

RECIPROCAL CHANGES IN PLASMA PROTEIN AND PLASMA ACACIA AS RESULT OF HIGH AND LOW PROTEIN DIETS

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It has been demonstrated that intravenous injection of gum acacia solution produces a definite decrease of both the concentration and total amount of circulating plasma protein. This phenomenon has been of interest not only from the standpoint of plasma protein formation and release, but also in regard to the mechanism of the acacia effect. It has been suggested (1, 2) that one reason for the decrease in plasma protein is a displacement of the proteins by the acacia. That plasma protein does not return promptly to the circulation is thought to be associated with the presence of acacia in the blood, plus the deposit of acacia in liver cells which interferes with the production or release of the protein. Further experiments (3) have shown that by repeated injections of gum acacia in dogs, it is possible to reduce plasma protein concentration and total circulating protein to markedly low levels and to maintain them below normal limits for relatively long periods of time. During the course of such investigations, it was noted that *following cessation of acacia injections* one dog on a high protein diet showed a more rapid rise in plasma protein than occurred in another dog receiving a diet of lower but adequate protein content. As a result of these findings, it was suggested that perhaps diets might be adjusted so that the acacia animal could be maintained at a constant low plasma protein content, or that if a low protein diet were given, the total content of plasma protein might be further reduced, even though it was already at a low level. At that time, the possibility of an increase in acacia in the blood was not considered.

The data presented here concern the effects of varying the protein content of the diets of animals to which acacia injections have been discontinued, and they demonstrate the changes not only in plasma protein but also in the acacia content of the blood.

Methods

Three mongrel dogs were used. Each had previously received weekly injections of gum acacia as indicated in the history. The acacia injected (Lilly, minus sodium chloride) was

diluted to a concentration of 12 per cent in distilled water, and was injected intravenously. At varying intervals following discontinuance of acacia injections, the animals were started on diets of known protein content, and alternate high and low protein adjustments were made. In some instances, there were periods of fasting. At the onset and end of each dietary period, blood for analysis was collected. Plasma protein, blood volume, plasma acacia, albumin and globulin determinations were done by methods previously described (1).

Histories

Dog 1.—A small short haired female mongrel weighing 7.9 kilos which received 327 gm. or 41.7 gm per kilo of gum acacia over a period of 16 weeks. During the entire period of injection the dog received a diet of salmon bread and raw lean beef which contained protein equivalent to 2.8 gm. per kilo body weight per day. The first experiment was started 1 week after the last injection of gum acacia. After 3 weeks of this same diet, the animal was given raw lean beef alone, equivalent to 5.4 gm. of protein per kilo body weight per day. This was continued for 2 weeks. The dog was then fasted for 1 week, after which for 4 weeks the lean beef diet was given. During the next period of 2 weeks, the lean beef was reduced by half, and then increased to its original amount of 5.4 gm. per kilo per day for the ensuing final 2 week period. During the entire experiment the animal's weight varied 1 kilo, the low point being after the fasting period. The terminal weight was 0.5 kilo less than that at the start of the experiment. The animal was in good condition during the entire period.

Dog 2.—A short haired mongrel male weighing 24 kilos which, over a period of 22 weeks, received 671 gm. of gum acacia or 27.5 gm. per kilo. During the injection interval the dog was fed a mixed diet, the protein content of which was not known. In the 8th week following cessation of acacia injections, the diet was changed to a potato-ground lean beef diet containing 1 gm. of protein per kilo body weight. At this point the present experiments were started. The animal at this time weighed 16.5 kilos. After 2 weeks the diet was changed to 4 gm. of protein per kilo per day, the extra 3 gm. by addition of more ground lean beef. This was continued for 25 days, when the original diet containing 1 gm. of protein per kilo was resumed. During the entire experiment, the dog was in good condition and the weight remained constant.

