BLOOD PLASMA PROTEINS AS INFLUENCED BY INTRAVENOUS INJECTION OF GUM ACACIA

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Any experimental technique which involves disturbance of liver function is of particular interest in regard to plasma protein studies, for there is evidence that the liver is associated in the production of plasma protein. When a solution of gum acacia is injected intravenously in the dog, there is a marked decrease in the blood plasma protein concentration (5). That the liver is concerned with this process is suggested by the observation that injected gum acacia is rapidly removed from the blood and deposited to a large extent in the liver, whence it is slowly eliminated in the bile (4).

In any attempt to evaluate this phenomenon following gum injection, it is important to ascertain, first, whether decrease in plasma protein *concentration* is associated with a decrease in *total* circulating protein, and second, the degree of impairment of liver function under these conditions. The data given here represent such an attempt.[1] By means of plasma volume determinations it was possible to show a decrease in the total circulating protein. Determinations of plasma fibrinogen were made as a means of estimating the relative degree of hepatic function. Marked disturbance in fibrinogen concentration was demonstrated both in dogs receiving single injections of the gum, and in those in which repeated doses were given. It was possible by means of repeated weekly injections of acacia to maintain dogs at low plasma protein and fibrinogen levels for several weeks. Following

[1] We are grateful to Eli Lilly and Company for furnishing valuable material used in these experiments.

such procedure, the most marked anatomical changes were noted in the liver.

Methods

In experiments involving a single injection of gum acacia four normal dogs were used. These animals were given a standard potato-hamburger diet containing all food elements essential for health. The diet which was adjusted to furnish 2 gm. of protein per kilo of body weight, was usually started a few days before the proposed injection. Basal figures as indicated in the tables were obtained by one or more analyses previous to injection. When more than one analysis was made, the average is given. The dogs were put in metabolism cages. In two of the animals analysis of urine for nitrogen was done. The urine was collected in concentrated sulfuric acid, and nitrogen was determined by the micro-Kjeldahl method, to be described.

Blood for analysis was collected in 1.4 per cent sodium oxalate solution in hematocrit tubes. Appropriate factors for dilution were introduced into the various formulae.

Plasma volume determinations were made by two methods. In two of the dogs (36-21, 36-94), the method of Hooper, Smith, Belt, and Whipple (7) with modifications by Whipple and Robscheit-Robbins (13) was used. In dog 35-151 we employed a modification of Gibson and Evans' method (6) using T-1824 dye, and spectrophotometric quantitation. Nitrogen determinations were made in duplicate and triplicate by a modification of the method of Goebel, as described by Peters and Van Slyke (10).

Albumin and globulin were determined by Howe's method, as described by Peters and Van Slyke (10), using 22 per cent sodium sulfate at 37°C. Fibrinogen was estimated by the following method: To 1 ml. of oxalated plasma in a large test tube, 28 cc. of 0.8 per cent sodium chloride solution is added. After mixing, 1 ml. of 2.5 per cent calcium chloride solution is added, mixed, and allowed to stand for 1 hour. The resultant clot is washed several times and transferred to a micro-Kjeldahl flask, where nitrogen is determined by the usual method. Duplicate samples were determined.

Preparation of Acacia Solutions.—A sterile solution of gum acacia (Lilly—"without sodium chloride") of 30 per cent concentration is diluted with Locke's solution (minus calcium chloride) to yield the desired amount of acacia in the concentration desired. One dog (32-8) was given 6 per cent solution, while all other animals received the substance in 12 per cent concentration. The solution is heated to body temperature and administered slowly into the jugular vein.

To determine if the presence of acacia might have any possible interference with the precipitation of globulin, or with the precipitation or clotting of fibrin *in vitro*, tests were carried out using a mixture of the gum solution in various concentrations with plasma of determined fibrinogen and albumin content. Differences were negligible and entirely within the limits of experimental error for the various techniques.

EXPERIMENTAL OBSERVATIONS

Dogs Receiving Single Injections of Gum Acacia.—Four animals were studied in this group. All dogs were on the same diet, which with one exception (35-151) was continued throughout the course of the experiment. All dogs received the same relative amount of 12 per cent gum acacia solution (1 gm. of acacia per pound body weight).

TABLE 1

Single Injection of Gum Acacia

(A) Dog 36-21.

