Plasma proteins in hookworm disease

by

GILBERTO G. VILLELA and J. de CASTRO TEIXEIRA

The study of plasma has been for the last years of the past century, the object of numerous investigations. This, notwithstanding researches tending to determination of the proportions of each of the component fractions of proteins, are much more recent.

The albumin globulin ratio has been given more attention in pathology, in consequence of Csatary’s, Nya-Vigliezo’s, Limbeck and Pick’s, Hammarsten’s, von Jaksh and Freund’s studies. In pneumonia, nephritis, and diabetes, these authors have always found an increase of the rate of globulin. Lewinsky, quoted by Rowe, pointed out in eclampsia a decrease and in pregnancy an increase of the number of globulin. In syphilis, most authors report to have always observed percentages higher than normal averages. In infections, there is generally an increase of globulins, which, according to Gottwalt, is in relationship with the anti-toxic power of the serum.

In pernicious anemia, there seems to be only a decrease of the globulin number (Erben) whereas in parenchymatous nephritis, these are found increased against a decreasing number of albumin.

Normally, the proportion between albumin and globulin (albumin-globulin ratio), varies according to authors, or to the technique applied to. Hammarsten states the A/G relation to be, in normal plasma, 1.3 to 1.5. Lewinsky found 1 to 1.3. Epstein makes it 1.7, Rusznyak from 1.6 to 2.6 and Rowe 2.9. We have found in four normal cases, for the relation A/G an average of 2.5.

Hereunder we give a statement of our results, obtained in four normal instances, which will enable one to make an easy comparison with data observed in patients attacked with hookworm disease.

**TABLE N. 1**

(In grs. per 100 cc. of plasma)

<table>
<thead>
<tr>
<th></th>
<th>Albumin</th>
<th>Globulin</th>
<th>Total</th>
<th>Protein</th>
<th>Non protein nitrogen</th>
<th>Fibrinogen</th>
<th>A/G Ratio</th>
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<td></td>
<td><strong>Average</strong></td>
<td><strong>2.05</strong></td>
<td><strong>7.1</strong></td>
<td></td>
<td><strong>0.032</strong></td>
<td><strong>0.42</strong></td>
<td><strong>2.5</strong></td>
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</table>
Under pathologic conditions, these values may vary from 0.6 to 3.5. Rowe gives the following numbers, for cases studied by him:

<table>
<thead>
<tr>
<th></th>
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<th>Globulin</th>
<th>Total protein</th>
<th>A/G Ratio</th>
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<td>Pneumonia</td>
<td>3.7</td>
<td>2.5</td>
<td>7.2</td>
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</table>

In anemia determined by hookworm infection, we have no knowledge of any single work having been published till now, except perhaps a paper of Vadala (Clin. Med. Ita. VI, 1907) quoted by Rowe, of which however we were unable to procure an original leaflet. Vadala realized the dosage of albumin and globulin only in two cases of helminthic anemia, and found, as a result, 7.8% for the former and 2.4 for the latter. The data exposed by this author are, as may be seen, exaggeratedly high, except if realized on very benign cases. Even if compared with normal values, Vadala's figures are too high.

In nephrosis, a characteristic fact is the fall of the albumin fraction and the increase of the globulin percentage in plasma, which means inversion of the term A/G. Cunha Motta and Juvenal Meyer obtained experimentally in rabbits, with injection of cantharadin, changes of the nephrotic type with lowering of the albumin percentage in plasma. In some more severe instances, these authors observed the inversion of the albumin globulin ratio.

In a recent work on concentration of plasma proteins in nephritis, Linder, Lundsgaard and Van Slyke came, respecting nephrosis, to the following conclusions:

"In the glomerulo-tubular or nephrotic type, active or recently active, the total plasma proteins were less than 5 grs. per 100 cc. This decrease from the normal 5.5 to 7.5 occurred whether edema was present or absent. The decrease affected chiefly the albumin, the globulin being usually diminished but little, and sometimes slightly increased. Consequently the ratio albumin to globulin was reduced to less than 1, and occasionally to 0.6.

