Significant physiological changes take place during the deficiency of the anti-stiffness factor. The development of a stiffness at the wrist joint could be caused by changes in osmotic pressure, due to a redistribution of the proteins present in the body fluids. For this reason the protein distribution of normal, cured, and deficient guinea pigs was investigated.

The experimental evidence obtained showed an increase in the total nitrogen in the plasma during deficiency. This was due to an increase in the non-protein-nitrogen and the globulin nitrogen. The plasma albumin decreases, resulting in a decreased albumin-globulin ratio. Normal distribution of nitrogen in the blood can be maintained during the deficiency if supplements of the anti-stiffness factor are administered. The implications of these changes are discussed.
APPROVED:

Assistant Professor of Biochemistry
In Charge of Major

Head of Department of Chemistry

Chairman of School Graduate Committee

Chairman of State College Graduate Council
ACKNOWLEDGMENT

The author wishes to express his deep appreciation to Dr. W. J. van Wagendonk for his constant encouragement and interest.
"Up to the present time we have learned only to conjecture as to the cause of a great number of diseases and as to the means of their cure. Before hazarding a theory we propose to multiply our observations, to investigate the phenomena of digestion and to analyze the blood in both health and disease. We will draw upon medical records and the light and experience of learned physicians who are our contemporaries, and it will be only when we are thus completely armed that we will dare to attack a revered and antique colossus of prejudice and error."

Lavoisier 1790
TABLE OF CONTENTS

Introduction .......................................................................................................................... 1
Experimental ......................................................................................................................... 7
   (a) Method ......................................................................................................................... 7
   Diagram of Procedure ....................................................................................................... 10
   (b) Results ......................................................................................................................... 11
      Table I ................................................................................................................................ 12
      Table II ............................................................................................................................ 13
      Table III .......................................................................................................................... 14
      Table IV .......................................................................................................................... 17
Discussion ............................................................................................................................ 18
Summary ............................................................................................................................... 20
Bibliography ......................................................................................................................... 21
CHANGES IN THE DISTRIBUTION OF PLASMA PROTEINS IN GUINEA PIGS RAISED ON A DIET DEFICIENT IN THE ANTI-STIFFNESS FACTOR

INTRODUCTION

Chemical blood analysis as an intermediate measure of various metabolic and excretory processes has long been used. Disruption of regulatory mechanisms through malnutrition or pathological changes, resulting in distributory variations in the blood constituents, may be traced by blood analysis. The study of such variations can give an indication of abnormal conditions and provides a basis for further investigations. These studies have, in the main, been carried out on blood plasma, since the removal of the corpuscles voids the plasma of hemoglobin but leaves practically all of the physiologically important constituents found in whole blood, in the plasma.

Normally, the total solids of human blood amount to 19-23%, of which all but about 1.5% is protein. Of the whole blood proteins, hemoglobin comprises nearly three quarters, the plasma proteins one quarter. (12)

The normal osmotic relations which exist between the blood and tissues is maintained by the plasma proteins. Starling (15) was the first to recognize the significance of the protein content of the blood as a controlling factor in the distribution of the fluids between the plasma and tissues. The hydrostatic pressure in the capillaries
tends to force water into the tissues; the osmotic pressure of the plasma proteins tends to draw fluids from the tissue spaces into the blood. Normally these opposing forces are delicately balanced. Increased hydrostatic pressure in the capillaries may be accompanied by a loss of fluid to the tissues, and up to a certain point by an increased concentration of protein in the plasma because of a proportionately smaller loss of this constituent. The greater concentration of protein tends to balance the effect of the heightened hydrostatic pressure, so that within certain limits, a new balance is soon attained (2).

