The mechanism of the interrelationship of injected gum acacia and plasma protein in the blood stream involves principles which may lead to a better understanding of plasma protein both as to its production and its function as a blood colloid. In dogs, following single, or a few repeated injections of gum acacia, there are profound changes in plasma protein and fibrinogen concentration and in total circulating plasma protein (6). Since it has not been possible to account for the loss of protein from the blood stream by an increase of nitrogen in the urine, it has been suggested that the missing plasma protein has been replaced (or displaced) by acacia and deposited in "storage depots" in the body. The second possibility suggested is that the production of plasma protein is interfered with so that inadequate amounts are produced to supply the normal demand.

In order to study this problem adequately, it is desirable to maintain animals at levels of protein concentration as low as are consistent with a good clinical state, and to continue this condition for periods of time long enough for more complete studies than have been made heretofore. Previous experiments have been carried out with single injections of the gum, and with multiple injections over periods of a few weeks.

With these objectives in view, weekly injections of gum acacia have been given over periods of 4 to 5 months to dogs which have been studied from the standpoint of plasma protein and plasma acacia metabolism. Such animals have shown changes similar to those receiving the gum over shorter periods of time, but more marked drops in protein levels have been produced and maintained. In addition, it has been noted that upon cessation of acacia injections the plasma protein concentration and total circulating protein tend to remain at relatively constant low levels, rising very slowly over a period of months, depending upon the protein content of the diet. At the same time, the acacia concentration in the blood slowly diminishes.
At autopsy the liver may be 4 to 5 times normal weight and may contain as high as 8 to 10 per cent acacia.\(^1\)

Animals such as these, in a relatively chronic state of hypoproteinemia, but in otherwise good clinical condition have proved to be of value in studying the variations of their two blood colloids, protein and acacia, under various experimental conditions.

**Methods**

Normal dogs vaccinated against distemper were used for all experiments. Gum acacia\(^2\) (Lilly—minus sodium chloride) was diluted in Locke's solution (minus calcium chloride) to a concentration of 12 per cent, and injected intravenously.

During the periods of injection, except when otherwise indicated, weekly determinations of total plasma protein, fibrinogen, albumin, and globulin were made in all cases by methods described in a previous publication (6). Plasma volume determinations were made by a modification of the method of Gibson and Evans (5) using T-1824 dye. Blood for analysis was obtained by jugular puncture and added to measured amounts of 1.4 per cent sodium oxalate solution in hematocrit tubes. Appropriate dilution factors were introduced into all calculations.

Concentration of gum acacia solution in whole blood and plasma was determined spectrophotometrically following the principles of the method described by Peoples and Phatak (13).

**EXPERIMENTAL OBSERVATIONS**

Table I presents a summary of the dogs that have been given repeated injections of gum acacia. The average weekly dose approximated 2 gm. of the gum per kilo body weight. The total period of injection ranged from 15 to 22 weeks. During this period we attempted to give the injections weekly, and the only exceptions to this rule were prompted by the clinical condition of the animals. At all times it was attempted to keep the animals in a good clinical state, i.e., active, with a hearty appetite, even at the expense of following a strict routine injection program. It was not possible to control completely the dietary intake during the injection periods. No animal that has been subjected to this procedure has at all times consumed all of the diet offered. Only one animal, 36-94, however, was a chronic offender in this regard, the result of which is shown in the table by a loss of weight of 5 kg. during the 22 weeks period of injection.

The duration of the period of injection was arbitrary, with an attempt to have the animal at as low a plasma protein concentration as was con-

\(^1\) Unpublished data.

\(^2\) We are indebted to Eli Lilly and Company for an abundant supply of this valuable material.
sistent with a satisfactory clinical state. It would undoubtedly be possible
to give more of the gum over a longer period of time.

