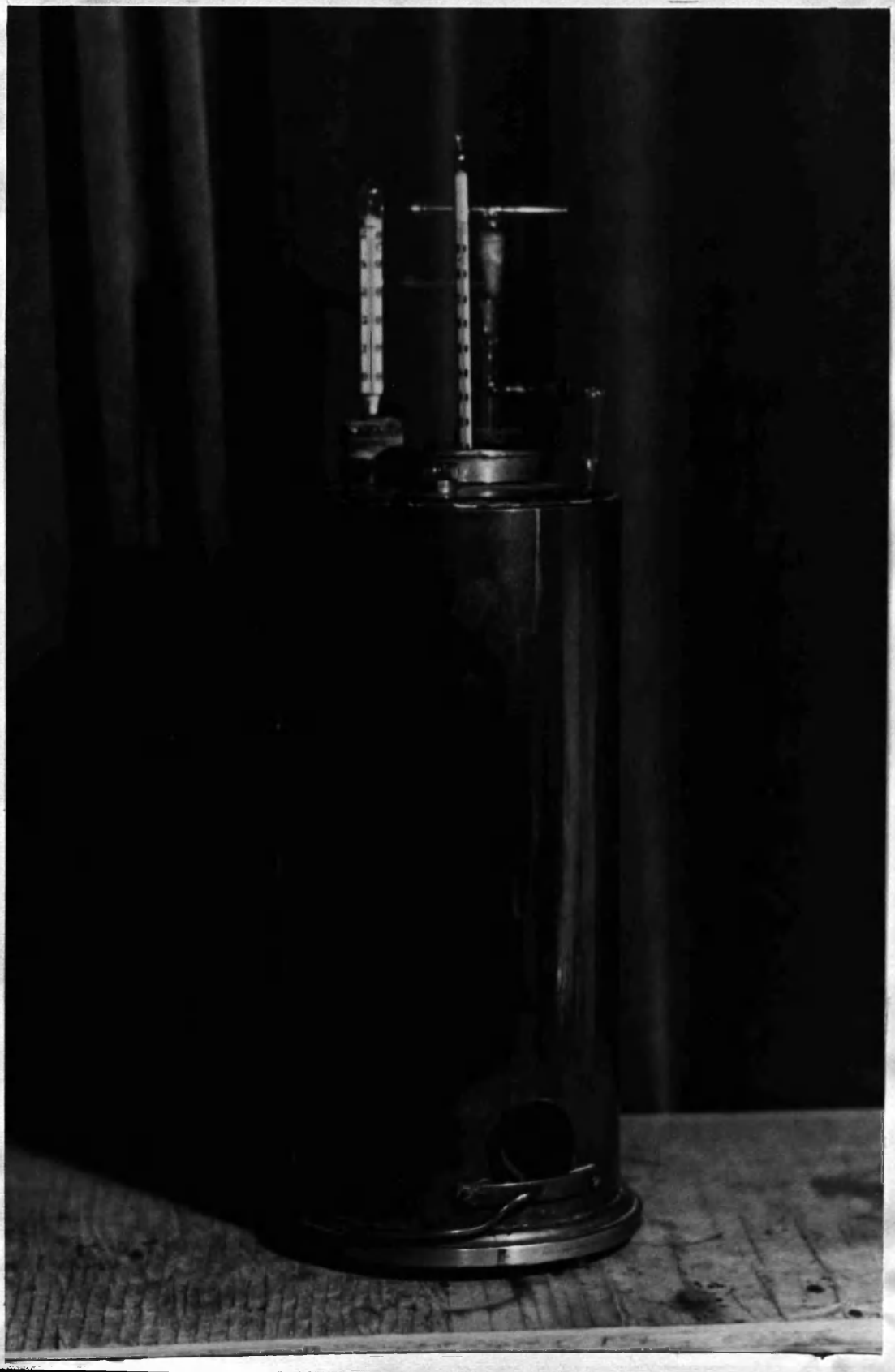
D. Opening for centrifuge tube.

E. Openings for Opsonic index pipettes.

Incubator



½ Scale.



The centrifuge used in this investigation, is shewn in the photographs.

With the exception of the large bucket head it was designed and made by myself twenty-five years ago.

The small bucket head was made by myself to take Wintrobe tubes and to get the necessary speed by cutting down air resistance. It brings down to constant volume in 35 to 40 minutes.

This centrifuge has never given any trouble.

It embodies a few ideas which have conduced to very good efficiency.