Dog 3.—A black haired mongrel weighing 13.3 kilos received over a period of 15 weeks 355 gm. or 26.7 gm. per kilo of gum acacia. During this period the animal was fed a diet composed of table scraps. Twenty-one days after the termination of gum acacia injections, the present experiments were started. The dog was fasted for a period of 7 days immediately preceding the first period. The following 9 days the dog was fed a raw lean beef diet containing 4.3 gm. of protein per kilo per day or a total of 53 gm. of protein per day. Fasting was carried out for 2 days and following this the diet resumed for 3 days. This was followed by 8 days of fasting, with a return to the lean beef diet for 10 days. The dietary intake was then reduced twice; first to 1 gm. protein per kilo per day for 10 days and then to 0.5 gm. for 8 days. Subsequent to this a third period of fasting was instituted, lasting 10 days and then during the last 8 days of the experiment, the diet consisted of 5 gm. of protein per kilo per day. The weight at the onset of the experiment was 12 kilos and this gradually dropped to 9.5 kilos over a period of 75 days. The dog's condition was good during the course of the experiment.

EXPERIMENTAL OBSERVATIONS

Table I shows plasma protein and acacia studies in one animal (dog 1) over a period of 95 days. During the course of the entire experiment except at one interval (period 3) when fasting was carried out, this dog at all times received diets containing more protein than is considered necessary for maintenance.

Although changes in plasma protein concentration were not marked during the periods of dietary change, it showed a tendency to fall when the intake was reduced from 5.4 gm. per kilo per day to the lower figures. This tendency was most marked after the fasting period. The acacia, on the other hand, showed more marked tendencies in the opposite direction, tending to fall when the protein in the diet was increased, and to rise when the reverse was done.

TABLE I
Summary of Acacia and Protein Determinations—Dog 1

Period	Day	Weight	Protein intake	Plasma protein				Acacia		Total circulating "colloid", gm.
				Concen- tration gm./kg./ day	Fibrin- ogen mg. per cent	Total cir- culating albumin gm.	Total cir- culating globulin gm.	Concen- tration gm. per cent	Total in circula- tion gm.	
1	1	6.5	1.7	2.4	57	7.6	3.7	3.0	13.9	25.2
	7	6.6	2.2	2.9	85	8.3	4.5	2.5	11.0	23.8
	14	6.5	2.0	2.9	75	10.6	2.5	2.2	9.9	23.0
2	21	6.5	4.8	3.3	70	10.6	4.3	2.1	9.6	24.5
	28	6.3	5.4	3.9	78	11.6	4.6	2.3	9.6	25.8
	35	6.7	5.4	3.7	96	10.6	5.2	1.7	7.3	23.1
3	42	5.5	0	3.1	49	7.1	5.1	2.4	9.2	21.4
	49	5.8	5.4	3.2	—	7.9	4.2	2.7	10.2	22.3
	56	6.0	5.4	3.4	89	7.9	5.6	2.4	9.6	23.1
	63	5.0	5.4	3.8	77	—	—	2.2	—	—
4	70	6.2	5.4	3.8	102	10.5	4.0	2.2	8.2	22.7
	77	6.1	2.7	3.8	69	9.5	6.3	2.5	10.2	26.0
	81	5.6	2.7	3.5	78	9.9	3.8	2.6	10.0	23.7
	88	5.7	5.4	4.1	227	10.7	8.3	2.0	9.1	28.1
6	95	—	5.4	4.3	131	10.5	7.8	2.0	8.3	26.6

Fibrinogen, as shown, remained low until the last period. Total circulating protein and acacia showed the same reciprocal tendencies.

Concerning the protein fractions involved, that is, albumin and globulin, one notes that during the first three periods the total circulating albumin roughly paralleled the total circulating protein, whereas the globulin paralleled the acacia. Later the globulin was irregular, following no definite pattern but tending to rise markedly. Total circulating "colloid" (total circulating protein plus total circulating acacia) was fairly constant until the period of fasting when it diminished. From that point on it gradually increased to about 20 per cent over this low point. It can be seen from the table that the total circulating plasma

protein and plasma protein concentrations were quite parallel, and the same parallelism existed with the concentration and total amounts of the gum.

