CHEMICAL CORRECTION OF STORED BANK BLOOD BY ION EXCHANGE RESIN MIXTURES

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Summary: A mixture (mixture 'M') of the sodium, potassium, calcium and magnesium forms of the strong cation exchange resin, Amberlite IR-120, was used for the correction of the cationic concentration changes of the ACD stored bank blood. Similarly, a mixture (mixture 'R') of the chloride and bicarbonate forms of the strong anion exchange resin, Amberlite IRA-410, was used for the correction of the anionic concentration changes and pH of the stored blood. The relative composition of the various forms of the resin, in the cation exchange and in the anion exchange resin mixtures 'M' and 'R' were worked out from their exchange behaviour with respect to the cationic and anionic concentrations of the stored bank blood.

Altered levels of blood electrolytes, metabolites and pH were restored closed to the normal values when the ACD bank blood, from each of the three storage periods respectively, was passed over the combined resin mixtures 'M' and 'R' columns. Exchange transfusions of the ACD stored canine blood, treated with the combined resin mixtures, produced no detectable harmful effects in the recipient dogs during the post-transfusion period. From these studies it was concluded that a combined use of the cation and anion exchanged resin mixtures would be useful in restoring a near normal chemical composition of the ACD stored bank blood prior to its transfusion.

INTRODUCTION

The major biochemical changes encountered in the acid-citrate-dextrose (ACD) stored bank blood have been a marked elevation in the potassium and ammonium levels and a sharp in the pH of the blood (13, 16, 19, 25, 33, 34). Acidosis, hypotension, cardiac arrythmias, hepatic coma and respiratory and cardiac arrest have all been reported as consequences of massive blood tranfusions with the ACD stored bank blood (2, 5, 14, 23, 26, 29). Over the past decade attempts have been made to correct the various biochemical changes in the stored bank blood by the use of both strong and weak cation and anion exchange resins either as single bed or as mixed bed resins (24, 26, 28, 31, 32). The weak cation and anion exchange resins, used by the various workers, have limited exchange potentials and are effective within a narrow range of *pH* (3). The sodium exchange resin, either used alone or in combination with the potassium exchange resin, besides removing ammonium and to some extent potassium, will also remove calcium and magnesium (20), from the stored blood which is not desirable. Furthermore, the various resins used have not been described quantitatively and their relative affinities for various ions of the stored blood have not been properly elucidated. In order to gain more experience with the cation and anion exchange resins and with their exchange behaviour detailed *in vitro* and *in vivo* studies were undertaken to develop suitable resin mixtures which would be

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efficient in restoring the chemical composition of the ACD stored blood close to its original state and thereby making it suitable for massive transfusions.

MATERIALS AND METHODS

ACD stored bank blood: Canine blood, obtained by exsanguinating healthy adult mongrel dogs under aseptic conditions, was used in all the experiments of this study. Prior to bleeding, 200 IU heparin per kg body weight was administered intravenously. 100 *ml* of the ACD solution, containing 0.47 *g* of citric acid, 1.32 *g* of trisodium citrate and 1.48 *g* of dextrose, per 400 *ml* of whole blood was employed as preservative. Blood thus collected in sterile glass blood transfusion bottles was stored at 4-6°C for 7, 14 and 21 days respectively. A heparinized sample of blood from each donor dog was analysed for the various anionic and cationic blood constitutents. An aliquot from each bottle was similarly analysed before and after storage and after passing through the ion exchange columns.

Preparation of cation exchange resins: Amberlite IR-120 (analytical grade), which is a monofunctional, sulphonated crosslinked polystyrene strong cation exchange resin in the bead form, was used. The exchange capacity of the resin was found to be 3.77 *meq/g* air dried resin. The details of the technique used for the preparation of resins and resin mixtures are described elsewhere (4, 17, 20).

Preparation of anion exchange resins: Strong action exchange resin, Amberlite IRA-410, which is a crosslinked polystryene resin in the bead form, was used. The resin was cycled three times between the hydroxyl and chloride forms using 1N sodium hydroxide and 1N hydrochloric acid solutions. The final chloride form was thorougly washed with deionized water and dried in air. The BSS 30/60 fraction of the air dried resin was selected for the present study. Its exchange capacity was found to be 3 *meq/g* air dried resin. The bicarbonate form of the resin was prepared by passing 5%solution of sodium bicarbonate through a column containing a known quantity (in *meq*) of the chloride form of the resin till the overnight effluent was free from chloride ions. The final bicarbonate from of the resin was dried in the similar way.

