THE INFLUENCE OF PREGNANCY AND LACTATION UPON
THE REGENERATION OF SERUM PROTEIN*

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It has long been known that there are changes in the blood volume
during pregnancy, and that these are associated with a hydremia due
almost entirely to an increase in the water content of the plasma
(1-6). Various fluctuations in the concentration of plasma proteins
(7-12, 6) occur during all stages of pregnancy and they appear in
large measure to be associated with this hydremia, although Peters
and Van Slyke (14) have suggested that loss of blood during labor
may play a rôle. Davis and Bodansky (13) have reported observa-
tions made on rabbits, a species which delivers large litters, from
which it appears that the delivery of large litters intensifies the
process by which the concentration of plasma proteins is lowered.
They reported that the lowest concentration occurred 12 hours after
parturition; the normal concentration was not attained during the
25 day period of lactation.

In the present investigation we have been interested primarily
in the transitory changes in the serum protein concentration which
take place during terminal pregnancy and early lactation. It seems
to us that animals, which deliver relatively large litters of young, are
better suited for such a study. Inasmuch as the "strain" of preg-
nancy and lactation in these species may be assumed to be greater
than that in cases of human pregnancy, one might expect to ob-
tain values which because of their distinct differences would be of
greater significance. Such has been the case in the studies conducted

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by Davis and Bodansky (13) on rabbits during pregnancy and lactation. Furthermore, we have been impressed with the relatively low serum protein concentrations recorded at parturition, which in the case of the rabbit may actually approximate the low values recorded for significant hypoproteinemias. In view of the remarkable ability of the normal organism to regenerate serum protein (15), the possibility suggests itself that in conditions of pregnancy and early lactation there may be a limited store of reserve serum protein or an impairment of the ability of the organism to form this blood protein or an involvement of both of these factors. Indeed, the synthesis of body proteins in the fetus during pregnancy and the milk proteins during lactation may be considered to be actually an internal plasmapheresis, leading to a depletion of the serum protein by the preferential utilization of the material from which this complex is made. In the present study by means of rigid control of the dietary intake and by use of the technique of quantitative plasmapheresis we have attempted to evaluate the ability of the organism to regenerate serum protein during pregnancy and lactation.

Methods and Material

The methods followed were practically the same as those used in our previous investigations of serum protein regeneration (16-18, 15). The concentrations of the serum protein were determined for the most part by the nephelometric procedure of Rona and Kleinmann (19), modified as described in our previous communication (15). Periodically, these values were checked by determining the concentration of the blood protein by the direct gravimetric procedure (20, 21), especially on those days when the serum protein concentration showed dramatic changes. The gravimetric method was always used for determining the concentration of the serum protein in the pooled plasma aliquots, after the fibrin had been removed according to the procedure described elsewhere (17). For the estimation of the blood volume, the dye method as improved by Hooper, Smith, Belt and Whipple (22) was employed. Because obstruction to the venous return very quickly increases the protein concentration in the plasma (23), the samples used for the determination of the serum protein concentration and blood volume were taken in most cases from the femoral artery. Since we were very much interested in the blood volume changes and did not want to affect them appreciably by our methods of analyses, the blood samples used for the various determinations were as small as possible. Thus, in the case of a serum protein estimation 2 to 3 cc. of blood were withdrawn, while in conducting a blood volume determination the animal lost a total of only 10 cc. of blood. Plasmapheresis, plasma depletion
effected by the repeated bleedings of the animal, followed immediately by the
reinjection of the corpuscular elements of the blood suspended in Locke's solution,
was conducted according to the standardized technique described in our previous
publication (16).

In the present investigation three diets were employed, a natural stock diet,
a "synthetic" artificial protein ration, and a "synthetic" artificial protein-free diet.
The natural stock ration\(^1\) consisted of a mixture of natural foods and was occa-
sionally supplemented with meat scraps obtained from the New Haven Hospital.
The "synthetic" artificial protein diet was the casein III ration described by Cow-
gill (24), which contains casein\(^2\) as the sole source of protein, with exception that a
modified Osborne and Mendel salt mixture (25) replaced that described in the
original formula. In our laboratory dogs have subsisted on this diet for as long
as 18 months and throughout this period appeared to have been in an excellent
nutritive condition. The protein-free diet is described in detail elsewhere (17).
The same vitamin supplements\(^3\) and the same quantities as described in that
paper (17) were administered to the dogs, when they subsisted on either of the
two "synthetic" rations.