Chart 1 shows plasma protein and gum acacia concentrations in one animal

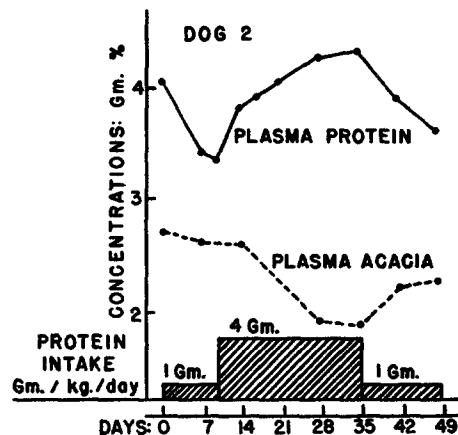


CHART 1. Effect of dietary protein changes on plasma protein and plasma acacia concentration (dog 2).

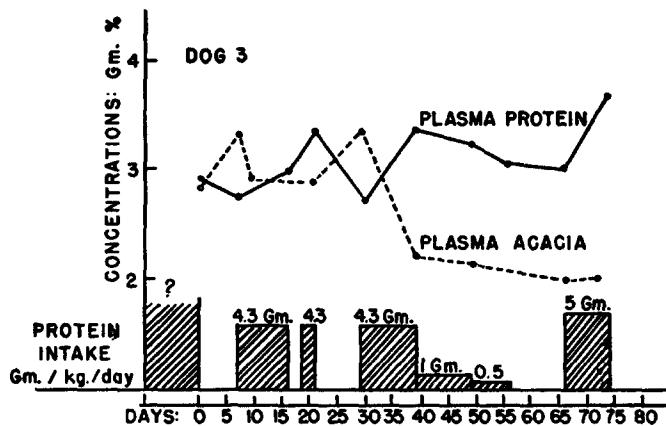


CHART 2. Effect of dietary protein changes and fasting on plasma protein and plasma acacia concentration (dog 3).

(dog 2) over a period of 7 weeks. During the first period on a relatively low protein diet, the plasma protein concentration decreased. During the next period of 25 days, following dietary protein increase, there was a gain in plasma protein concentration, reaching a maximum of 4.3 gm. per cent while the plasma acacia concentration fell from 2.5 gm. per cent to 1.8 gm. per cent. Following this period the diet was again reduced and in 2 weeks the plasma protein concentra-

tion had changed from 4.3 gm. per cent to 3.6 gm. per cent, while the acacia concentration rose from 1.8 gm. per cent to 2.3 gm. per cent. This portion of the experiment was then discontinued. It is to be noted that at the end of the experimental period, the plasma acacia was still lower than the initial figure, as was the plasma protein. The total circulating "colloid" was similarly lower (5.9 gm. per cent compared to an initial level of 6.9 gm. per cent). The third animal (dog 3) (Chart 2) was subjected to a similar type of procedure in ten experimental periods. The initial protein concentration was lower and the plasma acacia concentration was higher than that of the first. The first period was one of fasting for 7 days. The plasma protein (Chart 2) diminished slightly, perhaps not significantly, whereas the plasma acacia concentration showed a definite rise—from 2.8 gm. per cent to 3.3 gm. per cent.

Further periods are indicated in the chart. It can be seen that in general, during periods of fasting the protein concentration diminished. The plasma acacia concentration increased during the first two periods of fasting, but in the last period, from the 56th to the 66th day, it did not respond in this manner, but continued to diminish slightly after the period of fasting. In the periods of high protein feeding, the reverse usually took place, the acacia concentration diminishing and the plasma protein concentration rising. Thus a roughly reciprocal relationship between the two colloids is noted, with the total circulating colloid remaining more or less constant, except at the very end. Again the acacia concentration was lower at the end than at the beginning of the experiment and the protein concentration was higher.

DISCUSSION

The original premise of the experiments has been fulfilled within certain limits. It has been possible to lower consistently the plasma protein by reducing the dietary protein intake. As to how long this could be continued, there are no data, since the variations in acacia and the reciprocal relationship between it and plasma protein absorbed our interest in the current experiments. One variable that has not been measured is the rate of excretion of the gum from the body. This is accomplished chiefly *via* the bile, but the gum has been demonstrated in the urine of animals, such as the three under discussion, which have received large amounts of this substance. In addition all three of these dogs received varying amounts of the gum over varying periods of time. It has been found that it takes more gum acacia to lower the plasma protein to a given level in some animals than it does in others. This may be associated with individual differences in the total protein nutritional state at the outset.

