Effect of Plasmapheresis on Blood Cholesterol Levels in the Dog.*† (23122)

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The hypercholesterolemia of nephrotic syndrome is regularly associated with heavy proteinuria and diminished plasma albumin concentration. Clinically, proteinuria precedes alterations in plasma cholesterol and albumin levels. Recently, Rosenman and his co-workers(1) have presented evidence indicating that plasma cholesterol elevation in nephrotic rats occurs as a consequence of heavy proteinuria and subsequent plasma albumin depletion. We have reasoned that if hypercholesterolemia is a result of plasma protein depletion, it should occur if the plasma proteins are lost via a diseased kidney, or by some other route in the presence of substantially normal renal function. In the present experiments the levels of blood cholesterol have been studied following depletion of plasma protein by the technic of plasmapheresis.

Methods. Adult male dogs were used in all experiments. The animals were trained to lie on their backs with legs restrained. A 16-gauge needle was inserted into the external jugular vein and the effluent blood was collected in a sterile vacuum flask containing heparin. One hundred fifty to 300 ml of blood was collected daily, Monday through Friday each week. Immediately after collecting the blood, its temperature was lowered to 2°C. The erythrocytes were separated from plasma by centrifugation in the cold and were washed once with cold sterile 0.89% sodium chloride solution. The washed erythrocytes were then suspended in 0.89% sodium chloride solution equal to the volume of plasma removed and were kept in the cold until reinfused into the dog from which they were taken. The warmed, washed erythrocytes taken the day before were given intravenously through the needle in the jugular vein after the day's bleeding was completed. The animals tolerated this procedure without noticeable ill effects. If the hematocrit began to fall, additional washed erythrocytes from a donor animal were given. Animals were weighed daily, and weight was maintained as closely as possible by increasing food intake. All glassware and tubing were kept sterile. Rectal temperatures were taken daily and did not deviate from the normal range. *Diet*: Animals were initially given a protein free 7% fat diet of the following composition in the form of a baked cake: corn starch 38.6%, sucrose 19.3%, crisco 12.9%, dextrose 6.4%, corn oil 2.4%, baking powder 1.6%, salt mixture(2) 1.2%, bone ash 4%, kaolin 4%, water 9.6%. After one week, 0.5 g/kilo body weight of protein (horsemeat) was added and the animals were continued on this diet for duration of experiment. Vitamin supplements were added to the diet daily. *Chemical determinations*: Plasma albumin and globulins were determined by Kingsley's Method(3). Cholesterol was determined by modification of the method of Schoenheimer and Sperry (4).

Results. Results are shown in Fig. 1 (A-D). The calculated regression line is indicated on each plot as is the correlation coefficient "R". The significance of the correlation coefficient has been calculated according to the formulae: $Z = 1.1513 \times \log_{10} \frac{1+r}{1-r}$. Standard deviation of Z = Fishers "t" = $\frac{1}{\sqrt{N-3}}$ where N = number of paired observations. P values are obtained by reference to Fishers Table of t, where t has an infinite number of degrees of freedom. Thus, for dog A, P = 0.1, for dog B<0.01, for dog C 0.02 – 0.01 and for dog D<0.001. This indicates a

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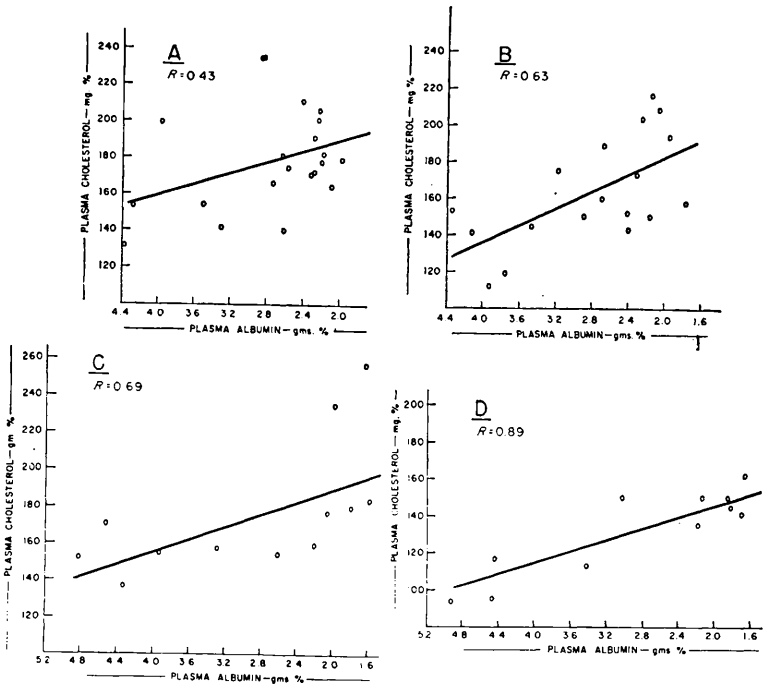

FIG. 1. Relation between plasma cholesterol and plasma albumin during plasmapheresis in each of 4 separate animals. R = Correlation coefficient.

satisfactory correlation of the plotted data from dogs B, C and D, but not from dog A. Inspection of Fig. 1-A indicates that a single determination that departs radically from the remainder (at plasma albumin level of 4) keeps this plot from attaining a greater degree of significance.