In order to study the relative affinities of the chloride and bicarbonate forms of the resin for the various anions of the stored blood, artificial serum was prepared on the similar lines as described by Hald (15). The cationic and the anionic composition of the artificial serum 'A', artificial serum 'B' and artificial serum 'C' used in the present study, was identical to that of the ACD blood stored for 7 days, 14 day and 21 days respectively (16, 19).

Passage of the stored blood through the ion exchange columns: The resin column used was a heamorepellent polyvinyl plastic column of 4 *cm* internal diameter and 30 *cm* long and fitted with a 100 mesh filter at the distal end. The column was filled with the desired resin mixture and the column and the tubings were sterilized in an autoclave before use. The

column was flushed with 5% sterilized glucose solution so as to make the liquid contents of the column isotonic with blood and to keep the resin moist and swollen. The volume of the glucose solution in the column was never more than 20 *ml*. Heparin (2000 IU) was added to each bottle when it was removed from storage. The stored blood was then passed through the resin column, containing the desired resin mixture, at a flow rate of 20 *ml/min* under aspectic conditions. The effluent blood traversed a drip chamber, fitted with a 100 mesh nylon filter, before being collected into a sterilized blood transfusion bottle. Samples of the influent and the effluent blood were taken for analysis.

For studies with the artificial serum, 500 *ml* of each of the artificial serum A, B and C (corresponding to the volume of the stored blood) was passed through the glass columns, filled with the desired resin, at a flow rate of 20 *ml/min*. Samples of the influent and the effluent artificial serum were taken for analysis.

Technique of exchange transfusions: Nine recipient dogs, weight 15-20 *kg*, were divided into three groups of three dogs each. Exchange transfusions, with the combined resin mixtures 'M' and 'R' treated 7 days, 14 days and 21 days ACD stored canine blood, were carried out on the dogs of group one, two and three respectively under aspectic conditions. The animals were anaesthetized with pentobarbital sodium and exchange transfusions were started at a flow rate of 20 *ml/min* through the exposed femoral vein. Concomitantly, equal amount of blood was withdrawn from the exposed femoral artery of the dogs. This process was continued until the dogs had received 200% of their calculated total blood volume (27). Pre-transfusion, end-transfusion and post-transfusion (24 *hr*) samples of blood were withdrawn for analysis.

Chemical determinations on the samples of blood and artificial serum were made by standard analytical techniques (1, 35). Blood ammonium was estimated by the microdiffusion method of Conway and Cook (7) and plasma haemoglobin was estimated by the benzedine method (9).

RESULTS

In Vitro Studies

Effect of mixture of various forms of IR-120 resin: The exchange characteristics and relative affinities of the various forms of Amberlite IR-120 resin have been reported earlier (Chaudhry 4; Juggi, 16, 17, 20). From these studies, methods were evolved whereby suitable cation exchange resin mixtures could be developed for adjusting the cationic composition of the influent blood. By trials with different proportions of various forms of the resin, a mixture (mixture 'M') containing 65 *meq* of the sodium form, 3 *meq* of the potassium form, 4 *meq* of the calcium form and 3 *meq* of the magnesium form (total 75 *meq*) was developed. The proportions of various resin forms in the mixture were adjusted for correcting the cationic concentrations of one bottle (500 *ml*) of stored blood. The results of passage of one bottle

of the ACD stored blood, from each of the three storage periods, through the resin mixture columns are shown in Table I. As seen, the resin mixture 'M' is efficient in removing excessive ammonium and potassium from the blood. In addition, levels of plasma calcium and magnesium were adjusted close to their pre-storage values.

TABLE I : Cationic concentration of the ACD stored canine blood before and after treatment with the resin mixture 'M'.