Table I shows marked changes in the plasma protein concentration in all
animals studied, which decreased from 42 to 71 per cent below the basal
levels. There is apparently no correlation between the amount of gum
received and the percentage drop in the plasma protein concentration.
The fibrinogen concentration shows in every case a greater fall than does
the plasma protein concentration, an observation which agrees with those
made in dogs receiving a single injection of gum acacia. The weight
variation is very slight except in the case of one dog, 36-94, mentioned
above. It is of interest that the one dog, 37-187, which gained weight,

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Average dose</th>
<th>Injection period</th>
<th>Total number of injections</th>
<th>Total gum acacia received</th>
<th>Gum received per kg.</th>
<th>Weight variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>36-94</td>
<td>30.5</td>
<td>22</td>
<td>22</td>
<td>671</td>
<td>27.8</td>
<td>24.1</td>
</tr>
<tr>
<td>35-151</td>
<td>25.6</td>
<td>20</td>
<td>15</td>
<td>385</td>
<td>32.4</td>
<td>10.9</td>
</tr>
<tr>
<td>37-117</td>
<td>20.2</td>
<td>16</td>
<td>16</td>
<td>327</td>
<td>41.5</td>
<td>7.9</td>
</tr>
<tr>
<td>37-187</td>
<td>26.2</td>
<td>21</td>
<td>17</td>
<td>445</td>
<td>40.4</td>
<td>11.0</td>
</tr>
<tr>
<td>38-123</td>
<td>22.6</td>
<td>15</td>
<td>15</td>
<td>340</td>
<td>38.2</td>
<td>8.9</td>
</tr>
</tbody>
</table>

showed the least decrease in plasma protein concentration, and received
next to the largest amount of acacia per kilo of the group. Various types
of diets of known protein content were fed to these animals, but they were
irregularly consumed.

Table II shows the characteristic picture of an animal during the period
of injection. Figures given as "basal" in the first column represent the
mean value of weekly determinations for a period of 9 weeks previous to the
first injection of gum acacia. The following periods represent consecutive
weekly intervals, injections being given the first day, determinations
being made on the last day thereof. In addition to the data represented
on the table, it can be stated that the plasma per cent, as measured by the
hematocrit, increased, and there was a definite diminution in the red blood
cell per cent. It has been shown that change in plasma per cent in such
animals is not an index of the degree of dilution of the blood. The diet
was irregularly consumed during the entire injection interval. It will be
noted that following the intervals when acacia was not injected, there was
always a tendency for the plasma protein concentration to increase. The total circulating protein for the most part reacted in like manner. The fibrinogen concentration in this animal as well as in all dogs studied shows greater fluctuations than the total plasma protein concentration. Blood volume determinations are also characteristic. After a tendency for the plasma volume to decrease slightly during the first few weeks, there is a gradual increase, sometimes as high as 20 or 25 per cent in the later periods. The total blood volume also shows a slight tendency to increase, but proportionately less than that of the plasma volume. This is due to a decrease in the red blood cell volume, another finding which has been uniformly consistent in all of the dogs.

The sum of plasma protein and gum acacia concentration is of interest. If the figures given were in terms of plasma protein concentration alone,
they would all be within normal limits of plasma protein concentration for the dog. The highest variation above the basal value amounts to a difference of 0.7 gm. per cent or an 11 per cent increase. The lowest variation is 1.0 gm. per cent or 16 per cent below the basal. This is of interest when compared with the variations in the total circulating colloid. In the case

### TABLE III

*Changes in Albumin and Globulin Concentrations Following Single and Multiple Injections of Gum Acacia*

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Basal albumin-globulin ratio</th>
<th>Albumin-globulin ratio 7 days after injection</th>
<th>Albumin-globulin ratio 14-16 weeks after injection</th>
<th>Percentage drop in 7 days</th>
<th>Percentage drop in 14-16 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>37-67</td>
<td>2.0</td>
<td>2.6</td>
<td>25</td>
<td>45</td>
<td>61</td>
</tr>
<tr>
<td>37-56</td>
<td>0.8</td>
<td>1.1</td>
<td>15</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>36-100</td>
<td>1.3</td>
<td>1.4</td>
<td>31</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>37-117</td>
<td>1.7</td>
<td>1.9</td>
<td>10</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>35-151</td>
<td>1.1</td>
<td>0.9</td>
<td>28</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>37-187</td>
<td>1.6</td>
<td>1.8</td>
<td>10</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>36-94</td>
<td>1.7</td>
<td>1.7</td>
<td>19</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>38-123</td>
<td>1.7</td>
<td>3.1</td>
<td>5</td>
<td>47</td>
<td>59</td>
</tr>
</tbody>
</table>