RESULTS

The initial investigations are concerned with the variations in the
serum protein concentration, blood volume, hematocrit, and plasma
volume in the normal dog subsisting on the adequate "synthetic"
casein diet, and in another animal fed the same ration during terminal
pregnancy and early lactation.

Dog 1-0, a fox-terrier with an optimal weight of 8.95 kilos, calculated for the
animal when adjusted to an optimal nutritive condition (24), was used as the
normal control dog in this study. Dog 5-6, a mongrel, was estimated to have the
same optimal weight and was employed to investigate the effects of pregnancy and
lactation upon the composition of the blood. Prior to the present experiments
both animals were fed the natural stock diet \textit{ad libitum}. Subsequently the casein
ration was administered, also \textit{ad libitum}, supplemented with the calculated
amounts of the vitamin adjuvants. Dog 1-0 subsisted on the "synthetic" diet
for a period of 8 weeks during which time it evidenced a slow but progressive gain
in weight until the final value was 10.2 kilos. Dog 5-6 was fed the casein ration

\(^1\) Tioga dog food, formerly Baloration, obtained from the Tioga Mills, Inc.,
Waverly, New York.

\(^2\) Obtained from the Lister Bros., New York.

\(^3\) We are indebted to the Health Products Corporation, Newark, and to The
Eli Lilly and Company, Indianapolis, for furnishing us with generous supplies of
their cod liver oil concentrate tablets and liver 343 powder, respectively.
5 days before term, subsisted on it for the first 2 weeks of lactation and finally was returned to the natural stock diet. This animal weighed 9.2 kilos before the litter was cast, 7.0 kilos post partum and finally 8.1 kilos when the normal blood picture was again attained. The effects of such a dietary régime upon the composition of the blood are presented in Chart 1.

Examination of the chart clearly indicates that the casein ration, when fed to the normal non-gravid dog, is adequate for the main-
tenance of a normal serum protein concentration and a normal blood and plasma volume. In the case of the pregnant animal there was a marked plasma hydration but no increase in the total blood volume, due to the fact that the hematocrit was extremely low. In human beings there is also a reduction of the hemoglobin concentration, cell count and cell volume somewhat too large to be explained simply by the hydremia (14). However, in the case of dog 5-6 the total cell volume showed actually a maximal decrease of approximately 40 per cent. As in the studies by Davis and Bodansky (13) of the composition of blood in rabbits during pregnancy and lactation, marked changes in the serum protein concentration were also recorded for this dog. Thus, the concentration of the blood protein 5 days before term was three-fourths and at parturition actually two-thirds of the normal value. However, when expressed as total circulating serum protein, there was no appreciable decrease except at parturition and early lactation, and then these values were only about 10 per cent less than normal. With human beings the changes in the serum protein concentration are not nearly as great and with respect to the total amount of serum protein there is generally an increase during pregnancy and a return to normal during the 1st week post partum (3, 6). The findings recorded with dog 5-6 indicate that the dog as well as the rabbit undergoes a considerably greater physiological strain during pregnancy and lactation than the human being. In this connection it is pertinent to point out that despite the fact that dog 5-6 subsisted on a diet which contains approximately 34 per cent protein it evidenced a significant hypoproteinemia during the periods of pregnancy and lactation which were under observation. This becomes even more significant when one considers that the normal dog subsisting on a ration which contains only from 15 to 16 per cent of the very same protein exhibits a remarkable ability to regenerate serum protein (18, 15). In the dog the duration of pregnancy is usually 63 days; the puppies are generally weaned after 42 days. In view of the markedly short period of gestation in this species and in view of the relatively large litters that are cast and nursed, the dog appears to be ideally suited for the experiments devised to evaluate the effects of normal pregnancy and lactation upon the ability of the organism to form serum protein.
<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Breed and sex</th>
<th>Optimal weight*</th>
<th>SP removed†</th>
<th>Initial total circulating SP</th>
<th>Reserve SP stores‡</th>
<th>Endogenous SP regeneration per week‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>kg.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Depletion</td>
<td>Equilibrium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Hound ♂</td>
<td>16.7</td>
<td>49.2</td>
<td>13.4</td>
<td>44.4</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>Collie ♂</td>
<td>17.3</td>
<td>56.4</td>
<td>9.6</td>
<td>52.1</td>
<td>52</td>
</tr>
<tr>
<td>4-3</td>
<td>Mongrel ♂</td>
<td>12.6</td>
<td>38.4</td>
<td>7.6</td>
<td>36.9</td>
<td>39</td>
</tr>
<tr>
<td>4-4</td>
<td>Fox-terrier ♂</td>
<td>10.0</td>
<td>38.0</td>
<td>9.2</td>
<td>37.2</td>
<td>36</td>
</tr>
<tr>
<td>4-6</td>
<td>Hound ♂</td>
<td>14.2</td>
<td>47.7</td>
<td>9.8</td>
<td>43.3</td>
<td>45</td>
</tr>
<tr>
<td>4-7</td>
<td>Terrier ♂</td>
<td>12.2</td>
<td>31.6</td>
<td>5.3</td>
<td>38.0</td>
<td>31</td>
</tr>
<tr>
<td>4-8</td>
<td>Terrier ♂</td>
<td>9.5</td>
<td>23.3</td>
<td>6.3</td>
<td>26.6</td>
<td>26</td>
</tr>
<tr>
<td>5-1</td>
<td>Airedale ♂</td>
<td>14.8</td>
<td>38.4</td>
<td>5.7</td>
<td>40.8</td>
<td>37</td>
</tr>
<tr>
<td>5-2</td>
<td>Hound ♂</td>
<td>15.9</td>
<td>46.4</td>
<td>9.6</td>
<td>46.2</td>
<td>35</td>
</tr>
</tbody>
</table>