Plasma constituent	0 *day heparinized*	7 *days*		14 *days*		21 *days*	
		Inf	*Eff*	*Inf*	*Eff*	*Inf*	*Eff*
Ammonium Blood $\mu g.$ NH_3-N%	18.0	98.0	0	150	15.0	240	46
Sodium *meq/l*	141.5	168.0	171	166	172.5	166	711
Potassium *meq/l*	4.4	4.6	4.8	8.4	4.65	12.6	5.6
Calcium *meq/l*	5.0	3.4	6.0	3.4	5.9	3.5	5.7
Magnesium *meq/l*	2.0	1.0	1.9	1.2	2.2	1.25	1.7
Haemoglobin *mg%*	4.0	18.0	26.0	25.0	44.5	40.8	60.0
pH Blood	7.4	6.6	6.6i	6.58	6.55	6.2	6.3

Inf = Influent Eff = Effluent

Effect of chloride form of IRA-410 resin: The results of passage of the artificial serum A B and C respectively through separate columns containing 150 *meq* of the chloride form of the resin are shown in Table II. As seen, the chloride concentration of the effluents is increased at the expense of a marked decrease in the bicarbonate concentration, resulting in the shift of pH more towards the acidic side.

TABLE II: Relative affinity of the chloride form (150 *meq*) of IRA-410 resin for the anions of the artificial serum A, B and C when separately passed through it.

	A		*B*		*C*	
	Inf	*Eff*	*Inf*	*Eff*	*Inf*	*Eff*
Chloride *meq/l*	78.5	88.0	71.0	87.0	71.0	88.5
Bicarbonate *meq/l*	21.6	6.0	19.2	4.0	14.55	5.0
Phosphate *mg%*	3.5	1.0	5.0	1.6	7.0	2.7
Sulphate *mg%*	2.0	0.5	3.5	1.3	4.8	2.5
Pyruvate *mg %*	2.7	0.5	4.5	1.0	8.5	1.5
Lactate *mg %*	71	43	100	47	132	53
Citrate *mg %*	300	26	298	25	300	38
pH	6.6	6.1	6.5	6.0	6.35	5.8

Inf = Influent Eff = Effluent

Effect of bicarbonate form of IRA-410 resin: Table 3 shows the results for the artificial serum A, B and C when passed separately through columns containing 150 *meq* of the bicarbonate form of the resin. As seen, the bicarbonate concentration is very much increased coupled with a marked decrease in the chloride concentration of the effluents, resulting in the shift of pH to a highly alkaline side.

TABLE III: Relative affinity of the bicarbonate form (150 *meq*) of IRA-410 resin for the anions of the artificial serum A, B and C when separately passed through it.

	A		*B*		*C*	
	Inf	*Eff*	*Inf*	*Eff*	*Inf*	*Eff*
Chloride *meq/l*	78.5	7.5	71.0	4.5	71.0	8.4
Bicarbonate *meq/l*	21.6	130	19.2	124	14.5	130
Phosphate *mg* %	3.5	0.4	5.0	0.2	7.0	1.7
Sulphate *mg* %	2.0	0.2	3.5	0.2	4.8	1.2
Pyruvate *mg* %	2.7	2.0	4.5	2.0	8.5	3.3
Lactate *mg* %	71	56	100	75	132	93
Citrate *mg* %	300	32	298	29	300	28
pH	6.6	9.3	6.5	9.3	6.35	9.3

Inf=Influent Eff=Effluent

Both the chloride and the bicarbonate forms of the strong anion exchange rasin were, however, found to be almost equally efficient in extracting various acidic ions and metabolities from the artificial serum (Table II, III).

Effect of mixture of two forms of IRA-410 resin: The results in Tables II and III show the relative affinities of the chloride and the bicarbonate forms of the resin for the various anions of the artificial serum. The chloride form of the resin tends to lower the pH, whereas the bicarbonate form tends to elevate it. However, from their relative exchange behaviour, it was possible to mix these two forms of the resin in suitable proportions in order to maintain the pH of the effluents close to the normal values. After a number of preliminary trials with varying quantities of the chloride and the bicarbonate forms of the resin, a suitable final resin mixture (mixture 'R') was worked out. The mixture 'R' (150 *meq*) contained 135 *meq* of the chloride form and 15 *meq* of the bicarbonate form of the resin. The results of separate passage of the artificial serum A, B and C through the resin mixture 'R' columns are shown in Table IV. As seen, the mixture 'R' is efficient in adjusting the pH of the effluents close to the normal value by removing excess of the acidic anions and metabolities and by increasing the bicarbonate and chloride levels of the effiuents. These results were confirmed when the ACD stored canine blood, one bottle from each of the three storage periods, was passed separately through the columns containing the resin mixture 'R' (Table V).