Although the factor of hydrostatic capillary pressure is significant, a disturbance in the equilibrium is usually due to changes in the plasma proteins. The osmotic pressure of plasma is about 6.5 atmospheres. This tremendous force is due to dissolved electrolytes and organic colloids, but does not produce the calculated effect, inasmuch as the tissues and tissue fluids likewise contain about the same concentration of these constituents. Such osmotic effect as is produced by the blood is however of great importance, although it amounts to only a very small fraction of the total. This effect is due to the difference in the concentration of protein in the plasma and tissue fluids, a difference which is maintained because of the relative impermeability of the capillary endothelium to protein (2).
Of the plasma proteins, albumin is osmotically more active than the globulin because of its relatively smaller molecular size. Govaerts (4,5) found that a 1g% solution of albumin in serum gave an osmotic pressure of 5.54 mm. of mercury, whereas 1g% of globulin exerted a pressure of only 1.43 mm. of mercury. According to these figures, the osmotic activity of plasma albumin is nearly four times that of globulin. Using these figures for calculation, Govaerts was able to predict the osmometric reading from the chemical analysis of the plasma for albumin and globulin.

Wulzen and Bahrs (1,19,20) during investigations on the nutritional requirements of planarian worms, found that raw cream contained a dietary factor which had not been previously described. van Wagendonk and Wulzen (17) described the isolation of a factor from raw cream which was able to cure the stiffness induced by a diet deficient in the factor (which will be referred to as the anti-stiffness factor).

The physiological changes in guinea pigs raised on a diet deficient in this anti-stiffness factor indicated a widespread disruption of both regulatory mechanisms and normal functions of certain organs. The first external sign of the deficiency was the development of a stiffness in the wrists. This increased in severity during the syndrome until it was impossible to bend the wrist. Autopsy revealed that the muscles were extremely atrophied and in
most cases were streaked with closely packed fine white lines of calcium deposits running parallel to the muscle fibers. There were often lumps of calcium phosphate deposited under the skin, in the joint regions, between the ribs, and in many body organs including the heart and aorta.

van Wagendonk (18) in studies on the distribution of acid-soluble phosphorus in the liver and kidneys during the anti-stiffness factor deficiency found the following: The concentration of the easily hydrolyzable phosphorus in both organs was decreased very markedly during the deficiency. The inorganic phosphorus showed sharp increases in the liver and kidneys. The anti-stiffness factor prevented an abnormal distribution of the acid-soluble phosphorus or restored it to normal after it had developed.

These physiological changes indicate a derangements in the calcium or phosphorus distribution which normally exists. In the blood, calcium is found almost exclusively in the plasma, where it occurs to the extent of about 8.4-10.4 mg. per 100 cc. blood (guinea pig) (14).

The calcium in the blood is represented by at least two types of components. These are commonly classified as diffusible and non-diffusible calcium. These are distinguished by the ability of the diffusible fraction to pass through membranes impermeable to colloids, while the non-diffusible calcium is held back by such membranes. The
non-diffusible calcium does not exist as a firmly bound molecular protein complex, but on the contrary, is present in an easily dissociable form apparently capable of coming into fairly ready equilibrium with the diffusible calcium. At constant pH, the amount of non-diffusible calcium is determined by the protein content and the height of the diffusible calcium concentration in the blood stream (9,11).

The maintenance of normal neuromuscular irritability and probably the deposition of bone salts are due to the concentration of calcium ion and inorganic phosphate in the blood plasma. Abnormal levels may be manifested in rickets, parathyroid dysfunction, renal disease, and other conditions which modify the utilization and excretion of calcium and phosphorus. It is in blood and bone that calcium and phosphorus manifest their interdependence most strongly, particularly through the calcium ion and phosphate ion concentration (3). Furthermore, calcium ion and calcium proteinate have a fluid relationship which can be approximately represented by a simple mass law equation (10,14).

Peters and Eiserson (13) express the opinion that any abnormality of the calcium concentration can only be properly interpreted if the amounts of phosphate and protein have been simultaneously determined. A low or high serum calcium can be considered as evidence of an abnormality in calcium metabolism only if the phosphate and protein con-
tent are normal. However, Greenwald (7) feels that the supposed reciprocal relation between the amount of serum calcium and inorganic phosphate is probably non-existent and has attempted to find a relationship for the serum calcium dependent only upon the content of serum protein.

Nitrogen that is found in the blood, other than the protein nitrogen, is broadly classified as non-protein-nitrogen. The nitrogen in this group is subject to variation in distribution with changes in the level of non-protein-nitrogen (8). With a decrease in the value of the total non-protein-nitrogen there is a general tendency for a simultaneous decrease in the absolute amounts of the urea, creatine, uric acid, and amino acid nitrogen, of which the urea reduction is the most uniform. There are differences in the degree to which these constituents decrease with the non-protein-nitrogen, the urea showing relatively the greater diminution. The rest of the nitrogen seems to be less affected by a change in the non-protein-nitrogen level.

