HAEMOLYSIS OF STORED BLOOD MIXED WITH ISOTONIC DEXTROSE- CONTAINING SOLUTIONS IN TRANSFUSION APPARATUS

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In the past few months haemolysis has been seen to occur within transfusion apparatus on two occasions. In both cases the patients were infants who had been given transfusions of stored whole blood followed by solutions of 4.3% dextrose with 0.18% saline. The blood filters were transferred direct from the bottles of blood to the flasks containing the dextrose-saline solutions, and inevitably there were mixtures of blood and solution within the flasks. The rates of infusion were slow and the environmental temperatures were high. *In vitro* haemolysis was noticed after about 12 hours, when the solutions in the flasks became a clear pink.

It is the purpose of this paper to show that such haemolysis is consistent, the degree depending on the temperature of the solution, the length of time the mixture is allowed to stand, the age of the stored blood, and the extent of its dilution in the isotonic dextrose-containing solution.

Before the following experiments were carried out various proprietary preparations of electrolyte and dextrose solution for intravenous infusion were analysed, and the accuracy of their advertised contents was confirmed. The dilution of stored blood in the various solutions was chosen to represent that amount of blood which might be transferred on a blood-transfusion filter to a flask containing 1 litre of solution. The amount of blood haemolysed, after incubation at various temperatures for different times, was determined in a colorimeter.

The Table below shows haemolysis of stored blood in different solutions. Blood, in acid-citrate-dextrose solution, was taken from the donor two days previously. 0.05 ml. of blood was mixed with 4 ml. of solution. The figures in the Table represent percentages of blood haemolysed.

The experiment was repeated with other variables and showed that: (a) donor blood stored for longer periods underwent slightly increased haemolysis; (b) a greater dilution than one part of stored blood in 80 parts of solution A resulted in increased haemolysis, while a mixture of equal parts of stored blood and solution A, incubated for 12 hours at 27° C., showed no appreciable haemolysis; (c) bacteriological sterility in the conduction of the experiment did not alter the results; and (d) the

amount of haemolysis in solution A was consistently greater than that in solution B throughout five different experiments.

Discussion

It is probable that isotonic infusion solutions were originally produced to avoid the chance of intravascular haemolysis during rapid transfusion, but it has become common practice to allow such solutions to mix with stored blood in transfusion apparatus. This appears to be safe where the isotonic solution contains electrolytes only, but haemolysis is apt to occur within the apparatus if dextrose is incorporated in the solution, and for some obscure reason is greater in isotonic glucose-saline solution than in isotonic glucose alone. Such haemolysis does not occur within six hours at room temperature, and is therefore unlikely to be encountered in the transfusion of adult patients.

In paediatric practice it is often necessary to give to an infant a transfusion of stored blood followed by isotonic glucose-saline solution, and it is here that a flask containing a litre of solution may be in use for 24 hours or more. Moreover, a special effort may be made to warm up the environment if the infant's temperature is low. Thus if any of the stored blood is introduced into the flask containing the glucose-saline solution the stage is set for *in vitro* haemolysis to occur.

It might be argued that the amount of haemoglobin liberated by the haemolysis of a few millilitres of blood, if transfused slowly over a period of 24 hours or more, would be unlikely to cause any harm, but it is probably better to eliminate the risk, especially when the infant's renal function may be impaired by shock or dehydration. The danger can be prevented by renewing the blood-contaminated filter during the changeover from stored blood to isotonic glucose-containing solution during continuous intravenous transfusion.

We have not attempted to investigate the cause of the haemolysis described, but the papers of Ebaugh *et al.* (1957a, 1957b) suggest that it is due to the passage of glucose into the red-blood cells with disturbance of osmotic equilibrium. These workers demonstrated reduced survival, after transfusion, of radioactively labelled red-blood cells which had been diluted with a solution of 5% dextrose and 0.225% saline for varying times before injection into patients. They also found a great increase in the glucose content of the red-blood cells, and a progressive increase in cell volumes, reaching a maximum of 180% of normal after two hours' exposure to the glucose-saline solution *in vitro*.

Summary

Mixtures of stored blood and isotonic dextrose-containing solutions within transfusion apparatus will result in haemolysis of the stored blood in certain circumstances.

Such haemolysis depends on the presence of dextrose in the isotonic solution, and the amount of haemolysis varies with the dilution of the blood in the dextrose-

Hours:	Solution A				Solution B				Solution C				Solution D			
	3	6	12	24	3	6	12	24	3	6	12	24	3	6	12	24
Room temp. . .	0	0	56	92	0	0	10	58	0	0	0	0	0	0	0	0
27° C. . .	7.5	60	85	100	0	17	71	96	0	0	0	0	0	0	0	0
37° C. . .	85	87	90	92	77	77	87	100	0	0	0	2	0	0	0	0

Solution A = 0.18% saline with 4.3% dextrose. Solution B = 5% dextrose in water. Solution C = Ringer's solution. Solution D = Normal saline.

containing solution, the length of time the mixture is allowed to stand, the temperature, and the age of the stored blood.