Day	Plasma protein		Blood plasma volume	Red blood cell volume	Total blood volume	Hematocrit plasma	Urine (48 hrs.)	
	Concentration	Total in circulation					Volume	Nitrogen
	gm. per cent	*gm.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*gm.*
Basal	5.95	32.3	544	643	1187	43.1		
3	4.26	23.3	547	545	1092	50.4		
5	4.90					45.7		
6	4.84	31.9	661	535	1196	45.1		
8	4.73	25.2	533	511	1044	51.0		
10	6.05	26.7	422	476	918	48.1		
(B) Dog 36-94.								
Basal	6.60	54.4	828	1061	1889	44.3	1650	12.8
1	4.73	43.6	923	926	1849	49.9		
2	4.80					51.2	1400	12.4
3	4.53	48.0	1059	1051	2110	51.7		
4	4.76					50.8	1750	12.2
5	4.97	51.8	1138	1201	2339	48.6		
6	5.10					50.6	1400	13.5
7	5.06					47.0		
8	5.38					48.0		
10	5.27	45.6	865	865	1730	50.0		
14	5.86	47.8	816	729	1545	52.8		

Table 1 (A) shows the reaction of dog 36-21 to the injection. Daily determinations were not done on this animal, the interest at the time being in blood volume and total protein changes at time intervals that had previously been shown to correspond to maximum and minimum levels. It will be noted that there was practically no in-

crease in the plasma volume on the 3rd day following injection, at which time the plasma protein concentration was at the lowest point. On the 6th day, however, there was an appreciable increase in plasma volume, the plasma protein concentration having risen in the meantime. On the 10th day, when the plasma protein concentration was up to the normal range, the plasma volume and blood volume were below their original levels. This ultimate shrinkage of blood volume is a phenomenon which has been observed to a greater or less extent in all three of the dogs on which blood volume determinations have been made. We attempt to explain it on the basis of diminution of total colloid, both plasma protein and acacia. That there is a diminution of total plasma protein, is indicated by the results. Acacia at this time should (3) be largely gone from the blood stream. In this event, then, the lowered amount of colloid would carry with it less fluid, thus accounting for the lowered plasma volume.

An interesting observation brought out by this and other tables has to do with the relationship between the hematocrit plasma percentage and the estimated plasma volume. It is to be noted that changes in plasma percentage were not proportional to changes of the plasma volume. For example in Table 1, from the 6th to the 8th days, the plasma per cent increased from 45 to 50 per cent, while the plasma volume diminished from 661 to 533 cc. There was also a slight decrease in total blood volume and in the red cell volume. The significance of this change is not understood but it has been so constant that it cannot be overlooked. Studies of numbers of red cells and of the mean corpuscular volume should be of interest in this regard. It is possible that there may be changes in the size of the red cells, or interference with their release into the peripheral circulation. At any rate, it is obvious that the hematocrit under such circumstances is unreliable as an index to any relative degree of change in the total plasma or red cell volume.

Table 1 (B) shows the reaction of dog 36-94 to the injection. This experiment differed from the others in that plasma volume determinations were made on the day following injection of the acacia solution. At this time there was an increase of plasma volume from 828 to 923 cc., enough to be considered significant. Plasma protein concentration fell from 6.6 to 4.7 and there was a definite decrease in the amount of total circulating protein, amounting to about 10 gm., or approximately 20 per cent. This was not associated with an increase of the urinary nitrogen. On the 3rd day there was an increase in plasma volume of a little over 200 cc. or about 25 per cent over the basal. The

plasma protein concentration diminished from a basal average of 6.60 to 4.53, or about 31 per cent. The total circulating protein in 3 days decreased from 54 gm. to 48 gm. On the 14th day there was an increase of 0.7 gm. of urinary nitrogen, which would be equivalent to about 4 gm. of protein. At this time, the total circulating protein was increasing. Unfortunately, later urine specimens were contaminated with fecal material. This dog also illustrates two points which have been mentioned before, namely the disproportion between plasma

TABLE 2

Single Injection of Gum Acacia

Dog 36-100.