Average values per kilo of body weight ........ 3.0 (±0.3) (av.d.) 0.62 (±0.1) (av.d.) 3.0 (±0.2) (av.d.) 38 (±5.4) (av.d.) 21 (±3.8) (av.d.)

* These values are calculated for the animals when adjusted to an optimal nutritive condition as estimated by the nutritive index formula (24).
† The symbol SP is used as an abbreviation for serum protein. During these periods the dogs subsisted on the protein-free ration and were subjected to the standardized plasmapheresis procedure described in the text.
‡ Expressed in terms of the per cent of the total amount of circulating serum protein normally present. 

The amount of reserve serum protein is calculated by subtracting from the total amount of the blood protein removed during the initial depletion week both that amount required simply to reduce the serum protein concentration from the normal to the basal level and that amount which the animal regenerates when fed the protein-free diet during the equilibrium week. By endogenous SP regeneration we refer to the ability of the dog under our experimental conditions to utilize the products of tissue protein catabolism for the formation of serum protein.
Previous studies (17, 18, 15) have given some index of the amount of reserve serum protein, stored as such or potentially so, in the normal dog and the ability of such an organism to utilize the products of tissue protein catabolism for the formation of this blood protein. By the administration of the protein-free diet at a high level of caloric intake to the dog subjected to plasmapheresis during which one-fourth of the blood volume of the animal is withdrawn daily, it is possible to reduce the serum protein concentration to the basal level (3.5 to 4.2 per cent) and to deplete the organism of its reserve stores of this protein within 1 week. The subsequent week was demonstrated to be an equilibrium period, indicative of the amount of serum protein that can be regenerated with no protein in the diet and with tissue protein catabolism alone furnishing the materials from which the protein complex is made. Plasmapheresis during this latter period is performed quantitatively (16); whenever the serum protein concentration rises to 4.2 per cent or above, a calculated volume of blood is removed to reduce the level to 3.5 per cent. The results, obtained to date with nine dogs, are summarized in Table I, and indicate that the normal dog possesses a considerable reserve store of serum protein of approximately 38 per cent of the total amount normally present in the circulation. When fed the protein-free diet and when subjected to quantitative plasmapheresis the dog is able in 1 week to regenerate approximately 21 per cent of the total amount of the blood protein normally present in the plasma.

Dogs 4-3 and 4-4 were employed in the experiments devised to evaluate the effects of pregnancy and lactation upon the ability of the dog to regenerate serum protein. In order to present more clearly and in greater detail the normal control findings with these dogs, reference is made to Chart 2. The intensity and frequency of the plasmapheresis necessary to deplete these animals of their reserve serum protein stores, reduce their blood protein concentrations to the basal level and to maintain them there are indicated in the chart. The normal serum protein concentrations, blood and plasma volumes and hematocrit readings are presented, as well as the corresponding values when the dogs were subjected to plasmapheresis.

