also explain the curious variation in the amount of protamine insulin and soluble insulin to which I have referred, the rapid swing from hyperglycaemia to hypoglycaemia and back again to hyperglycaemia, and the difference in the size of the two doses of insulin. This hypothesis of the varying activity on the part of the hypophysis is a pleasing one, as it will explain so many difficulties; but there is at present no direct evidence for it. If the amount of the glycotrophic substance and of insulin in the blood and urine can be estimated it should be possible to prove or disprove this hypothesis. The only direct evidence for the varying activity of the hypophysis is that provided by Dr. Leonard Mark (1912) in his observations on his own case. He described what he called an acromegalic state. This might come on at any time of the day, but was most common during the latter part of the morning. Its duration was very variable: it might last only about half an hour or it might be present all day. When he was in the acromegalic state his general appearance altered; his hands and feet were bigger, cold, and moist; his eyes were more sunken, and his tears flowed abundantly—so much so that the silk lapel of his frock-coat rotted. I have not found any other reference to this condition, as Harvey Cushing (1932) and Atkinson (1932) do not mention it. If, however, Leonard Mark's observations are correct the acromegalic state can only be due to a variation in the activity of the hypophysis, and if this takes place in acromegaly it may also occur in the secretion of the glycotrophic factor, and so explain many of our present difficulties.

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Y. M. Vesval (*Thèse de Paris*, 1940, No. 182), who has collected 57 cases of agranulocytosis in children aged from 3 months to 14 years, including a personal case in a boy aged 3½ years, states that twenty-eight were boys and twenty-nine girls. The prognosis is very grave. As in the adult the aetiology is very obscure; the occurrence of the disease may sometimes be explained by a toxic or more frequently an infective factor perhaps associated with a special predisposition of the bone marrow. Agranulocytosis in the child very frequently masks an acute leukaemia, and an early and correct diagnosis can only be obtained by a study of the myelogram.

CHANGES OCCURRING IN BLOOD STORED IN DIFFERENT PRESERVATIVES

BY

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The object of the present investigation was to study the changes that occur in certain elements of the blood stored in different preservatives.

Methods of Investigation

The following characteristics were initially chosen for analysis: (i) total red cell count and red cell fragility in hypertonic saline; (ii) total white cell count and differential count; (iii) platelet count; (iv) sedimentation rate; (v) coagulation time. The red cells were counted in a Bürker counting chamber, using a 0.5% solution of gentian violet in normal saline as diluting fluid (Price-Jones, Vaughan, and Goddard, 1935), and the fragility was determined by the method of Dacie and Vaughan (1938). The platelets were counted by Lempert's modification of Kristenson's technique (Lempert, 1935); the sedimentation rate was done by Wintrobe's method (Wintrobe and Landsberg, 1935); and the coagulation time by a modification of Lee and White's (1913) technique, using a dry instead of a wet tube.

After a few observations it was found that the most striking effect of changing the preservative was upon the red cells. In subsequent experiments, therefore, the red cells only were studied. The blood was withdrawn by gravity (Vaughan, 1939). All examinations were made on small samples taken from the full bottle of blood at the time of examination. In many instances daily estimations were made, though for clarity only the weekly results are recorded in the tables. The solutions were:

1. Saline citrate solution (1.05% sodium citrate in 0.85% sodium citrate).
2. The same saline citrate with the addition of 0.3% glucose (giving a final concentration of 0.1%).
- 3 and 4. Both the above solutions fully oxygenated.
5. The saline citrate solution with 3% glucose (giving a final concentration of 1%).
6. The saline citrate solution with 6% glucose (giving a final concentration of 2%).
7. Caramelized 0.3% glucose saline citrate.

In all cases 2 parts of blood were added to 1 part diluent.

These solutions were chosen because, with the exception of No. 7, their use had been advocated by other workers.

If glucose is autoclaved with sodium citrate caramelization occurs. Although glucose can be added after the sodium citrate solution has been autoclaved, it is a rather cumbersome procedure. It therefore seemed worth while to determine whether the caramelized solution had the same preservative effect. Caramelization decreases the amount of reducing substance present.

Results

Sedimentation Rate.—As other observers have pointed out (Filatov, 1937; Macdonald and Stephen, 1939; Fahraeus, 1939), the sedimentation rate is retarded in stored blood. This appeared to be slightly less definite in solutions containing glucose, but the figures available allow of no definite conclusions on this point.

FIG. 1

TABLE I.—Changes in Total White Cells at Three-day Intervals

Preservative	No. of Samples	White Blood Cells—Thousands per c.mm.						
		1st Day	3rd Day	6th Day	9th Day	12th Day	15th Day	18th Day
Saline citrate	5	3.0	2.8	2.8	1.5	0.6	0.3	—
Final concentration 0.1% glucose-saline citrate	6	2.6	1.7	1.7	1.1	0.7	0.2	0.2

Coagulation Time.—The observations available on coagulation time suggest that it is definitely reduced by storage, the figures observed being in the neighbourhood of 3 to 6 minutes instead of the normal figure of 10 to 15 minutes: this reduction occurs within a few hours and remains approximately unaltered for a month. The results with M.R.C. saline citrate and 0.3% glucose-saline citrate were similar.

White Cell Count.—Daily white cell counts (Table I and Fig. 1) showed a rapid fall in white cells. In both the saline citrate and 0.1% glucose-saline citrate (final concentration) differential counts showed that the polymorphs were completely absent after ten days in all samples, disappearing in the majority of cases by the seventh day. Occasional eosinophils, basophils, and lymphocytes persisted for at least a month. These findings are in close agreement with those of most other workers (Filatov, 1937; Macdonald and Stephen, 1939).