Previously, Epstein had already observed that in nephrosis, total proteins are in decrease, which decrease however merely affects albumin. For normal cases, this author came to the following figures: albumins 4.6; globulins, 2.7; and total proteins 7.3. For nephrosis, these numbers were: albumin, 0.4; globulin 3.4; total proteins, 3.8. In nephritis, there is a slight decrease of total proteins which is owing to the fact of both figures, albumin and globulin lowering. Epstein admits, thus, a plain differentiation to exist between chronic interstitial nephritis and parenchymatous or nephrotic type, in respect with the chemical constitution of blood.

The dosages we realized of the plasma in 20 patients, with anemia caused by hookworm infection, revealed lowering of total proteins, as already stated by ourselves in a former paper 1, which lowering is inherent only to albumin, since the globulin number keeps steady

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1 Villela (G. G.) and Teixeira (J. C.) - Blood study in hookworm anemia. Suppl. Mem. Inst. Osw. Cruz n. 6 March 1929.
or suffers but a slight rise. This might lead to think of some trouble in kidney in helminthic anemia of the nephrotic type, that is, an anemia characterized by the same humoral frame, typical in fatty nephrosis. We also observed the increase of fibrinogen, which fact equally is accordant with the occurrence in nephrosis (Kilian).

Ashford and Igavaniish discovered fatty degeneration in kidney in hookworm disease, and one of us was able to detect the same histopathologic frame of fatty nephrosis in the mentioned disease.

The values we set up with plasma of patients suffering from hookworm disease, varied with regard to total proteins, from 5.25 to 8.13; for albumins, from 2.72 to 4.87 and for globulins from 1.04 to 3.83 (see Table II).

There is consequently a fall of the albumin figure, and in some instances, a slight increase of globulin, which result in lowering the proportion $A/G$.

It must be referred, nevertheless, that in hookworm disease, the inversion of the relation $A/G$ is not marked to such an extent as is the case in clinical and experimental nephrosis.

Whipple, Mason and Peightal state, for a normal individual, the rate of 0.30 to 0.40 gr. of fibrinogen in 100 cc. of plasma. In cases of intoxication (venoms, poisons, infections, toxins) these authors have found that a reaction may take place on the part of the liver, resulting into an increase of fibrinogen.

In experimental anemia in dogs, by means of acetylphenylhydrazin, Bodansky, Morse, Kiech and Bramkamp verified the increase of fibrinogen, as also the diminution of the relation $A/G$.

A. A. Villela (These, Rio de Janeiro, 1926) found, in 4 cases of pure hookworm disease, an increase of the fibrinogen (an average of 0.56 gr.) by using the refractometric method of Winternitz, which, however, does not lead to quite satisfactory results.

In the cases studied by ourselves, fibrinogen appeared generally increased, as may be easily seen, by comparison of the figures given in tables I and II.

The non protein nitrogen remained, in our observations, steadily within the normal averages, i.e., more or less 25 to 35 mgr. for each 100 cc. of plasma (tables I and II).

The dosage of hemoglobin and reckoning of red corpuscles were performed in every case as a test for the grade of anemia affecting patients. In some instances (Observations II. III. IV. V. VI, and VIII) besides having effected the hemoglobin dosage by Gowers-Sahli’s method, we had an opportunity to make use also of Barcroft’s, that is to say, the gazometric determination of oxygen with the micro-Barcroft’s apparatus, and the calculation of hemoglobin based on Haden’s values.

This author considers as more accurate the rate of 15.6 gr. of hemoglobin in normal individuals, with 5 million red corpuscles per mm$^3$, when determining the oxygen capacity of blood.

There has been quite a lot of methods advised for determination of the plasma proteins, not only those based on precipitation of proteins through acids or alcaline sulphates (sodium and ammonium) as also those in which refractometric technique is made use of (Reiss, Rowe, Robertson). Those are the most recent, in which the nitrogen percentage of the molecule of protein is made available, in such way that, the latter being known, it is sufficient to multiply the figure thus obtained by the coefficient 6.25, the product showing a figure corresponding to protein (Koenig and Kisch, Van Slyke and Howe).

