The Dilution of Blood Plasma by Calcium-Combining Anticoagulants

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THE CONCENTRATION DISCREPANCIES between serum and plasma from the same sample of blood raise the questions: (1) To what extent does a given anticoagulant cause dilution of the plasma? (2) If the dilution varies with the anticoagulant, what is the basis for the difference? Serum samples[1–4] were found to be more concentrated in their components than oxalated plasmas from the same blood sample. Specific gravities, and therefore concentrations, were reported[5, 6] to be higher in serum than oxalated plasma from the same blood sample. Further investigations on these anomalies seemed necessary.

The findings of Marenzi[7] had prepared the way for this study. Although his work was primarily concerned with the effect of anticoagulant upon cell volume it may be assumed that a reduction of cell volume is a function of plasma dilution. The results of Marenzi[7] show that lithium oxalate produced the greatest reduction in cell size at any given concentration by weight; sodium oxalate, potassium oxalate and sodium citrate at the same concentration by weight caused reductions which were progressively less. However, the order of these salts is also the order of decreasing molarity. If the weight concentrations in Marenzi's work are converted to a molar basis, little difference in cell size is found at equimolar concentrations.

The present investigations were devised to measure the dilution effect of anticoagulants as determined by total nitrogen rather than cell volume.

Experiments

Blood from 4 New Zealand white rabbits was used. In order to prevent the hemodilution and the rapid overcompensation which other investigators had observed[8, 9] the large sample was taken into a large syringe in one bleeding by means of a cardiac puncture.

The syringe and the two centrifuge tubes to receive whole blood for the preparation of anticoagulant-free plasma, and of serum from anticoagulant-free plasma, were coated with G. E. "Dry Film" 9987 to increase clotting time. A small amount of paraffin oil was added to reduce evaporation and prevent carbon dioxide loss.[10] The anticoagulant-free blood was centrifuged for ten minutes at 12,000 times gravity in a multispeed attachment cooled by dry ice. This treatment removed sufficient platelets to prevent immediate, yet permit ultimate, clotting of the plasma.

The anticoagulant solutions with one exception were used in the proportions 0.1 ml. per ml. of whole blood. This produced an anticoagulant concentration of 0.208 M/10 in the blood as a whole. The required volumes of anticoagulant were evaporated to dryness in the centrifuge tubes. The blood added was measured carefully, then thoroughly mixed with the anticoagulant by rolling the tube between the hands, and centrifuged for fifteen minutes at 18,000 times gravitational speed.

The digestion was carried out with hyrogen peroxide without a catalyst in the manner of Miller and Miller[11] as modified by Leitch.[12] Since it was found that Nessler's reagent is sensitive to ketones, primary octyl alcohol (ketone free) was used as an antifoaming agent.

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The same amount of "Superoxol" was added to blanks and standards so that no correction for nitrogen contamination in this reagent was necessary. The samples were digested six times for five minute periods, on a different burner each time, to produce even heating as well as to insure complete digestion.

The Nessler reagent, prepared according to Koch and McMeekin[13] was added to the digested samples on a stirring apparatus built in our shop according to the directions of Miller and Miller.[11]

Depth of color was read in a Klett-Summerson colorimeter. Filter No. 50 or No. 54 was used depending upon the concentration of the nitrogen in the samples. The Klett tubes were calibrated to correct for differences in optical density.

The samples were analyzed in triplicate to reduce error in colorimetry.

TABLE 1.—*Dilution Effects of Anticoagulants as Measured by Total Nitrogen*

	Sample A-3		Sample A-5		Sample B-4		Sample C-3		Sample D-2		Sample C-4	
	mg./ml.	hemol.	mg./ml.	hemol.	mg./ml.	hemol.	mg./ml.	hemol.	mg./ml.	hemol.	mg./ml.	hemol.
Anticoagulant 0.208 Molar												
Lithium Oxalate			9.3	o	9.9	o	8.0	s	9.1	o	9.1	o
Sodium Oxalate	10.1	o	9.1	s	9.9	o	8.0	s	9.1	o	8.9	o
Potassium Oxalate	10.2	h	9.4	h	9.9	h	7.9	h			9.1	h
Potassium Citrate	10.4	h	8.9*	h	9.9	h	7.9	h			9.1	h
Sodium Citrate	10.4	o										
Mixture					9.9	o			9.3	o		
Anticoagulant 0.416 Molar												
Potassium Oxalate			8.7	h								
Anticoagulant-free Plasma	11.1		9.9		11.3		9.5		10.6		9.8	
Serum from anticoagulant-free Plasma					10.8						9.5	
Whole Blood Serum			9.7									
Average Dilution	8%		8%		14%		20%		15%		9%	

* Error in pipetting suspected.

h—hemolysis, s—slight hemolysis, o—no hemolysis.