Platelets.—The platelet count (Table II) fell on storage, but after the end of the second week a fairly constant count of 40,000 per c.mm. was still present both in the saline citrate and in the glucose-saline citrate. This result is not in agreement with those of previous observers

(Filatov, 1937), who state that no platelets are found at the end of fifteen days.

Red Cell Fragility in Hypotonic Saline Solution.—Red cell fragility may conveniently be expressed as mean corpuscular fragility—i.e., that concentration of saline in which 50% of the cells are haemolysed (Table III).

TABLE II.—Platelet Count at Weekly Intervals

Preservative	No. of Samples	Platelets in 100,000 per c.mm.				
		1st Day	7 Days	14 Days	21 Days	28 Days
Saline citrate	5	1.3	0.6	0.4	0.4	0.3
Final concentration 0.1% glucose-saline citrate	6	1.0	1.0	0.6	0.4	—

TABLE III.—Changes in M.C.F. of Red Cells at Weekly Intervals

Preservative	No. of Samples	M.C.F.				
		1st Day	7 Days	14 Days	21 Days	28 Days
Saline citrate	6	0.36	0.44	0.54	0.72	0.90
Saline citrate oxygenated	3	0.37	0.43	0.51	0.70	0.82
Final concentration 0.1% glucose-saline citrate	6	0.38	0.42	0.48	0.54	0.61
Caramelized 0.1% saline citrate	2	0.38	0.43	0.45	0.55	0.65
Final concentration 0.1% glucose-saline citrate oxygenated	4	0.37	0.41	0.48	0.52	0.69
Final concentration 1% glucose-saline citrate	2	0.44	0.57	0.50	0.53	0.72
Final concentration 2% glucose-saline citrate	2	0.52	0.57	0.62	0.70	—

M.C.F. (Mean corpuscular fragility): % NaCl at which 50% of cells are haemolysed.

There is a steady increase in mean corpuscular fragility in all solutions hitherto investigated, but it is most marked in those which do not contain glucose (Fig. 2). The results obtained with 0.3% glucose (final concentration 0.1%) and 3% glucose (final concentration 1%) appear the same (Fig. 3). Caramelization of a 0.3% solution does not reduce its preserving qualities as measured by mean corpuscular fragility. An initial concentration of 6% glucose (final concentration 2%) causes increased haemolysis. Oxygenation of the red cells throughout the period of storage does not appreciably affect their fragility.

Red Cell Count.—In solutions that do not contain glucose the red cell count (Table IV, Fig. 4) falls below 3,000,000 per c.mm. at approximately the end of the second week; in solutions containing glucose up to 3% (final concentration 1%) the count is maintained at the 3,000,000 level in some instances for longer than a month. How-

FIG. 2

TABLE IV.—Changes in Total Red Cells at Weekly Intervals

Preservative	No. of Samples	Red Blood Cells (millions per c.mm.)				
		1 Day	7 Days	14 Days	21 Days	28 Days
Saline citrate	6	3.3	3.2	2.9	2.3	1.5
Saline citrate oxygenated	3	3.5	3.4	3.3	3.0	2.7
Final concentration 0.1% glucose-saline citrate	6	3.4	3.2	3.1	3.1	3.0
Caramelized 0.1% saline citrate	2	3.7	3.5	3.5	3.2	3.2
Final concentration 0.1% glucose-saline citrate oxygenated	4	3.4	3.4	3.3	3.2	2.9
Final concentration 1% glucose-saline citrate	2	3.5	3.4	3.1	3.1	2.7
Final concentration 2% glucose-saline citrate	2	3.5	3.3	3.3	3.2	2.6

ever, a possible source of error in such observation was pointed out to us by Dr. Maizels. Since normal saline is used as a diluent for counting red cells, and some degree

of haemolysis may occur at this concentration of saline, it is probable that cells are haemolysed in process of diluting, thus making the observed counts too low. A few obser-

TABLE V.—Effect of Diluent on Red Cell Counts

Age of Blood in Days	Red Cells (millions per c.mm.)		
	Diluting Fluid	Citrated Plasma	Fresh Plasma
22	2.1	2.6	2.6
24	1.6	3.2	3.2
25	3.3	3.6	3.6
25	3.3	3.8	3.6
40	2.0	3.0	2.7
43	2.4	3.7	3.4

vations made on using plasma as diluting fluid have shown that this is true. The divergence between the red cell count made in plasma citrate diluent from that made with gentian violet saline diluent becomes more marked with increasing fragility of the red cells. Fresh plasma of the same group not mixed with citrate saline has the same preserving effect (Table V). All previous data on red cell counts must therefore be regarded with suspicion, and in future red cell counts on stored blood should be made, using the plasma anti-coagulant mixture as diluent.

It might be thought that the marked changes in red cell fragility described are in part due to the trauma caused by daily shaking necessary to mix the blood. In order to test this point a sample of blood in saline citrate solution that had not been moved for one month was examined. The fragility curve and red cell count were strictly comparable to those obtained with six samples of blood that had been constantly mixed. The observation that red cell preservation is improved by the addition of glucose to the diluent is not new (Rous and Turner, 1916; Domanig, 1937; Durán Jordá and Aleu, 1937; Bagdassarov, 1937; Filatov, 1937). Since our experiments were begun Maizels and Whitaker (1940) have published similar findings. Our analysis of the effects on cell fragility are, however, more detailed than others available, and are therefore of interest.

Conclusions

Glucose in a final concentration of 0.1% and 1% favours the preservation of red cells in stored blood through its effect on red cell fragility.

Red cell counts on stored blood must be made using plasma as diluent.

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The American Museum of Health reports an unprecedented popularity for its medical and public health exhibit at the New York World's Fair. During the 1939 season seven and a half million visitors viewed this scientific exhibit.

FIG. 3

FIG. 4