In order to obtain a more complete picture of the anti-stiffness factor syndrome and to substantiate the calcium and phosphorus derangement it was deemed advisable to determine the albumin, globulin, and non-protein-nitrogen content in the plasma of guinea pigs. These determinations were carried out on normal, deficient, and "cured" guinea pigs.
EXPERIMENTAL

(a) Method

Guinea pigs were segregated as to sex and housed in large cages. Animals on the deficiency diet were bedded on autoclaved straw. The deficiency diet consisted of the following:

Skimmed milk powder---------16.00 g.
Copper sulphate-------------00.25 mg.
Ferric chloride-------------00.25 mg.
Water----------------------84.00 g.

This diet was fed ad libitum both in the morning and in the evening. The average food intake was found to be 280 g. and was approximately evenly divided over the morning and evening feeding. To the morning diet was added a solution of the water soluble vitamins in such a concentration that the average daily vitamin intake was as follows:

Thiamin hydrochloride--------00.2 mg.
Riboflavin------------------00.5 mg.
Pyridoxin hydrochloride------00.1 mg.
Nicotinic acid--------------01.0 mg.
Calcium pantothenate--------00.1 mg.
Inositol---------------------10.0 mg.
Para-amino benzoic acid-----02.0 mg.
Choline----------------------50.0 mg.
Biotin (S.M.A. conc. S-200)---00.01 mg.

A solution of the fat soluble vitamins was added to the evening feeding so that the average daily intake was as follows:
beta-carotene------------------150.0 I.U.
Viosterol---------------------- 40.0 I.U.
alpha-tocopherol------------- 0.1 mg.
2-methyl-1-4-naphthoquinone-- 0.1 mg.

A stock solution of the fat soluble vitamins was prepared by dissolving them in cottonseed oil. Crystalline l-ascorbic acid was given once a week. This was dissolved in water shortly before use and orally administered. The dose was 50 mg., which is well above that recommended by Zilva (21). Water and iodized salt were provided ad libitum. In the experiments where the anti-stiffness factor was added to the diet, the amount is as indicated in the tabulated results. The animals gained steadily in weight during the course of the deficiency syndrome and showed no signs of any other deficiency disease, except that of increasingly stiff wrist joints.

Normal animals were maintained as controls and received a 'stock' diet composed of rolled barley, greens, water, and iodized salt ad libitum.

Animals were anesthetized with a solution of 50 mg. of sodium pentobarbital by interperitoneal injection. Blood samples were obtained by cardiac puncture and immediately transferred to centrifuge tubes containing Heparin (Hynson Westcott and Dunning Inc.). One milligram of Heparin per 7.5 ml. of blood prevented coagulation of the samples. Immediately upon completion of the blood sampling, the tubes were centrifuged and the supernatant plasma was used
for subsequent analysis.

The analytical method followed is given in the schematic diagram. The nitrogen was determined by the micro-kjeldahl method. The different protein fractions were calculated as follows (16):

\[
\text{Albumin N} = (\text{Albumin N} + \text{NPN}^*) - (\text{NPN})
\]

\[
\text{Globulin N} = (\text{Total N}) - (\text{Albumin N} + \text{NPN})
\]

\[
\text{Total protein N} = (\text{Total N}) - (\text{NPN})
\]

The results were analyzed statistically according to Fischer (6).

* NPN denotes Non-Protein-Nitrogen
PROCEDURE FOR BLOOD NITROGEN ANALYSIS

**Blood Sample**
(Containing Heparin)

- Centrifuge
- 1 ml Plasma

**0.75 ml plasma diluted to 7.5 ml with isotonic NaCl solution (0.9%).** Represents 0.1 ml plasma per ml.

**Precipitation of globulin—0.25 ml plasma diluted to 8 ml with 22.5% Na₂SO₄ in 25 ml flask.** Crystal of thymol added as preservative. Incubate at 37°C for 5 hours and filter through Whatman #42 filter paper. Filtrate contains 0.031 ml plasma per ml.