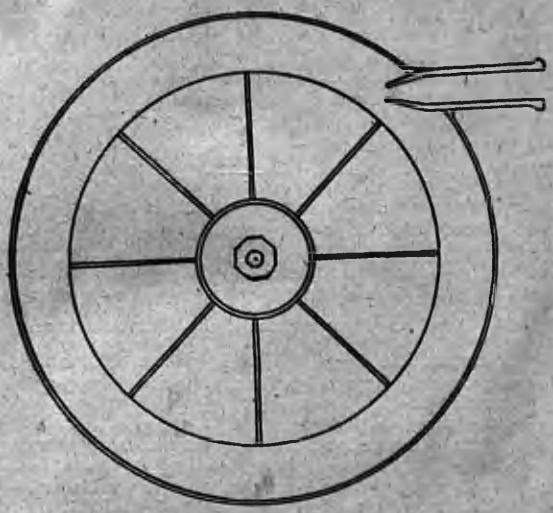
I am adding as a matter of interest a page of a magazine which shows one made locally and in use some years before.

I see from a note of mine in the B.M.J. on Opsonic index technique - April 13th. 1907, that it had been in use as far back as this.

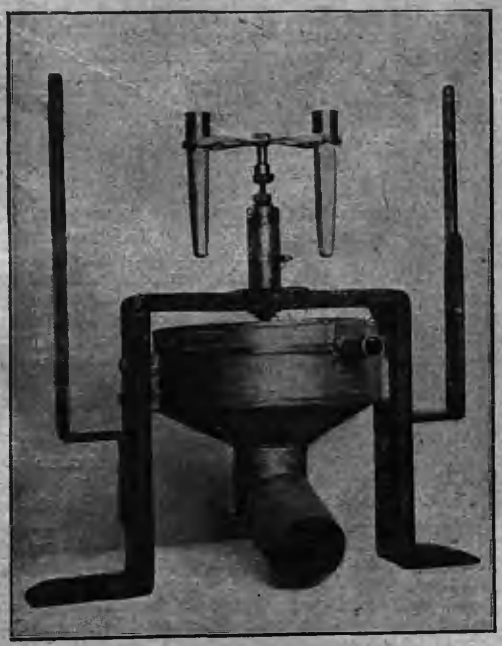
glass tubes, whence it can be easily obtained for examination.

The construction of the machine is seen in the two diagrams.

The vertical spindle runs in the ball bearings



PLAN OF WATER WHEEL.



A WATER-DRIVEN CENTRIFUGE.

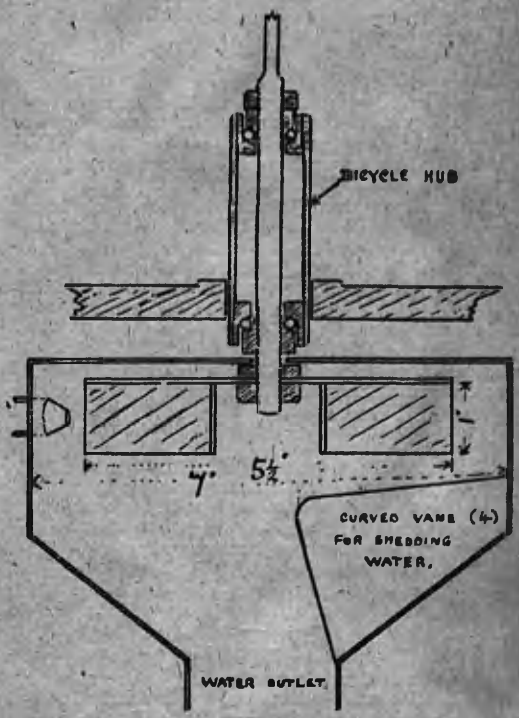
of a bicycle hub. The hub itself is a good fit in a hole in the cross member, where it is secured by a setscrew. The lower end of the spindle passes through a hole in the top of the casing and has attached to it the water wheel. The water wheel consists of a stout circular plate, on the under surface of which are fastened perpendicularly the flat, straight, radial vanes.

On the inside of the lower half of the casing are fixed four sloping curved vanes to stop swirl and give a quick lead off to the water.

The two side arms seen in the photograph carry a circular guard cage of stout wire mesh.

The machine was bolted to the top of a bench, and the water taken from an ordinary screw-down tap. The waste water was led by a tube slipping over the end of the exhaust opening into the sink.

It was fairly efficient and ran up to 2,400 revs. per minute. There was a little noise with it and unless the tubes were carefully balanced a good



SECTION OF EXISTING MACHINE.

deal of vibration; this did not matter much, as it was used in an outside house.

I am now requiring one for indoor use in my private room, and propose fastening it to the wall (solid stone) above the water sink.

Before starting it, and as I think it is possible to make one neater, quieter and more efficient, I should like the opinion of readers on one or two points.

First with regard to the form of the water wheel. Should this be—

(i) Flat disc with straight flat vanes fastened on the under surface; or

(ii) Flat disc with flat curved vanes on under surface; or

(iii) Flat disc with buckets fastened on under surface; or

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