Both dogs consumed the "synthetic" artificial protein-free diet at the high level of caloric intake of 90 calories per kilo of optimal body weight. Dogs maintained in metabolism cages under similar environmental conditions but fed an adequate diet usually require
in the neighborhood of 70 calories per kilo for maintenance. Dog 4-3 during this experimental period evidenced a slow but progressive loss

**Chart 2.** Serum protein depletion in the normal dog and the ability of the normal organism to utilize the products of tissue protein catabolism for the formation of serum protein. The dotted lines represent plasmapheresis.

*During this preliminary period the dogs subsisted on a natural stock diet followed by 1 week on an artificial “synthetic” casein ration.*

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Blood volume</th>
<th>Hematocrit</th>
<th>Plasma volume</th>
<th>Period</th>
<th>Total bleeding</th>
<th>Serum protein removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>%</td>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
<td>gm.</td>
</tr>
<tr>
<td>Dog 4-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1126</td>
<td>53.8</td>
<td>520</td>
<td>B-D</td>
<td>1577</td>
<td>773</td>
</tr>
<tr>
<td>C</td>
<td>942</td>
<td>48.0</td>
<td>490</td>
<td>D-F</td>
<td>343</td>
<td>186</td>
</tr>
<tr>
<td>E</td>
<td>965</td>
<td>44.4</td>
<td>537</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog 4-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>957</td>
<td>44.0</td>
<td>536</td>
<td>H-J</td>
<td>1322</td>
<td>748</td>
</tr>
<tr>
<td>I</td>
<td>890</td>
<td>38.4</td>
<td>548</td>
<td>J-L</td>
<td>374</td>
<td>226</td>
</tr>
<tr>
<td>K</td>
<td>846</td>
<td>41.8</td>
<td>492</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of body weight from 13.7 to 12.7 kilos; dog 4-4, from 10.6 to 10.2 kilos. The optimal body weights of these animals were calculated to be 12.6 and 10.0 kilos, respectively.
The influence of pregnancy and lactation upon the ability of these dogs to regenerate serum protein is presented in Charts 3 and 4.

In these experiments the animals were fed initially the natural stock diet ad libitum followed by the "synthetic" casein diet, also ad libitum. This was then replaced by the protein-free diet administered at the 90 calorie per kilo level.

* During this preliminary period the dog subsisted on a natural stock diet followed by 1 week on an adequate "synthetic" casein ration.

### Chart 3

The influence of pregnancy and lactation upon the ability of dog 4-3 to regenerate serum protein. The dotted lines represent plasmapheresis.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Blood volume</th>
<th>Hematocrit</th>
<th>Plasma volume</th>
<th>Period</th>
<th>Total bleeding</th>
<th>Serum protein removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>per cent</td>
<td>cc.</td>
<td></td>
<td>Blood</td>
<td>Plasma</td>
</tr>
</tbody>
</table>

Dog 4-3

<table>
<thead>
<tr>
<th></th>
<th>A 892</th>
<th>40.5</th>
<th>531</th>
<th>B-C</th>
<th>425</th>
<th>248</th>
<th>11.4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 920</td>
<td>40.7</td>
<td>546</td>
<td>C-D</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>D 763</td>
<td>40.0</td>
<td>458</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E 922</td>
<td>45.0</td>
<td>507</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

During the periods when the animals were offered this ration, they refused at times to eat the daily aliquots completely. This necessitated feeding the dogs forcibly, a procedure which was tolerated very well, the ingested food always being retained. The casein diet was administered during the period of lactation at a level of 120 calories per kilo of optimal body weight and was always consumed with great avidity. Dog 4-3 weighed 13.6 kilos prior to term, 11.4 subsequent to
parturition, 11.2 when the protein-free ration was replaced by the casein diet and 11.5 when the last observation recorded on Chart 3 was made. The corresponding weights listed for dog 4-4 were 15.8, 11.6, 10.6 and 10.2 kilos.

From the data given in the charts we note that these two dogs, as well as dog 5-6 described above, exhibited marked changes in the com-

![Chart 4](image-url)

**Chart 4.** The influence of pregnancy and lactation upon the ability of dog 4-4 to regenerate serum protein. The dotted lines represent plasmapheresis.