**2 ml sample for total plasma nitrogen determination by microkjeldahl.** Represents 0.20 ml plasma.

**Precipitation of total protein—Remainder (5.5 ml) treated with 5.5 ml of 10% trichloroacetic acid.** Allow to stand for 10 minutes and filter through Whatman #42 filter paper. Represents 0.55 ml plasma or 0.05 ml plasma per ml.

**5 ml sample for non-protein-nitrogen determination by microkjeldahl.** Represents 0.25 ml plasma.
EXPERIMENTAL

(b) Results

The distribution of nitrogen in the blood of normal guinea pigs is given in Table I. The figures represent the mean values obtained over a life span of 10-72 weeks. With the exception of the last experiment, all animals received a stock diet consisting of rolled barley, greens, water, and iodized salt ad libitum. The animals in the last experiment received a diet composed of raw milk, straw, water, and iodized salt ad libitum. During the time limit of the experiment no significant differences were observed except in the somewhat higher figures for non-protein-nitrogen and albumin-globulin ratios of the animals receiving the raw milk diet.

Table II is a representation of the nitrogen distribution in the blood of guinea pigs raised on a deficient diet. Animals were started on this diet at 13 weeks of age. The time on the diet varied from 1 to 57 weeks. Comparison with the data of Table I shows a significant increase in total plasma nitrogen due to the increase in non-protein-nitrogen and globulin fractions. The albumin fraction is decreased. The lowered albumin fraction and the raised globulin fraction result in a low albumin-globulin ratio.

It has been noted (17) that the symptoms of the deficiency can be alleviated by administration of the anti-
## Table I

### Distribution of Nitrogen in the Blood of Normal Guinea Pigs

<table>
<thead>
<tr>
<th>Age in Weeks</th>
<th>Diet</th>
<th>Ave. Bdywt. grams</th>
<th>No. of Detns.</th>
<th>Total Plasma Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Total Protein Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Non Protein Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Albumin Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Globulin Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Albumin Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Stock</td>
<td>284</td>
<td>5</td>
<td>719 ± 17</td>
<td>691 ± 16</td>
<td>28 ± 3</td>
<td>357 ± 36</td>
<td>334 ± 34</td>
<td>1.07</td>
</tr>
<tr>
<td>12</td>
<td>Stock</td>
<td>291</td>
<td>5</td>
<td>684 ± 13</td>
<td>663 ± 15</td>
<td>21 ± 3</td>
<td>308 ± 42</td>
<td>356 ± 52</td>
<td>0.87</td>
</tr>
<tr>
<td>16</td>
<td>Stock</td>
<td>380</td>
<td>15</td>
<td>749 ± 14</td>
<td>721 ± 16</td>
<td>28 ± 2</td>
<td>342 ± 20</td>
<td>380 ± 24</td>
<td>0.95</td>
</tr>
<tr>
<td>72</td>
<td>Stock</td>
<td>933</td>
<td>9</td>
<td>707 ± 52</td>
<td>639 ± 53</td>
<td>68 ± 4</td>
<td>319 ± 31</td>
<td>320 ± 55</td>
<td>1.00</td>
</tr>
<tr>
<td>72</td>
<td>Stock*</td>
<td>857</td>
<td>5</td>
<td>842 ± 69</td>
<td>759 ± 67</td>
<td>83 ± 5</td>
<td>410 ± 91</td>
<td>348 ± 37</td>
<td>1.18</td>
</tr>
</tbody>
</table>