### TABLE IV

*Rise in Plasma Protein Concentration Following Cessation of Acacia Injections*

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Length of experiment</th>
<th>Plasma protein concentration</th>
<th>Plasma acacia concentration</th>
<th>Average protein consumption during experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>At end of acacia injections</td>
<td>At end of experiment</td>
<td>At end of acacia injections</td>
</tr>
<tr>
<td>37-117</td>
<td>105</td>
<td>2.4</td>
<td>4.3</td>
<td>3.0</td>
</tr>
<tr>
<td>36-94</td>
<td>144</td>
<td>3.4</td>
<td>3.6</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* During the first 49 days of the experiment protein intake was not measured, but diet given was adequate in protein content for normal dogs.

of the latter, the highest variation above the control level amounts to 35 per cent, the lowest 19 per cent. Similar comparative variations have been observed in all dogs studied during the injection periods.

Table III shows comparative fluctuations in albumin and globulin in a group of 8 dogs. Three of these animals, 37-67, 37-56, 36-100, were used for single injections, while the other 5 received multiple injections. The interval of 14 to 16 weeks is chosen because some of the latter group were not injected longer than 15 weeks (Table I), whereas one dog, 37-187, missed an injection on the 15th week. Figures represent averages for this
period, and although 3 out of 5 dogs showed a more marked drop in albumin, there appears to be no significant difference between the two fractions, except in the case of the one dog (37-117). It would appear, then, that there is a differential effect only in the first weeks of injection (Tables I and III).

Table IV illustrates the point that if dogs are given enough gum acacia over a sufficient time interval, not only will acacia remain in the blood for long periods of time, but plasma protein concentrations will return toward normal levels very slowly. The plasma protein concentration of one dog (37-117), in 105 days, during which time no acacia was given, rose from 2.4 gm. per cent to 4.3 gm. per cent. This animal was receiving a relatively high protein diet, on the average of 4 gm. per kilo body weight per day. The other animal (36-94) which received somewhat less protein, 2.8 gm. per kilo per day, in 144 days rose from 3.4 gm. per cent to 3.6 gm. per cent. Both of these figures border on the edema level for dogs, but clinically during the entire period, both animals were in good condition as far as appetite and relative activity were concerned. The plasma acacia diminished in both animals, more markedly in the dog (37-117) whose plasma protein showed the greatest rise. At the end of the experiments, however, there was still considerable gum in the blood. Other dogs in this series were used during the post-injection period for other types of experiments, to be reported later.

DISCUSSION

The foregoing experimental data indicate a method whereby a state of marked hypoproteinemia can be induced and maintained over long periods of time. In the later intervals of this state following cessation of injections, animals remain in a satisfactory clinical condition and can be used for metabolic studies without further interference.

Comparison of the course of the two dogs (Table IV) following the termination of acacia injections is noteworthy. Both of these animals were on protein diets, which for a normal animal would be considered definitely more than adequate for the maintenance of nitrogen equilibrium. The animal which was on the lower protein diet (2.8 gm. per kilo per day) showed but slight tendency to increase the plasma protein concentration. The dog which was on the higher diet showed a more rapid rise. If this phenomenon is constant, it should be possible to adjust the protein intake, so that by manipulation of the diet alone, not only the rate of rise could be controlled but plasma protein concentration could be maintained at a constant low level. This has been accomplished, within certain limits, as will be shown in a later publication.
Albumin and globulin changes are of interest, particularly in the first week following the initial injection of gum acacia. Evidence heretofore presented (9, 10) indicates that the liver plays a part in the production of the plasma proteins, and that fibrinogen (3, 8, 11) is produced only by the liver. Dogs which have been subjected to the procedure described here have shown the most profound anatomical changes in the liver, and these are considerably more marked than those noted in any other organ. On the other hand, dogs with Eck fistulas have shown consistently low albumin-globulin ratios, due to lowering of albumin concentration. In addition, dogs whose livers have been injured by carbon tetrachloride (2, 4) also show diminution of albumin concentration. These latter observations are in distinct contrast to the reduction of globulin reported here. It is not thought that acacia injures the liver in the sense of causing acute necrosis of liver cells, but it has been suggested that there may be a physical "clogging" of liver cells by the gum, to the extent that the efficiency of the liver is greatly impaired. This would be substantiated by the findings in the liver itself. The polygonal cells are swollen with deposits of acacia. Its weight may be increased 5 to 6 times and it may contain 8 to 10 per cent gum acacia by weight.*