Significant haemolysis is most likely to occur in paediatric practice, where, owing to slow infusion, stored blood and dextrose-containing solution may remain in mixture for some hours.

All such haemolysis can be avoided by renewing the filter when stored blood is followed by glucose solutions in intravenous transfusion.

The haemolysis is explained by the entry of dextrose into the red blood cells with increased cell volume and fragility.

REFERENCES

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ORAL THEOPHYLLINE COMPOUNDS IN CHRONIC ASTHMA

A BLIND CLINICAL TRIAL

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The alkaloid theophylline is not very soluble (1/180 in water at 20° C.). It is also a gastric irritant (Martindale, 1958). The unmodified drug has not achieved a reputation of substantial therapeutic value in the treatment of asthma. The combination of theophylline with ethylenediamine (aminophylline) has been in use since 1908. This is a freely soluble compound, and the value of its parenteral administration, especially intravenously, in the treatment of severe asthma is undoubted and generally accepted (*British Medical Journal*, 1957). Its superiority by intravenous injection in the treatment of chronic bronchitis and asthma over theophylline ethanoate of piperazine ("etophylate") and dihydroxypropyl theophylline ("neutrathylline") has been demonstrated by Phear (1957).

Aminophylline, however, when dissolved, makes a strongly alkaline solution and is hydrolysed in the stomach, with the production of free theophylline (Turner-Warwick, 1958), and is therefore assumed frequently to cause symptoms of gastric irritation after oral administration.

Within recent years a large number of compounds of theophylline have been introduced for oral administration which are readily soluble in water, making a neutral solution stable over a pH of wide range. It has been considered that their use may be freer from the gastro-intestinal side-effects produced by theophylline itself or aminophylline (Turner-Warwick, 1958). Plasma levels of theophylline have been found higher after oral aminophylline than after the same dose of theophylline administered as theophylline sodium glycinate (Turner-Warwick, 1957), and this work also suggested that the therapeutic effect of these and other theophylline

compounds depended on the plasma level of theophylline achieved.

Method and Trial

Clinical comparisons of the efficacy of the new oral theophylline compounds with that of aminophylline in blind trials do not appear to have been made. It was thought that such a comparison might be interesting, and a blind trial was therefore undertaken, using tablets of identical appearance containing either aminophylline or theophylline sodium glycinate. The amount of theophylline in each tablet was 150 mg.

The tablets were put into bottles by the chief pharmacist. Each bottle contained a month's supply for one patient. The bottles were allotted numbers at random, and the key to the identity of the tablets was kept by the chief pharmacist until the trial had been completed.

The patients used in the trial were all chronic asthmatics whose ages ranged from 21 to 66 years, with an average of 41. All were using antispasmodics by mouth or inhalation regularly. They were each observed for one week at least before being included in the trial. No patient was given the tablets if spontaneous improvement had taken place during the initial period of observation. Those patients actually used were each given a bottle of tablets and instructed to take two tablets three times daily just before meals. They were seen at fortnightly intervals for reassessment, and again one month after the tablets had all been taken. A patient was considered "much improved" if the need for all antispasmodics vanished; "improved" if the need for antispasmodics decreased and the exercise tolerance improved; and a "failure" either if there was no improvement in the asthma or if severe side-effects precluded continuation of the course of tablets. Thirty-four patients were included in the trial. After every individual patient had been finally assessed the key was opened and the identity of the tablets given to each was discovered.

Results

The results are shown in Table I. It is obvious that the patients taking the theophylline sodium glycinate fared no better than those taking the aminophylline; in

TABLE I.—Results of Treatment

Result	No. on Aminophylline	No. on Theophylline Sodium Glycinate	Total
Much improved ..	6 (50%)	6 (50%)	12
Improved ..	5 (62.5%)	3 (37.5%)	8
Failure ..	5 (35.5%)	9 (64.5%)	14

fact, they fared rather worse, but the difference is not statistically significant. Partial or complete relapse occurred within a month of the end of the trial in all the "improved" and "much improved" patients except four, two having received aminophylline and two theophylline sodium glycinate.

The incidence of side-effects is shown in Table II. Again there was no obvious difference between the two groups. Purely gastro-intestinal symptoms were noted

TABLE II.—Side-effects

Results	No. on Aminophylline	No. on Theophylline Sodium Glycinate	Total
Side-effects present ..	8 (42%)	11 (58%)	19
No side-effects noted	8 (53%)	7 (47%)	15