* During this preliminary period the dog subsisted on a natural stock diet followed by 1 week on an adequate “synthetic” casein ration.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Blood volume</th>
<th>Hematocrit</th>
<th>Plasma volume</th>
<th>Period</th>
<th>Total bleeding</th>
<th>Serum protein removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cc.</td>
<td>per cent</td>
<td>cc.</td>
<td></td>
<td>Blood</td>
<td>Plasma</td>
</tr>
<tr>
<td>A</td>
<td>904</td>
<td>30.8</td>
<td>626</td>
<td>B-C</td>
<td>683</td>
<td>455</td>
</tr>
<tr>
<td>C</td>
<td>1016</td>
<td>30.2</td>
<td>709</td>
<td>C-D</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>884</td>
<td>31.0</td>
<td>610</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>873</td>
<td>30.5</td>
<td>607</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>867</td>
<td>35.4</td>
<td>560</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

position of the blood as a result of pregnancy. Thus, dog 4-3 just before plasmapheresis was inaugurated, 2 days prior to parturition, had a serum protein concentration actually 26 per cent less than the normal value but despite this managed to have a normal plasma volume. In the case of dog 4-4 there was a comparable decrease in
the serum protein concentration but actually a significant plasma hydration, so that the total amount of serum protein present in circulation was decreased by only about one-half as much (11 per cent) as in the case of dog 4-3. In both animals there were marked reductions in the hematocrit values and total cell volumes when comparisons are made with the normal findings recorded in Chart 2.

Of primary significance are the results which were obtained when the dogs subsisted on the protein-free diet and were subjected to the same standardized plasmapheresis procedures employed in the study described with the same animals in the normal state. In the case of dog 4-3 plasmapheresis was begun 2 days prior to parturition. Only two hemorrhages, resulting in the withdrawal of only 11.4 gm. of serum protein, were required to deplete this animal of its reserve serum protein and to lower the concentration of the blood protein to the basal level. These results should be compared with the normal findings (see Chart 2) which show that fully six bleedings, resulting in the removal of 38.4 gm. of serum protein, were essential for this purpose. During the subsequent week, period of early lactation, the serum protein concentration never did attain the basal bleeding level of 4.2 per cent but showed a gradual fall to the very low level of 3.2 per cent, when the animal's diet was changed to the adequate casein ration. In the normal state during a comparable period of time this animal had to be bled that amount of blood containing 7.6 gm. of serum protein in order to keep the concentration of the blood protein below the basal bleeding level. In like fashion with dog 4-4 only three hemorrhages were required to reduce the serum protein concentration to the basal level and, once attained, it was found not necessary to bleed the animal to maintain it there. The actual amounts of serum protein removed from this animal in the normal, in the pregnant and in the lactating states are given in Charts 2 and 4. These results are confirmatory of those obtained with dog 4-3. When both animals were fed the adequate casein ration and subjected to no bleedings the serum protein concentrations rose promptly during the period of lactation until normal values were approximated.

The data obtained from these experiments seem to gain in significance, since not only was each dog its own control but also because the
normal picture was obtained on one dog at the same time the effects
of pregnancy and lactation were being evaluated on the other animal
and vice versa. A period of approximately 7 months separated one
study from the other. It is also pertinent to point out that experi-
mental data are available to indicate that the results recorded for
the initial depletion week and subsequent equilibrium periods are
reproducible in the normal dog (15, 17).

One may feel that during the pregnancy study it was much easier
to reduce the serum protein concentration to the basal level simply
because the initial concentration values were already markedly less
than normal. However, perusal of the protocols (26) of a study
conducted with dog 3 to determine the reproducibility of our pro-
cedures indicates that this could not be the only factor involved. The
serum protein concentration of this animal was initially 6.21 per cent.
One bleeding resulted in it being reduced to 4.82 per cent. How-
ever, in order to lower it to the basal level, it was essential to bleed
the animal on the 5 successive days so that a total of 47.2 gm. of
serum protein were withdrawn during this depletion period. Com-
parison of this value with that recorded initially with dog 3 (see
Table I) indicates excellent agreement.

With both dogs the greatest change in the serum protein concen-
tration occurred just at parturition. In the case of dog 4-3 it is ob-
vious from the change in the plasma volume on that day that the
decrease in the concentration of the blood protein cannot be at-
tributed to hydremia. The blood volume estimation conducted on
dog 4-4, however, did indicate a significant plasma hydration. We
can find no reason to suspect that this particular determination is
incorrect due to faulty technique; nevertheless, we feel that a signi-
ficant decrease in the hematocrit value should also result if the hy-
dremia were unquestionably so great. Since such did not occur
and in view of the relationship between hematocrit and plasma vol-
ume in the subsequent determinations, it is concluded that the
changes in the serum protein concentration in this dog were probably
due primarily to our experimental procedures and not simply to a
dilution of the plasma.