*Raw milk
### Table 2

**Distribution of Nitrogen in the Blood of Deficient Guinea Pigs**

<table>
<thead>
<tr>
<th>Age in Weeks</th>
<th>Time on Diet Weeks</th>
<th>Ave. Bdywt. Grams</th>
<th>No. of Defts.</th>
<th>Total Plasma Nitrogen mg per 100ml mean and s.e.</th>
<th>Total Protein Nitrogen mg per 100ml mean and s.e.</th>
<th>Non Protein Nitrogen mg per 100ml mean and s.e.</th>
<th>Albumin Nitrogen mg per 100ml mean and s.e.</th>
<th>Globulin Nitrogen mg per 100ml mean and s.e.</th>
<th>Albumin Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>1</td>
<td>280</td>
<td>15</td>
<td>749 ± 23</td>
<td>684 ± 27</td>
<td>56 ± 4</td>
<td>228 ± 27</td>
<td>466 ± 31</td>
<td>0.49</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>315</td>
<td>15</td>
<td>844 ± 16</td>
<td>781 ± 19</td>
<td>58 ± 3</td>
<td>345 ± 16</td>
<td>441 ± 26</td>
<td>0.65</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>394</td>
<td>11</td>
<td>795 ± 15</td>
<td>750 ± 17</td>
<td>44 ± 4</td>
<td>204 ± 22</td>
<td>546 ± 32</td>
<td>0.37</td>
</tr>
<tr>
<td>20</td>
<td>7</td>
<td>482</td>
<td>8</td>
<td>911 ± 26</td>
<td>827 ± 26</td>
<td>84 ± 13</td>
<td>215 ± 31</td>
<td>612 ± 38</td>
<td>0.35</td>
</tr>
<tr>
<td>41</td>
<td>28</td>
<td>711</td>
<td>11</td>
<td>923 ± 22</td>
<td>855 ± 23</td>
<td>67 ± 3</td>
<td>224 ± 42</td>
<td>631 ± 48</td>
<td>0.36</td>
</tr>
<tr>
<td>70</td>
<td>57</td>
<td>747</td>
<td>12</td>
<td>890 ± 26</td>
<td>816 ± 29</td>
<td>82 ± 3</td>
<td>298 ± 37</td>
<td>533 ± 45</td>
<td>0.56</td>
</tr>
</tbody>
</table>
### Table 3

**Distribution of Nitrogen in the Blood of 'Cured' Guinea Pigs**

<table>
<thead>
<tr>
<th>Age in Weeks</th>
<th>Time on Diet Weeks</th>
<th>Ave. Bdywt. Grams</th>
<th>No. of Dets.</th>
<th>Total Plasma Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Total Protein Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Non Protein Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Albumin Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Globulin Nitrogen mg. per 100 ml. mean and s.e.</th>
<th>Albumin Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>7</td>
<td>461</td>
<td>7</td>
<td>910 ± 11</td>
<td>823 ± 47</td>
<td>86 ± 9</td>
<td>224 ± 49</td>
<td>599 ± 45</td>
<td>0.37</td>
</tr>
<tr>
<td>29</td>
<td>19</td>
<td>576</td>
<td>5</td>
<td>870 ± 18</td>
<td>779 ± 20</td>
<td>91 ± 8</td>
<td>213 ± 69</td>
<td>567 ± 74</td>
<td>0.38</td>
</tr>
<tr>
<td>29</td>
<td>19</td>
<td>576</td>
<td>5</td>
<td>901 ± 33</td>
<td>821 ± 34</td>
<td>80 ± 3</td>
<td>337 ± 95</td>
<td>480 ± 85</td>
<td>0.70</td>
</tr>
<tr>
<td>29</td>
<td>19</td>
<td>576</td>
<td>10</td>
<td>920 ± 22</td>
<td>841 ± 22</td>
<td>79 ± 1</td>
<td>296 ± 40</td>
<td>544 ± 31</td>
<td>0.54</td>
</tr>
</tbody>
</table>
stiffness factor over a period of approximately one week. It was of interest to determine whether the upset in nitrogen distribution could be brought back to normal through a similar procedure. The results of such an experiment are given in Table III. All animals in this group were reared on deficient diets. In Experiment I the deficient diet was supplemented with 1000 units (1 unit = 0.01¥) of the anti-stiffness factor per day for the last week of the deficiency. Animals in Experiment II received no supplement of the anti-stiffness factor. Animals in Experiment III received 1 unit of the anti-stiffness factor per day for the last 5 days. The animals in Experiment IV received 1000 units of the anti-stiffness factor per day for the last 5 days of deficiency. From the results obtained it is apparent that an abnormal distribution of the blood nitrogen is not rapidly restored under the influence of the anti-stiffness factor. This is in contrast to the action of this factor upon the phosphorus metabolism. It was reported by van Wagendonk (18) that the acid soluble phosphorus of the liver and kidney reverted to normal after administration of the anti-stiffness factor.