Day	Plasma protein					Hematocrit plasma
	Concentration	Albumin	Globulin	A/G ratio	Fibrinogen	
	gm. per cent	*gm. per cent*	*gm. per cent*		*mg. per cent*	*per cent*
Basal	6.85	3.82	3.03	1.5	307	47.8
1	4.37	2.43	1.94	1.3	176	49.8
2	4.19	2.27	1.92	1.2	204	51.3
3	4.15	2.25	1.90	1.2	82	52.8
4	3.98	—	—	—	109	53.7
5	4.41	2.55	1.86	1.4	119	55.3
7	4.45	2.63	1.83	1.4	118	57.0
9	4.93	2.82	2.11	1.3	125	53.8
11	4.99	2.68	2.31	1.2	154	53.0
13	6.14	—	—	—	317	52.4
20	6.34	3.33	3.01	1.1	286	51.0
27	6.64	3.79	2.86	1.3	250	49.9

per cent and plasma volume, and ultimate diminution of plasma and red cell volume.

Table 2, dog 36-100, represents an animal in which an attempt was made to correlate fibrinogen concentration with plasma protein concentration. It is of interest to note that although the general reaction is the same, the ratio between the two substances is inconstant. For example the fibrinogen reached the lowest level of 82 on the 3rd day, whereas the minimum protein concentration was attained on the 4th day. Likewise, between the 1st and 2nd days, plasma protein concentration decreased, whereas fibrinogen concentration increased. These

changes are not great, but indicate a trend which, it will be seen later, occurs to a greater degree in dogs receiving frequent injections. There was a tendency for the albumin-globulin ratio to decrease a trifle. This same tendency is noted in the following animal, and at the present, its significance if any, is not known.

TABLE 3

Single Injection of Gum Acacia

Dog 35-151.

Day	Plasma protein						Blood plasma volume	R. B. C. volume	Total blood volume	Hematocrit plasma	Urine (48 hrs.)	
	Concentration	Total in circulation	Fibrinogen	A/G ratio	Albumin	Globulin					Volume	Nitrogen
	gm. per cent	*gm.*	*mg. per cent*		*gm. per cent*	*gm. per cent*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*gm.*
Basal	6.04	26.0	337	1.3	3.39	2.65	437	521	958	46.4	538	4.40
1	4.41		248	1.0	2.24	2.16				54.6		
2	4.31		111	1.3	2.43	1.88				52.4	630	4.98
3	4.34	22.4	174	1.1	2.28	2.06	516	509	1025	52.9		
4	4.05		173	1.2	2.24	1.81				55.0	280	3.66
5*	4.57		169	1.2	2.48	2.09				52.7		
6*	4.52		161	1.2	2.45	2.07				53.4	340	4.36
7*	4.69		227	1.2	2.60	2.09				52.6		
9*	4.30	17.0	293	1.3	2.39	1.91	395	286	681	58.0	360	4.14
11†	4.81		406	1.1	2.47	2.34				54.3	266	4.29
14	4.89		396	1.0	2.48	2.41				56.7	266	4.44
18	4.98		338	1.0	2.45	2.53				60.3	320	6.20
22	5.84		359	1.1	3.07	2.77				54.7		
29	5.69		242	1.2	3.14	2.55				56.2		
37	5.89		259	1.2	3.23	2.66				51.9		

* Refused part of diet.

† Diet changed.

Table 3, dog 35-151, shows essentially the same changes noted in the other animals in this group. The change in plasma and red cell volume shrinkage on the 9th day was more marked than that noted in other animals. Changes in fibrinogen concentration were somewhat less marked. On the 1st and 2nd days there were slight in-

creases in urinary nitrogen, followed up to the 11th day by decreases. During this interval, however, the animal was not eating well, and the

TABLE 4

Repeated Injections of Gum Acacia

Dog 32-8.

Date	Plasma protein		Hematocrit plasma	Weight	Acacia given
	Concentration	Fibrinogen			
	gm. per cent	*mg. per cent*	*per cent*	*kg.*	*gm.*
Apr. 13	6.69	217	44.7		33
14	4.43	246	54.2	15.5	
15	4.69	[illegible]	55.0		
16	5.11		55.2		
17	5.03	251	55.9		
18	5.28		52.5		
20	5.68	167	51.2		8
21	4.80		56.5	15.4	
22	5.16	212	52.5		
24	5.56		53.9		16
26	5.12	271	54.8		
28	5.20		54.2	15.5	18
29	3.98	195	59.2		
May 2	4.59	168	53.3		
4	4.91	138	53.8		16
5	4.04	150	56.5	15.3	
6	4.58	142	56.3		
7	4.62	143	54.3		
10	4.98	174	50.6		30
11	3.49	110	58.4		
12	4.00	106	58.2	15.4	
13	4.19	99	56.0		
14	4.38	97	55.6		30
15*	3.46	76	59.5		
16	3.48	77	58.8		18
17	3.44	73	61.1		18
18	3.03	59	61.6		18
19	2.73	30	63.2	14.9*	30
20†	2.48	61	61.9		

* Animal consumed all of diet during course of experiment.