It is apparent, that as the plasma albumin concentration is experimentally reduced, the plasma cholesterol concentration rises. Except for Fig. 1-D, a curve with increasing slope at lower plasma albumin levels would better fit the experimental observations. It may be that significant increases in plasma cholesterol do not occur until the plasma albumin falls below a concentration of approximately 2.5 g %. It is also possible that the increased rise in plasma cholesterol at lower albumin levels is related to some factor not accounted for in these plots, such as time. There may be a lag in the maximal elevation of plasma cholesterol in response to a given level of reduced plasma albumin.

Total plasma globulin concentrations were not significantly altered during the experiments. Two control dogs were fed the same diet as the experimental animals, but they were not plasmapheresed. Although their plasma albumin concentrations fell from levels of about 4.5 g % to approximately 3.5 g %, no consistent alteration was observed in the levels of plasma cholesterol.

Discussion. It is possible that chronic plasmapheresis depletes the animal of a variety of substances only a few of which may have been measured in these studies. However from the data at hand, the rise in plasma cholesterol that occurs following plasmapheresis appears to depend on depletion of plasma albumin. Total plasma globulin concentrations were not significantly altered by plasmapheresis. The animals were kept at nearly constant weight, and great care was exercised in maintaining erythrocyte counts and hemoglobin concentrations at normal levels.

These data support the earlier findings of Boggs and Martin(5) and Fishberg and Fishberg(6) and recent observations of Rosenman, Friedman and Byers(1). Furthermore, it is demonstrated that albumin loss need not occur through a diseased kidney to initiate elevations in plasma cholesterol. If sufficient

plasma albumin is lost from the animal to deplete plasma albumin concentration, elevation of plasma cholesterol will occur whether the protein is lost via a heavy proteinuria, or whether it is withdrawn directly from the blood stream in the presence of substantially normal kidneys. The corollary to this experiment has already been performed in the rat by Rosenman *et al.*(1) when they demonstrated that plasma cholesterol values fell toward normal following the infusion of bovine albumin into nephrotic animals.

Summary. Chronic plasmapheresis in the dog resulted in a fall in plasma albumin and an elevation of plasma cholesterol concentration.

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Boundary Electrophoresis of Human Parotid Saliva. (23123)

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The ultraviolet spectrum of human parotid saliva has been shown to possess bands characteristic for protein(1). Recently, Pigman *et al.*(2) stated that parotid saliva contained 8 electrophoretic components with a major peak of low mobility at pH 8.6. The present study presents electrophoretic patterns of 6 specimens of parotid saliva from 5 individuals.

Methods. Six samples of parotid saliva of 60 ml each were collected from 5 individuals by means of a modified* Lashley cup (3). They were concentrtaed by ultrafiltration(4) at 4°C, dialyzed against 0.1 ionic strength veronal at pH 8.6, filtered, made up to 11 ml, and subjected to boundary electrophoresis in the standard cell of the Aminco Model B Electrophoresis Apparatus. Duplicate specimens of 60 ml of parotid saliva were concentrated(4), made up to 20 ml and samples were taken before and after ultrafiltration for protein analysis by the biuret reaction(5). Mobilities were determined according to standard procedures(6) and the relative concentrations were calculated from the Rayleigh fringes(7) shown in Fig. 1 with the customary assumption that the refractive index increment of all the components was the same. The minor components II and IV (Fig. 1) were included with component III in the calculation of the relative concentrations.

Results. As shown in Table I, the extent of concentration of protein after ultrafiltration (5.4 fold) closely paralleled concentration in volume (5.5 fold, or 60 ml to 11 ml), indicating that essentially no protein was lost by ultrafiltration. Before ultrafiltration the parotid salivas contained 18.7-58.9 mg %

TABLE I. Protein Content of Parotid Saliva before and after Ultrafiltration (mg %).

Subject	Before	After	Degree of conc. Volume	Protein
A	41.0	243.5	5.5	5.9
B	52.0	269.2	"	5.2
C	18.7	98.3	"	5.3
D	51.5	259.4	"	5.0
E	58.9	323.8	"	5.5
		Mean	5.5	5.4

* Prepared in Instrument Shop, N.I.H.