4 Melnick (26), page 224.
The experiments described and discussed thus far indicate among other things that the pregnant dog possesses a limited store of reserve serum protein. In order to evaluate more completely the influence of pregnancy upon the regeneration of this blood protein additional tests were conducted on another animal. For this purpose plasmapheresis was initiated 2 weeks prior to term. It was also expected that this study would enable one to ascertain more fully whether or not the precipitous drop in the serum protein concentration observed previously is dependent specifically upon some peculiar effect of the parturition process. The results of the experiments are present in Chart 5.

The animal subsisted initially on the natural stock diet followed by 1 week on the synthetic casein ration. This was followed by use of the protein-free diet fed at the level of 90 calories per kilo of optimal body weight for a period of 17 days, when the litter was cast. Occasionally the diet had to be administered forcibly but here as in the case of dogs 4-3 and 4-4 the animal did not object to being fed in such a manner. Subsequent to parturition the casein ration was offered at a 120 calorie per kilo level and was always consumed with great eagerness. The weight of this dog was initially 10.8 kilos, rose to 12.3 at the time when the protein-free diet was initiated, continued to rise in spite of the inadequate ration to the peak value of 13.1 kilos but finally dropped to 12.4 just prior to parturition. Immediately after the litter was cast the dog weighed 10.7 kilos, which weight was maintained during the period of lactation. The optimal weight (24) of this dog was calculated to be 10.9 kilos.

The data presented in Chart 5 were obtained over a longer period of time than in the case of the other experiments. The finding of a plasma hydration and of marked decreases in the hematocrit value, in the total cell volume, in the serum protein concentration, and a negligible change in the total amount of serum protein were also recorded for this dog during pregnancy.

When subjected to plasmapheresis this animal showed precisely the same response as the pregnant dogs 4-3 and 4-4 when fed the same diet. Only three bleedings, resulting in the removal of 19.8 gm. of serum protein, were essential to deplete this dog of its reserve store of this protein and to reduce its concentration to the basal level. Subsequently for fully 12 days the serum protein concentration failed to reach the basal bleeding level of 4.2 per cent. It was not con-
considered essential to obtain the normal control picture with this dog. On the basis of the average data presented in Table I, calculations indicate that this animal may be expected normally to lose about 33

![](chart.png)

**Chart 5.** The influence of pregnancy and lactation upon the ability of dog 5-7 to regenerate serum protein. The dotted lines represent plasmapheresis.

* During this preliminary period the dog subsisted on a natural stock diet followed by 1 week on an adequate "synthetic" casein ration.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood volume, cc</td>
<td>1046</td>
<td>1014</td>
<td>940</td>
<td>918</td>
<td>935</td>
<td>939</td>
<td>848</td>
<td>926</td>
<td>915</td>
<td>925</td>
<td>951</td>
</tr>
<tr>
<td>Hematocrit, per cent</td>
<td>48.6</td>
<td>43.0</td>
<td>38.6</td>
<td>35.5</td>
<td>35.8</td>
<td>34.2</td>
<td>34.2</td>
<td>38.8</td>
<td>40.5</td>
<td>49.5</td>
<td>49.0</td>
</tr>
<tr>
<td>Plasma volume, cc</td>
<td>537</td>
<td>578</td>
<td>577</td>
<td>592</td>
<td>600</td>
<td>618</td>
<td>558</td>
<td>567</td>
<td>545</td>
<td>467</td>
<td>485</td>
</tr>
<tr>
<td>Period</td>
<td>D-E</td>
<td>E-H</td>
<td>703</td>
<td>0</td>
<td>452</td>
<td>0</td>
<td>19.8</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total bleeding, cc</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

... gm. of serum protein during the initial depletion week and approximately 7 gm. during the subsequent equilibrium period of 7 days. Of particular interest is the fact that this dog failed to show any greater increase in the hydration of the plasma at parturition. Obvi-
ously then the ease, with which the serum protein concentration can be reduced during pregnancy and lactation to the basal level and maintained there, must be due solely to the parasitic effects on the maternal organism of bearing the young and nursing them during early life. In this connection it is interesting to note that dog 5-7 still evidenced during lactation a significant decrease of about 16 per cent in the serum protein concentration, even though it had subsisted for almost 3 weeks on the adequate casein diet. The fact that the total amount of serum protein during this period was normal does not seem to be an adequate explanation; it is difficult to think of an equilibrium between tissue protein and serum protein which depends upon total quantities rather than upon concentrations.