Studies of urinary nitrogen during injection periods have in these animals been unsatisfactory, since the dietary intake cannot be controlled. In post-injection periods, such studies may be of interest.

The results show that a considerable amount of acacia has remained in the blood of dogs for as long as 144 days. Other workers (7, 12, 13) have indicated that gum acacia in appreciable quantities disappears from the blood of various animals in relatively short periods of time up to 5 to 7 days. All of such observations, the present included, obviously are dependent upon the quantity of the gum injected, the number of injections, and the elapsed time from the first to the last injection. The idea as expressed by one author (1) that "gum-saline injections cannot function by themselves to maintain blood volume and blood pressure for much more than 48 hours" would also fall under this criticism. In dogs such as these whose plasma protein concentration remains below the "edema level" for long periods of time without exhibiting edema, it would seem reasonable to assume that acacia is exerting some osmotic effect. This is further substantiated by the observations that the plasma volume is increased for certain intervals following injections, during which intervals the plasma protein concentration is quite low. Under these conditions, the extra

* Unpublished data.
demand for maintaining osmotic pressure above the "edema level" must be borne by the acacia.

Many measurements of the osmotic pressure of gum acacia have been recorded, with a wide range of results. It would seem probable that the aggregation of acacia molecules may vary, not only in different states of solution, but under other conditions as well. It is quite possible that a given concentration of acacia in the circulation may, under varying conditions of the blood, exert different osmotic pressures. Furthermore, it is not known whether or not the addition of acacia has any effect upon the state of aggregation of plasma proteins themselves. On the other hand, it is presumptuous to assume without measurements of pressure that gram for gram acacia exerts as much osmotic pressure as plasma protein in spite of total colloid concentrations (acacia plus protein) which fall within limits of normal plasma protein concentration.

SUMMARY

By repeated weekly intravenous injections of gum acacia solution in dogs over periods of 4 to 5 months, it has been possible to maintain plasma protein concentration and total circulating protein at very low levels.

If sufficient numbers of such injections are given and then discontinued, plasma protein concentration will remain below the normal limits for several more months. Acacia remains in the blood during this time.

Reduction of fibrinogen concentration in such animals is out of proportion to and more marked than the changes in plasma protein concentration. This would indicate interference with liver function.

Plasma volume when determined at 7 day intervals during injection periods at first diminishes, then rises 20 to 25 per cent above basal levels. The total blood volume does not show such marked changes because of a decrease in red cell volume.

Globulins are reduced to a greater extent than albumin after a single injection of gum acacia, although both albumin and the globulins diminish. This cannot be accounted for by a decrease in fibrinogen alone. After 14 to 16 weekly injections, both albumin and globulins are more profoundly reduced.

During injection periods in such animals, it has not been possible to control quantitatively the dietary intake, a complication which has made it difficult to ascertain the effect of various protein diets upon the protein acacia balance. The changes described, however, have taken place regardless of various types of animal protein diets.

Following periods of injection, in spite of very low plasma protein con-
centration and high acacia concentration in the blood, most of the dogs eat well and therefore they can be used during this period for controlled dietary experiments which may be of value in investigating the mechanism of the production and function of the plasma proteins.

BIBLIOGRAPHY