RESULTS

Effect of Equimolar Anticoagulants Upon Plasma Dilution as Measured by Total Nitrogen

When equimolar quantities of anticoagulant were added to aliquot portions of a given sample of blood, the plasmas showed little variation in nitrogen content. Most of the results fell within the limits of error, ± 1.6 per cent, for triplicate analysis.[11] The only exception was plasma A-5, prepared with 0.208 molar potassium citrate; here an error in pipetting is suspected. A mixture of sodium and potassium oxalates of total molarity 0.208 produced the same dilution as either substance alone (B-4 and D-2). On the other hand, doubling the concentration of anticoagulant increased the dilution (A-5). Other unpublished experiments confirm this finding.

Effect of Type of Anticoagulant upon Cell Destruction

All the potassium anticoagulants produced hemolysis (table 1) while plasmas prepared with lithium and sodium anticoagulants were hemolysis free. A mixture of equivalent quantities of sodium and potassium oxalates of total molarity 0.208 failed to cause the hemolysis produced by potassium oxalate alone. The potassium ion thus appears to exert on the erythrocyte a type of hemolytic action which either vanishes at concentrations 0.104 M/10 or less in the blood as a whole, or else is prevented by the presence of adequate concentrations of sodium ion.

The Dilution Caused by Calcium-combining Anticoagulants

Plasmas prepared without anticoagulant served as a standard for determining the dilution produced by the anticoagulants. The minimum dilution observed was 8 per cent, the maximum 20 per cent. This range is in agreement with Marenzi,[7] Nichols[14] and Elman,[15] who observed the effect of anticoagulant on cell size by hematocrit measurements.

Discussion

The calcium ions of the blood combine with the oxalate and citrate ions of the anticoagulants. Since each calcium ion is replaced by two monovalent cations, and since an excess of anticoagulant is required to insure adequate removal of calcium from the blood, the osmotic pressure of the extracellular fluid is increased. The red cell membrane is apparently more permeable to water than to the ions,[16] for the osmotic pressure difference across the membrane appears to be equalized by the passage of water from erythrocytes to plasma. For any given sample of blood and any given concentration of anticoagulant the extent of plasma dilution may be expected to vary with the ratio of plasma volume to cell volume, with the concentration of free calcium, and possibly with the concentration of hemoglobin within the cell and of plasma proteins outside the cell.

Equimolar solutions of lithium, sodium and potassium oxalates and of sodium and potassium citrates have been shown to produce identical dilution of the plasma in aliquot portions of a given sample of blood. The colligative properties of these anticoagulants, rather than any special characteristics, thus appear to be responsible for the dilution effects. The experiments emphasize the desirability of measuring and comparing anticoagulants on a molar rather than on a weight basis.

The potassium ion was found to produce hemolysis at a concentration of 0.208 M/10 in the blood as a whole. Lithium and sodium anticoagulants are therefore recommended instead. Tyler[17] has presented evidence for the presence of an antihemolytic agent in fresh plasma. Perhaps the potassium ion inactivates this inhibitor, or in some way interferes with its action.

Serum was prepared from blood A-5, and from the anticoagulant-free plasmas obtained from bloods B-4 and C-4. In each instance the nitrogen content was less than that of the corresponding anticoagulant-free plasma, and the difference was about what might be expected for the removal of fibrinogen.

The degree of dilution produced by 0.208 molar anticoagulants varied from

8 per cent to 20 per cent in the bloods studied. The breadth of this range, and the dependence of the dilution on the anticoagulant concentration in the blood as a whole, stress the importance of using serum or unoxalated plasma for comparing the plasma protein concentrations of individual blood samples.

Summary

When used in equimolar concentrations, five different calcium-combining anticoagulants produced uniform dilution of a given blood sample.

The potassium anticoagulants seem to exert a hemolytic effect on the erythrocytes.

The plasma dilution caused by a given concentration of the calcium-combining anticoagulants ranged from 8 to 20 per cent in the bloods examined. This shows the importance of using serum or unoxalated plasma for comparing the plasma protein concentrations of individual blood samples.

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