† Dog sacrificed (gas anesthesia).

diet was changed to one containing more protein, which lessens the significance of the urinary findings.

Table 4 shows the record of a normal dog (32-8) which received frequent injections of a 6 per cent gum solution. The first injection contained the same amount of acacia per pound as that given the previous four dogs. The amount of fluid injected, though, was twice

TABLE 5

Repeated Injections of Gum Acacia

Dog 36-94.

Date	Plasma protein					Hematocrit plasma	Weight	Food consumed	Acacia given
	Concentration	Albumin	Globulin	A/G ratio	Fibrinogen				
	gm. per cent	*gm. per cent*	*gm. per cent*		*mg. per cent*	*per cent*	*kg.*	*per cent*	*gm.*
June 3	6.65	4.19	2.46	1.7	181	45.2	24.1	100	52
10	5.37	3.40	1.97	1.7	144	46.5	24.1	95	26
17	4.66	3.55	1.11	3.2	75	50.5	23.5	100	26
24	4.51	3.24	1.27	2.6	75	51.9	24.1	90	13
July 1	4.97	3.70	1.27	2.9	94	54.0	23.3	95	13
8	5.16	3.73	1.33	2.8	91	54.9	23.5	100	26
15	4.95	3.61	1.34	2.7	—	54.8	23.2	100	25
23	4.84	—	—	—	109	57.4	23.3	100	25
29	5.13	3.65	1.48	2.5	113	55.0	22.9	100	35
Aug. 5	4.83	3.28	1.55	2.1	62	58.6	23.1	100	35
12	4.61	3.30	1.31	2.5	98	58.7	23.5	100	35
19	4.58	3.11	1.47	2.1	91	58.8	23.0	100	20
26	4.74	3.23	1.51	2.1	96	58.2	22.8	100	20
Sept. 2	4.88	3.23	1.64	2.0	125	57.5	22.8	100	20
9	4.94	3.22	1.72	1.9	127	57.0	22.5	100	35
16	4.64	3.07	1.57	2.0	97	55.2	22.7	90	25
23	4.64	2.88	1.76	1.6	118	57.2	21.9	90	40
30	4.36	2.56	1.79	1.4	77	58.0	21.9	80	40
Oct. 7	4.14	2.86	1.28	2.2	74	55.9	21.6	80	40
14	3.94	2.78	1.16	2.4	67	56.5	20.9	70	40
21	3.64	2.38	1.26	1.9	63	56.8	20.1	50	40
28	3.43	2.15	1.28	1.7	84	61.1	19.8	50	40
Nov. 4	3.13	1.92	1.21	1.6	76	61.9	19.1	40	0
11	3.48	2.41	1.07	2.3	101	58.9	18.7	30	0

as large as that given the former animals. It is of interest that the lowest point in plasma protein concentration was reached on the 1st day following injection as compared to the 3rd and 4th days in the other dogs. The fibrinogen did not show the marked variation pre-

viously described. It seems probable that the concentration of acacia and the total amount of fluid given are responsible for the variation exhibited in this animal. Finally, after several injections at relatively frequent intervals, it was possible to reduce the plasma protein concentration to below 3 gm. per cent. The fibrinogen in the meantime had been below 100 mg. per cent for a week, preceding the time the animal was sacrificed. Throughout the course of this experiment which lasted a little over a month, the dog ate well and showed no untoward clinical signs until the last few days when there was slight edema of one leg. There was loss of weight of a little over a pound during the period.

In order to produce a longer period of low plasma protein concentration, another normal dog (36-94, *Table 5*) was given weekly injections of 12 per cent gum acacia, the dose from week to week being graduated according to the dog's reaction during that period. This dog had previously had a single acacia injection. It has been possible to maintain the plasma protein concentration below 4.5 gm. per cent for a period of 9 weeks. The animal showed a steady weight loss until injections were stopped. In a period of 5 months about 660 gm. of gum acacia were introduced into the animal. The fibrinogen maintained a constant low level from the start of the procedure. It is of interest to note that for the most part fibrinogen and plasma protein concentration closely paralleled each other, fibrinogen changes being more marked, however, than plasma protein deviations. The albumin-globulin ratio in this animal had a tendency to be higher than normal, as contrasted with an opposite tendency in dogs receiving single injections. This was due to a relatively greater fall in albumin concentration, although there was a decrease in the concentration of both albumin and globulin.