It is of interest that in these animals, "saturated" with gum acacia, a dietary protein intake of more than 1 gm. per kilo of body weight per day is necessary to maintain the plasma protein concentration at any given level. In one instance (dog 3, period 5) the plasma protein concentration as well as total blood plasma

protein dropped during a period when the diet contained as much as 2.7 gm. per kilo per day. The reasons for this are obscure. It is doubtful whether lack of absorption from the intestinal tract is responsible. Lack of synthesis by a liver filled up with the gum is a possibility. It is difficult, however, to explain why the synthesis could take place in the presence of extra large amounts of the necessary dietary protein and not in the presence of what is usually considered a normal diet. It has been shown (1) that during a single injection of gum acacia on a given constant diet, there is no increase in urinary nitrogen, in spite of marked diminution of plasma protein. Because of this, it has been argued that the plasma protein is displaced and possibly returned to cells. In other words, the presence of more than a sufficient amount of colloid in the blood may result in a deposition of that colloid in certain storage sites. In the case of the two colloids gum acacia and plasma protein, such a deposition of gum acacia is known to take place, and the results given here indicate that this behavior of acacia or of protein may be selective, depending probably on (1) the amount of protein being formed or available for release into the blood, (2) the amount of acacia present both in the blood and in sites of deposit.

It would be hazardous to draw too close a parallel between the behavior of gum acacia and plasma protein, but there is some evidence indicating that these two colloids behave in much the same way under certain conditions. When together in the blood stream, although no figures as to oncotic pressure are available, their total concentration in grams per cent approximates the normal limits of the concentration of plasma protein alone. Whipple's evidence (4) that the plasma protein molecule can enter cells and leave them is pertinent to these observations. Acacia, according to the results given, apparently behaves in the same manner. The argument as to whether plasma protein in its departure from a cell into the blood is broken down on passage through the cell membrane and reconstituted outside the cell would not be expected to be considered in the case of gum acacia. There is certainly no evidence to support any suggestion that this colloid, foreign to the body, could be broken down at the time of its exit from the cell and reassembled either on the surface of the cell or in the blood stream. Presumably we are dealing in this instance with pure colloidal passage. Further experiments are being carried on in an attempt to verify the belief that acacia can actually be liberated from its storage sites.

It would seem that a distinction must be made between plasma protein "formation" and plasma protein "release." The technics used in the present experiments give no information as to the amounts of plasma protein formed. An acacia animal on an "adequate" protein diet may be forming sufficient protein for normal maintenance of cells and plasma, but its release into the blood appears to be dependent on the situation (probably colloid osmotic pressure) which exists in the blood itself. Hence if sufficient stores are present, and there is a "need" for colloid in the blood, it is released from the stores, perhaps by

differences in intra- and extracellular osmotic pressures. It is possible, therefore, that plasma protein may be formed and stored with no measurable differences noted in the circulating plasma protein, as in the case of normal animals which have an adequate protein reserve. Experiments based on protein content of viscera, particularly the liver, under these conditions might throw light on this question.

SUMMARY AND CONCLUSIONS

Dogs were made hypoproteinemic by repeated injections of gum acacia, and the acacia injections were discontinued. Diets of varying protein content were then given. When a high protein diet is provided the plasma protein concentration increases; with a low protein diet, or under conditions of fasting, the plasma protein concentration diminishes. Similarly, plasma acacia concentration shows increases and decreases which are reciprocal to the protein variations. Total circulating plasma protein and total circulating plasma acacia show similar changes. In all instances total circulating colloid (acacia plus protein) concentration adds up to an amount within normal limits for protein alone.

The results indicate that under these conditions, acacia stored in the body (principally in the liver) can be removed from its site of deposit and returned to the blood.

The data also show that dogs in which acacia is deposited in large quantities, require a larger amount of protein in the diet to maintain a constant plasma protein content than do normal dogs.

It appears that the mechanism for maintenance of peripheral colloidal material may be dependent on differences in intracellular and extracellular colloidal osmotic pressure. The experiments also support the idea that plasma protein molecules, as well as gum acacia, may pass in and out of cells through the cell membranes.

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