**DISCUSSION**

From the evidence presented in our previous publications (17, 18, 15) a hypothesis was presented explaining the mechanism by which serum protein formation takes place. This was illustrated by the following diagram.

![Diagram](image)

This hypothesis emphasizes the rôle of tissue protein catabolism as an essential factor in the *normal* production of serum protein.

The results of our present investigations harmonize very well with this concept. It has been reported that the amino acids of the blood appear to be the precursors of the milk proteins (27) and that the amino acids or polypeptides of the blood are utilized in the nutrition of the fetus, since the placenta is apparently impermeable to the plasma proteins (28). In our experiments the normal non-gravid dog was shown repeatedly to be able to utilize the products of tissue protein catabolism for the formation of serum protein. When fed the protein-free diet and when subjected to quantitative plasmapheresis after the basal serum protein level is attained, the dog was
found still to be able to regenerate each week approximately 21 per cent of the total amount of protein normally present in the plasma. However, in the case of the pregnant and lactating animal subsisting on the same ration no appreciable regeneration of serum protein was observed to take place. Evidently under these experimental conditions, where there are no amino acids or polypeptides being absorbed from the gastrointestinal tract and where such products of tissue protein catabolism are utilized on the one hand for the nutrition of the fetus and on the other for the production of the milk proteins, the *Bausteine* of the serum protein complex are no longer available. It does not appear likely to us that the serum protein complex is the precursor of the milk proteins or the source of the amino acids essential for the nutrition of the fetus. It would seem to be very poor physiological economy for the organism to synthesize first the serum protein complex from the amino acids liberated as a result of tissue protein catabolism and then destroy it again to supply the amino acids for the functions of pregnancy and lactation involving additional protein synthesis.

In these studies of pregnancy in the dog we have observed no increase in the total blood volume, but in most cases a significant plasma hydration. This increase in the volume of the plasma occurred in spite of the markedly lowered serum protein concentrations. We believe one of the contributory factors for such a change in the plasma volume is the great reduction in the hematocrit and total cell volume. We have shown (29) with the *normal* dog that when there is a concomitant and significant decrease in the hematocrit resulting in a passive reduction of the blood volume, a lowered serum protein concentration is negligible in effecting a further decrease in the blood volume. In such conditions the plasma volumes were frequently greater than normal. Precisely the same variations in the composition of the blood appear to occur in the dog during normal pregnancy, so that the above explanation may very well be applied to explain in part such changes observed in the present study. Obviously some other factor must also be involved since shortly after parturition there is, especially in human beings, a marked decrease in the blood volume as a result of the removal of water from the plasma with a concomitant increase in the hematocrit due
simply to the hemoconcentration and not to any increase in the total cell volume.

SUMMARY

1. In view of the markedly short period of gestation in the dog and in view of the relatively large litters that are cast and nursed, this species when compared with the human being undergoes a much greater physiological strain during pregnancy and lactation. This is evidenced by marked decreases in the hematocrit values, in total cell volumes and in the serum protein concentrations, by an appreciable plasma hydration, and in some cases by significant reductions in the total circulating serum protein.

2. When pregnant dogs are fed a protein-free diet at a high level of caloric intake and are subjected to our standardized plasmapheresis technique, it is possible to deplete the animal of its reserve serum protein stores and reduce the serum protein concentration to the basal level (3.5 to 4.2 per cent) within the extremely short period of from 2 to 3 days. This indicates that the dog during pregnancy possesses a very limited amount of reserve serum protein.

3. Once the basal serum protein level is attained, the pregnant or lactating dog exhibits a marked impairment in its ability to regenerate serum protein. The synthesis of body proteins in the fetus during pregnancy and the milk proteins during lactation is considered to be actually an internal plasmapheresis, leading to a depletion of the serum protein by the preferential utilization of the materials from which this complex is made. These parasitic effects on the maternal organism are believed to be of primary importance, over and above any hydremia, in causing the lowered serum protein concentrations characteristic of pregnancy.

BIBLIOGRAPHY