Clinical Histories

Dog 32-8. Female coach-bull in good condition. Had been previously used in anemia colony. Was given a diet of meat scraps. On Apr. 13, 1937, the first injection of 6 per cent gum acacia solution was given. Thereafter over a period of 38 days, ten intravenous doses of varying amounts of gum acacia solution were given. No untoward clinical signs were noted until 1 month after the initial injection, at which time the animal had received a total of 151 gm. of acacia. At this time bleeding from the needle puncture wound was sustained, and pressure

had to be applied to the vein for some time until it was stopped. The plasma protein concentration was 3.48 gm. per cent, the fibrinogen concentration 77 mg. per cent. On May 20, slight pitting edema of the right leg was noted. The plasma protein concentration at this time was 2.48. The following day the dog was killed with gas and postmortem examination was carried out immediately. At no time was there evidence of jaundiced plasma. The animal ate well at all times and never appeared to be sick.

Autopsy.—An obese dog with moderate pitting edema of hind legs. Bleeding from neck puncture wound which had been made just before death. The peritoneal cavity contains 50 to 60 cc. of clear watery fluid. The pericardial cavity is filled with similar fluid. The blood runs freely from vessels and heart and after 10 to 20 minutes forms rather soft, semi-elastic light red clots. Heart shows a few small reddish granulations along the free margins of the mitral leaflets. Lungs are negative. Spleen is rubbery and large. The cut surface is deep red and large diffuse gray foci, apparently Malpighian bodies, are readily seen. Gastro-intestinal tract and pancreas are essentially negative.

Liver weighs 810 gm. It is swollen and tense; the capsule is smooth, glistening, and the liver parenchyma beneath shows large confluent interlacing grayish colored lobulations. The organ tends to be friable in consistency and cuts with ease. The cut margins evert and bulge. The very congested red color of the cut surface appears limited to small central areas that tend to be obscured by coalescing large dull gray peripheral zones. The main bile ducts and vessels are negative.

Suprarenals and genito-urinary tract are negative. Main arteries and veins are negative. There is no lymphatic enlargement. The subcutaneous tissues of the neck show extravasated bloody fluid. Bone marrow and brain are not remarkable.

Microscopic Examination.—Heart negative. *Lungs:* Numerous large mononuclear cells with abundant clear cytoplasm are present in many alveoli.

Liver: All the polygonal cells are greatly swollen and have a foamy appearance due to the presence in the cytoplasm of numerous small vacuoles (Fig. A). Many of the nuclei are pyknotic and irregularly stellate in shape. The majority of the Kupffer cells appear unaltered but some have thick blunt clear cytoplasmic processes. The intracellular material does not stain with Sudan III (fat) nor Best's carmine (glycogen). This appearance is quite uniform, but there is a suggestion of slightly greater involvement in the mid-zonal regions and in the cells about portal areas. The bile ducts are essentially negative.

Spleen: The pulp, in scattered areas, shows many large clear cells filled with vacuoles similar to those seen in the liver cells. *Kidney:* The epithelium of the convoluted tubules is not remarkable. The epithelium of most of the collecting tubules appears to be finely vacuolated. An occasional glomerular tuft in both kidneys is partially or completely composed of large, irregular, vacuolated, clear cells. Scattered small foci of lymphocytes, plasma cells, and neutrophils are present.

Anatomical Diagnosis.—Congestion of, and infiltration of acacia into liver cells. Infiltration of acacia into epithelium of collecting renal tubules. Pitting edema of lower posterior extremities. Fluid in peritoneal and pericardial cavities. Acute (early) endocarditis.

Dog 36-94. A short-haired black and white spotted male mongrel weighing 19.3 kg. Vaccinated against distemper. On Dec. 31, 1936, put on hamburger-