TABLE IV: Effect of mixture (mixture R) of the the chloride and the bicarbonate forms of the IRA-410 resin on the anions and pH of the artificial serum A, B and C when separately passed through it.

	A		*B*		*C*	
	Inf	*Eff*	*Inf*	*Eff*	*Inf*	*Eff*
Chloride *meq/l*	78.5	95.2	71.0	87.7	71.0	83.0
Bicarbonate *meq/l*	22.0	32.0	19.2	25.0	14.5	25.0
Phosphate *mg %*	3.5	0.62	5.0	0.62	7.0	1.1
Sulphate *mg %*	2.0	0.3	3.5	1.2	4.8	2.0
Pyruvate *mg %*	2.7	0.5	4.5	0.8	8.5	2.0
Lactate *mg %*	71.0	42.0	100	43.0	132	53.0
Citrate *mg %*	305	20.0	295	20.0	300	28.0
pH	6.6	7.42	6.5	7.4	6.3	7.4

Inf = Influent Efl = Effluent

TABLE V: Anionic concentration of the ACD stored canine blood before and after treatment with the resin mixture 'R'.

Plasma constituent	0 *day*	7 *days*		14 *days*		21 *days*	
	heparinized	*Inf*	*Eff*	*Inf*	*Eff*	*Inf*	*Eff*
Chloride *meq/l*	106	74.2	95.5	80.0	98	75.0	109.2
Phosphate *mg %*	4.5	4.1	0.4	6.0	1.0	6.9	1.4
Sulphate *mg %*	2.0	2.1	0.2	3.5	0	4.3	0.1
Pyruvate *mg %*	3.5	5.0	0	6.0	1.5	9.0	2.3
Lactate *mg%*	19.0	60.5	0	100.5	11.0	143.5	51.3
Citrate *mg%*	1.4	318	15.0	306	29.5	305	24.2
Haemoglobin *mg%*	5.0	13.5	55.0	25.0	70.0	35.0	110.5
pH Blood	7.4	6.65	7.58	6.42	7.5	6.3	7.41

Inf = Influent Eff = Effluent

Effect of combined 'M' (cation exchange) and 'R' (anion exchange) resin mixtures: The resin mixture 'M' (75 *meq*) and the resin mixture 'R' (150 *meq*) were combined together and transferred to the ion exchange columns. Six bottles of the ACD stored canine blood, from each of the three storage periods respectively, were passed separately through the resin columns. The mean values of the results for the influents and the effluents are shown in Table VI. As seen, the combination of both the resin mixtures is effective in correcting the various cationic and anionic changes of the stored blood and in restoring its pH close to the original values.

In vivo studies: None of the nine dogs, which received exchange transfusions with the resin mixtures treated blood, showed any ill effects in the post-transfusion period. Fig. 1