This is clearly demonstrated in the experiment reported in Table IV. Two experiments are reported here in which the diet was supplemented with the anti-stiffness factor over a longer period of time. In the first experiment the animals received no factor the first week, one supplement
of 1000 units the second week, 3 supplements of 1000 units the third week, and 4 supplements of 1000 units the fourth week. The animals in the second experiment received 1000 units of the factor every other day while on the deficient diet. The results in Experiment I illustrate the rapid onset of the redistribution of nitrogen and show the slow reversion of blood protein to normal. In Experiment II the nitrogen distribution remained normal.
Table 4

Distribution of Nitrogen in the Blood of Guinea Pigs Raised on a Deficient Diet Supplemented with the Anti-Stiffness Factor.

<table>
<thead>
<tr>
<th>Age in Weeks</th>
<th>Time on Diet Weeks</th>
<th>Ave. Body wt. Grams</th>
<th>No. of Detns.</th>
<th>Total Plasma Nitrogen mg per 100ml mean and s.e.</th>
<th>Total Protein Nitrogen mg per 100ml mean and s.e.</th>
<th>Non Protein Nitrogen mg per 100ml mean and s.e.</th>
<th>Albumin Nitrogen mg per 100ml mean and s.e.</th>
<th>Globulin Nitrogen mg per 100ml mean and s.e.</th>
<th>Albumin Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>4</td>
<td>318</td>
<td>12</td>
<td>816 ± 12</td>
<td>754 ± 13</td>
<td>61 ± 3</td>
<td>241 ± 45</td>
<td>499 ± 42</td>
<td>0.48</td>
</tr>
<tr>
<td>23</td>
<td>10</td>
<td>509</td>
<td>14</td>
<td>887 ± 11</td>
<td>816 ± 11</td>
<td>71 ± 3</td>
<td>402 ± 21</td>
<td>413 ± 19</td>
<td>0.97</td>
</tr>
</tbody>
</table>
Distinct abnormalities in the distribution of nitrogen in the blood of guinea pigs on a diet deficient in the anti-stiffness factor have been observed. One of the most significant changes in the blood is the decrease of the albumin fraction and the increase in the globulin fraction. The decrease in the albumin fraction is an indication of at least two derangements in equilibria in the blood. The diffusible and non-diffusible calcium in the blood are present as an equilibrium mixture of calcium albuminate and calcium and protein ions respectively. Since the total albumin is decreased, there must be a corresponding shift towards calcium and protein ions. There exists another equilibrium between colloidal calcium phosphate and its ions. This must also change since it has ions in common with the calcium albuminate. The net result of these two shifts is the disruption of the Donnan equilibrium, which normally exists through the capillary endothelium.

The decrease in albumin would indicate an increase in calcium ions in the blood. There are then presented two avenues of equilibrium readjustment. Either the concentration of calcium ions could be decreased by shifting to colloidal calcium phosphate, or they could be transferred through the capillary wall and thereby readjust the Donnan equilibria. The latter case would seem more logical,
since deposits of calcium phosphate are found to a great extent in the tissues. Since the fluid content of the tissues is very much less than the blood, the solubility product of calcium phosphate would be exceeded with the accompanying depositions of calcium phosphate in the tissues. The increase in the globulin fraction is insufficient to offset the osmotic changes due to the decrease in the albumin. It is evident that the equilibrium changes discussed are accompanied by an osmotic disruption.
SUMMARY

Experimental evidence has been presented showing an increase in the total nitrogen in the plasma during deficiency. This is due to an increase in the non-protein-nitrogen and the globulin nitrogen. The plasma albumin decreases, resulting in a decreased albumin-globulin ratio. Normal distribution of nitrogen in the blood can be maintained during the deficiency if supplements of the anti-stiffness factor are administered. The implications of these changes are discussed.
BIBLIOGRAPHY

5. Govaerts, P., Ibid., 95, 724 (1926).
15. Starling, E.H., J. Physiol. 12, 312 (1895-6).
18. van Wagendonk, W.J., In press.