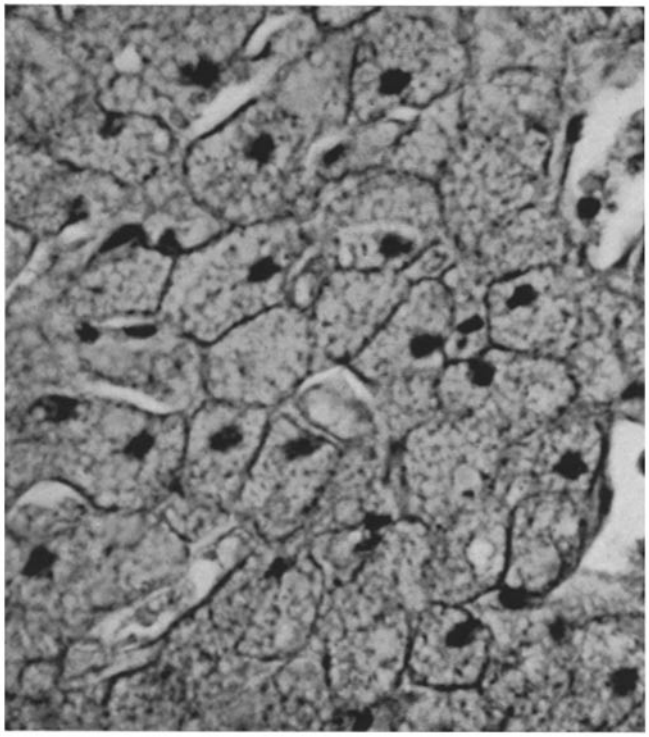

FIG. A. Photomicrograph of section of liver of a dog (32-8, Table 4) which had received repeated injections of gum acacia solution. Swelling and vacuolization of liver cells is marked. Hematoxylin-eosin stain. ×650.

potato basal diet containing 2 gm. protein per kilo body weight. Samples of blood were taken over a period of 9 days. On Jan. 9, 1937, was given 42 gm. of gum acacia made up to a 12 per cent solution with Locke's solution. Dog stood injection well, did not vomit. The animal was studied for a period of 16 days. It was then returned to the animal house and kept on kennel diet until June 3, 1937, when potato-hamburger diet was instituted. On the same day 52 gm. of gum

acacia solution was given. (In the meantime the weight had increased 4.8 kg.) From that time until Oct. 28, 1937, the dog was given an intravenous injection of gum acacia every 7 days, blood being removed for analysis previous to each injection. The dog gradually lost weight, and ate less and less food. At the present the dog has not received acacia injections for 3 weeks, is eating all food, and is gaining weight. Plasma protein and fibrinogen have increased a little but are still far below normal limits.

Dog 35-151. Short-haired male mongrel, vaccinated against distemper. The animal had been on kennel diet for some months previous to start of experiment. On Aug. 23, 1937, it was put in a metabolism cage, given potato-hamburger diet. Three basal determinations were made during a period of 4 days. On Aug. 27 the dog was given a single injection of gum acacia, 1 gm. per pound weight. The dog was studied for a period of 37 days. From the 4th to the 11th days the animal did not eat well, so the diet was changed to one of table scraps. Except for this episode, the animal at all times remained in excellent clinical condition.

Dog 36-100. A long-haired female brown mongrel vaccinated against distemper. Put in a metabolism cage Aug. 23, 1937, and given potato-hamburger diet. The animal was given a single injection of gum acacia solution on Aug. 27. It was studied for 15 days following this procedure. At all times the animal ate well, no untoward clinical signs were noted.

Dog 36-21. A long-haired mongrel female collie vaccinated against distemper. On Oct. 21, 1936, the present study was started. On Oct. 30 a single injection of gum acacia solution was given. The animal was studied for 10 days following this procedure. At all times it remained in excellent clinical condition.

DISCUSSION

The question arises as to what becomes of the protein which has disappeared from the blood. As far as studies of the urine are concerned, although they are not entirely satisfactory we have not been able to demonstrate enough of an excess of nitrogen to account for that which has disappeared from the blood stream. Is the plasma protein then deposited in storage places in the body? If we assume that the osmotic pressure of the blood plasma of a normal animal cannot be altered for long, and that the circulating plasma volume cannot be increased above a certain point, it is apparent that, if a colloid substance be added to the plasma in large amounts, some colloid must leave the blood stream. It is quite probable that the plasma proteins

are more rapidly withdrawn than acacia. The initial plasma protein diminution following gum acacia injection may represent merely the colloid adjustment necessary to keep the osmotic pressure of the plasma constant. The plasma protein would then return to the circulating plasma as quickly as the acacia could be removed. The work of Stanbury, Warweg, and Amberson (12) in which both plasma protein and acacia determinations were made following total plasmapheresis may be consistent with this thesis, for they have shown that the plasma protein concentration in the blood rapidly rises as that of acacia falls.