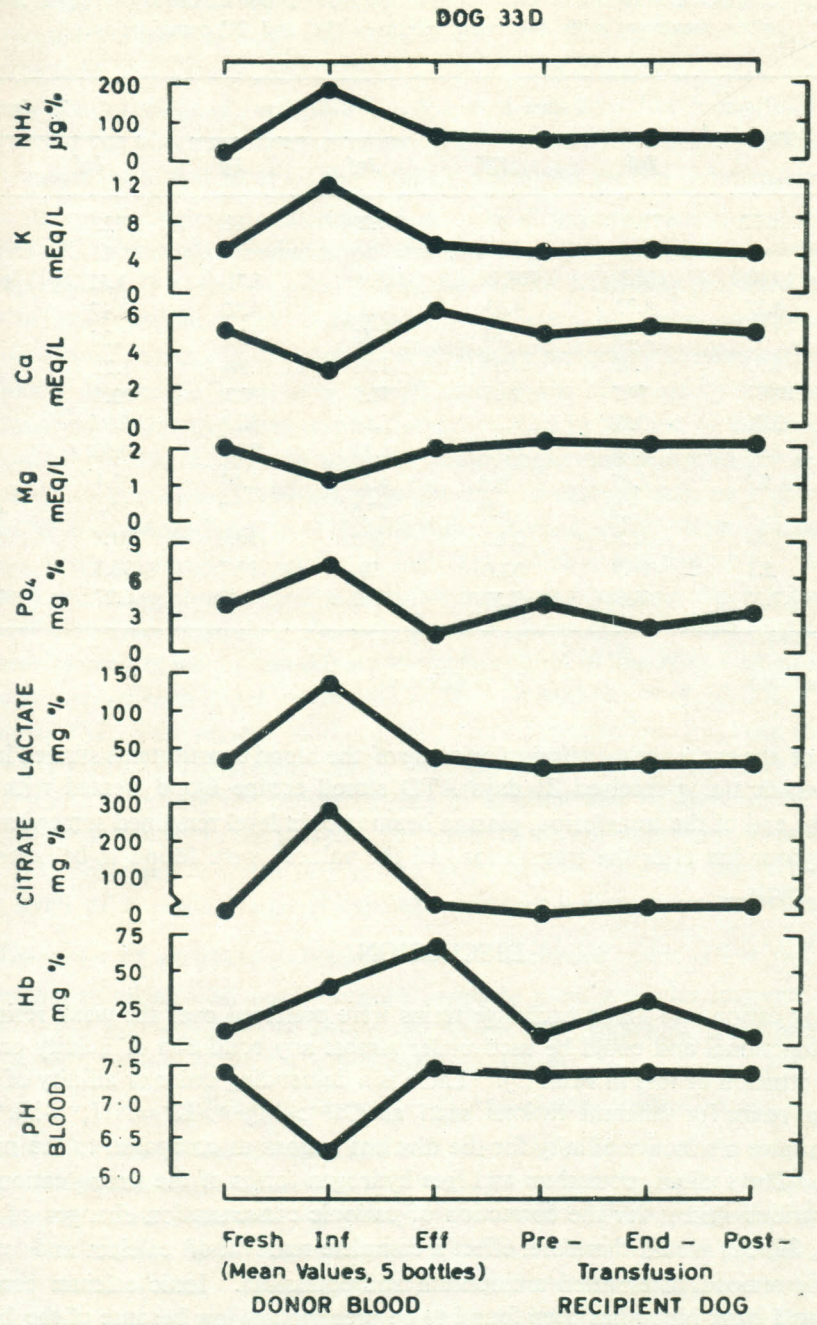

Fig. 1: The effect of exchange transfusion of 21 days ACD stored canine blood, treated with the resin mixtures M and R, on the chemical composition of blood of the recipient dog. Inf = Influent Eff = Effluent

TABLE VI: Cationic and anionic concentrations of the ACD stored canine blood before and after treatment with the resin mixtures 'M' and 'R' combination.

Plasma constituents	0 *day heparinized*	7 *days*		14 *days*		21 *days*	
		Inf	*Eff*	*Inf*	*Eff*	*Inf*	*Eff*
Ammonium Blood $\mu g.$ NH_3-N%	22	78.8	0	137.2	12	223	52
Sodium *meq/l*	140	166.3	168.8	166.4	1.71	1.62	168.7
Potassium *meq/l*	4.12	4.73	4.63	8.5	4.62	12.47	5.53
Calcium *meq/l*	5.03	3.58	5.81	3.43	5.77	3.27	5.74
Magnesium *meq/l*	1.93	1.05	1.95	1.13	1.98	1.21	1.77
Chloride *meq/l*	109.7	79.7	100.5	77.5	97.7	73.3	102.7
Phosphate *mg%*	3.76	3.98	0.29	5.54	0.69	6.97	1.33
Sulphate *mg%*	1.54	2.03	0.13	3.35	0.18	4.52	0.26
Pyruvate *mg%*	2.1	4.47	0.4	6.27	1.67	9.4	2.36
Lactate *mg%*	23.8	62.2	4.0	97.5	17	134	43.4
Citrate *mg%*	1.44	301.3	23.3	308	30.6	301.8	27.4
Haemoglobin *mg%*	5.3	16.9	52.8	30	80.7	36.3	86.3
pH Blood	7.38	6.64	7.51	6.42	7.43	6.27	7.37

Inf = Influent Eff = Effluent

shows the effect of the exchange transfusion on some of the blood constitutents studied in a typical experiment in which a dog received 21 days ATD stored canine blood treated with the resin mixtures. At the end of the transfusion, plasma heamoglobin level remained somewhat elevated. However, twentyfour hrs after the transfusion, all the values were found to be close to their pre-transfusion levels.