In spite of the difference of method, our work and theirs may be comparable. The total amounts of acacia remaining in the circulation following total plasmapheresis were not over twice as much as those given by us in single injections. It seems not unlikely that the organism could handle the two quantities with almost equal facility, since the liver appears to be the organ most involved, and its reserve is known to be great. If this is so, plasma protein levels in the two cases, although quite unlike at first, should, at a given time following injection, be similar. In the case of *total plasmapheresis*, plasma protein must be poured into the circulation starting from a concentration of almost zero as the acacia is removed. In *acacia injection*, the protein must be first withdrawn from the circulation in order to maintain the osmotic pressure and then replaced as the acacia is removed. In the total plasmapheresis experiments normal plasma protein levels were reached within 10 days after substitution of acacia for plasma. In one of our animals (36-21) the curve of the plasma protein concentration from the 5th through the 10th days was quite similar to this. In three out of four of our dogs normal levels were not reached in over 2 weeks. The 5th day, however, is of considerable significance. The plasma protein concentrations of all the dogs in both groups of experiments were quite similar ranging between 4 and 5 gm. per cent.

Another possible explanation of the diminution of plasma protein following injection of gum acacia must be considered. Andersch and Gibson (4) have shown that the liver is responsible in great part for the removal of the acacia from the blood. There is considerable evidence (1, 2, 9) that the liver constitutes the chief warehouse for

reserve protein. If the liver is substantially filled with acacia, it is conceivable that this may be at the expense, in part at least, of reserve protein. It has been further pointed out that there exists a constant utilization or *wear and tear* of plasma protein that may amount to as much as several grams of protein a day (8). In a normal animal, if there should be reduction in production of plasma protein, one would expect to find a decrease in the total amount of circulating plasma protein equivalent to the amount utilized. It is probable that acacia might interfere with the liver function and production of plasma protein to this extent.

Reticulo-endothelial structures have been implicated by various investigators as possible sites of plasma protein formation. While at present we have no specific method for positive identification of acacia histologically, in Eck fistula dogs which have received intravenous gum acacia[2] and in dogs receiving liver poisons such as carbon tetrachloride along with gum acacia,[2] we have noted marked depositions of what is apparently the gum in the spleen and lymph nodes. In the normal dog receiving the gum which came to autopsy in the present study, by far the most extensive and severe histological involvement was that of the liver (Fig. A). This finding fits in with the work of Andersch and Gibson (4) who showed that considerable quantities of gum acacia were deposited in, and excreted by, the liver. Evidence of liver damage is indicated by the marked fibrinogen reduction, for it is generally conceded that the liver alone is the source of this substance (11). On the basis of these observations, regardless of other factors that may enter into the picture, we cannot dismiss the idea that the liver function is markedly disturbed following such injections. By the use of such technique, it should be possible to investigate other phases of liver function. Such studies might throw added light on both the function of the liver and on the acacia-protein phenomenon itself.

SUMMARY

Lowered plasma protein concentration following single injections of gum acacia in the dog is due in some part to dilution, and in greater

[2] Unpublished data.

part to actual decrease in total circulating protein. The maximum decrease in the total circulating protein does not take place at the same time as the maximum decrease in concentration. Fluctuations in fibrinogen concentration are marked, and are not necessarily proportional to changes in plasma protein concentration. Plasma protein concentration returns to normal limits within 10 to 21 days after the injection, at which time total circulating protein and plasma volume are lower than normal. Loss of protein cannot be accounted for by increase in urinary nitrogen.

It is possible to maintain dogs at low levels of plasma protein concentration for several weeks by repeated injections of gum acacia solution. Anatomical changes following such a procedure in a normal dog are most conspicuous in the liver (Fig. A). These observations further implicate the liver as a source of plasma protein.

Two mechanisms for the diminution of plasma protein following gum injection are suggested. One of these is based on the possibility that the liver cells being engorged with gum acacia are not able to produce the necessary amount of plasma protein to supply the normal demand. The other possibility is that with the injection of the gum, since there is obviously a greatly increased amount of colloid in the blood, the more readily removable colloid, *i.e.* plasma protein, is taken out of the blood stream, in an attempt to return plasma volume and colloid osmotic pressure to the normal limits. It is probable that both of these mechanisms are involved.

Injection of gum acacia is suggested as a technique for further study of disturbed liver function.

It must be obvious that clinical use of gum acacia for intravenous injection is not without danger.

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