DISCUSSION

The strong cation and anion exchange resins were preferred over the weak resins because they are mono-functional and could be used under almost any condition of acidity or alkalinity without risk of attrition or loss of resin (3). There is a descending order or affinity of the strong cation exchange resins for different cations such as $Ca^{2+}>Mg^{2+}>K^{+}=NH_4^{+}>Na^{+}>H^{+}$ and these resins also have a selective affinity for the divalent cations as compared to the monovalent cations (20, 21). The sodium, potassium and free hydrogen forms of the strong cation exchange resins used in various studies for the correction of cationic concentration changes of the stored bank blood (24, 26, 32) would therefore, effect a complete removal of calcium and magnesium in addition to the removal of elevated ammonium and potassium. Ionic calcium concentration of the ACD stored bank blood has been found to be precariously low because of the binding of calcium with the citrate ions (16, 19, 27). Treatment of this blood with the sodium, potassium

or free hydrogen forms of the resin would make it a calcium and magnesium depleted blood which when transfused in large quantities would precipitate a serious hypocalcemia and hypomagnesemia. In fact, Nealon and associates (26, 27, 28) encountered these difficulties and found that massive transfusions of the stored blood treated with an admixture of sodium and potassium resin, lowered the plasma calcium concentration of the recipient dogs to as much as 50% of their original values such that most of the animals developed serious hypotension and died. These authors attempted to prevent calcium deficiency by giving intravenous calcium in the post-transfusion period. But this was found to be unsatisfactory since the exact amount of calcium required to be replaced by intravenous injections could not be accurately predicted owing to a variable amount of ionic calcium which is chelated with citrate (24). It is imperative to avoid elevated levels of calcium because of the risk of precipitating dangerous cardiac arrhythmias. Changes in the blood magnesium levels were neither studied nor corrected by these authors although a prompt and significant hypomagnesemia brought about by the use of cation exchange resins has been reported (10, 16, 18, 20). In addition, acute magnesium deficiency *per se* can prove to have undesirable consequences (11) which, as in the case of calcium deficiency, could not be effectively corrected by intravenous infusion of magnesium. Among other effects, magnesium infusions are known to increase urinary output of calcium (12), which is not desirable. Therefore, infusions of calcium and magnesium are not an effective means of correcting the deficiency of these cations in the post-transfusion period. The only possible alternative would be to prevent these changes in the resin treated blood by modifying the composition of the resin admixtures on the basis of affinities of various resin forms for the cations of the stored blood (4, 16, 20). The cation exchange resin mixture 'M' of the present study achieves these objectives and has been shown to be effective in removing excessive ammonium and in lowering elevated potassium levels of the stored blood in additions to adjusting the concentrations of calcium and magnesium of the effluents close to their normal values (Table I, VI). The presence of the potassium form of the resin in the mixture 'M' prevented the excessive uptake of potassium from the stored bank blood and the effluent levels of potassium were always kept between 4.65 to 5.6 *meq/l* (Table I, VI).

The order of exchange potentials of anion exchange resins is less well known than the cation exchange resins. However, valency seems to exert a similar influence as with the cation exchange resins and according to Kunin and Myers (22), following is the pattern of affinities of strong anion exchange resins for a few common anions:

$$SO_4^{2-} > \text{Citrate} > PO_4^{3-} > \text{acetate} > Cl^-.$$

The affinities of the chloride and the bicarbonate forms of the strong anion exchange resin for various anions of the stored blood were worked out by the *in vitro* studies (Table II,III). Both the resin forms when used separately, were found to be equally efficient in the removal of various acidic ions and metabolites from the artificial serum but at the expense of serious changes in the pH of the effluents. No single form of the resin was, therefore, concluded to be of use for the anionic and pH correction of the stored blood. However, from the relative exchange behaviour of the anions, it was possible to work out a suitable mixture (mixture 'R') of the