In our studies, we availed ourselves of Howe’s technique, modified in some

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2 Teixeira—Do rim na ankylostomose. 1929 (Kidney into okworm disease).
way by Prof. Carneiro Felippe. Howe's method is a variant of Cullen and Van Styke's, and contrarily to this latter, in which 5 cc. of serum or plasma is necessary, only minime quantities are needed (0.5 to 1 cc.). Howe advises to use sodium sulphate for precipitating globulin, instead of the ammonium sulphate adopted by Cullen and Van Styke. According to Howe's method, albumins are determined from the filtrate into which globulin has precipitated; the fibrinogen is coagulated by means of calcium chloride; globulins are determined by subtraction between total albumins and the albumin fraction, and the non protein nitrogen is dosed in the filtrate of the precipitated total proteins.

The improvements brought by Prof. Carneiro Felippe consist in: 1° dosing by Pregl's technique, nitrogen in filtrates and other separated material obtained by Howe's process; 2° using for precipitation of protein tungstic acid instead of trichloroacetic acid, when dosing non protein nitrogen; 3° using a rod with a small loop at one end, to afford an easier coagulation and adherence of fibrinogen.

Here below, we have stated the various stages of our experimentation for determination of plasma proteins by Howe's method, in accordance with the above mentioned Carneiro Felippe's improvements.

**Fibrinogen.**—Being poured into a small beaker 48 cc. of a 9 % sodium chloride solution, plus 1 cc. of a 2.5 % calcium chloride solution, we dropped 1 cc. of clear plasma.

We stirred up with a glass rod which was then left dipping in the fluid. The whole was brought into the ice box and left to stay there for 2 hours. After that time, the glass rod was turned along the inside wall of the beaker, the rod being kept revolving so as to collect around it, the whole quantity of coagulated fibrinogen. After a few minutes, the whole lot of fibrinogen is found sticking to the rod, which is by then suspended over the beaker to get free from any remaining droplet of fluid. The mass is then put to stay in the dry oven at 100/110°C., washed with distilled water by simple immersion and again left to dry between 100 to 110°C until a stable weight is reached at. The weight figure subtracted from the total weight of the rod when dried at the same temperature will give in grams the quantity of fibrinogen contained in 1 cc. of plasma. Our results are referred to 100 cc. of plasma.

**Albumin.**—Being poured, into a graduated eprouvette, 30 cc. of a 22.2 % sodium sulphate solution, prepared in complete accordance with Howe's recommendations (Journ. of Biol. Chem. 1921, vol. 49, p. 93), plus 1 cc. of clear plasma, the whole is stirred in a circular way and left to stand in the dry oven at 37°C for three hours, after which time it is filtered, the filtrate to serve for dosing nitrogen. For this purpose, 5 cc. of the filtrate are taken out, 1 cc. of pure sulphuric acid and a few drops of a 6 % copper sulphate solution are added. The whole is then exposed to fire to destroy the organic matter (digestion) then neutralized by 5 M soda (8 cc.). Distilled afterwards by a solution of hydrochloric acid N/70 (15 cc.) Hydrochloric acid is then titrated by N/70 soda, already containing the indicator (methyl red) according to the micro-Kjeldahl's technique.

From the figure, calculated on 100 cc. subtract first the non protein nitrogen, before multiplying by 6.25.

**Total proteins.**—The fluid, that separates from the coagulated fibrinogen, is used for the dosage of total proteins.
(albumins and globulins). 5 cc. of the fluid are taken and nitrogen determined, according to the above described technique. Before multiplying by 6,25 the above figure, referred to 100 cc., non proteic nitrogen should be subtracted.

Globulin.—In order to know the amount of globulin, it is sufficient to subtract from the result obtained for proteins the figure previously calculated for albumin.

Non protein nitrogen.—2 cc. of clear plasma should be taken; 14 cc. distilled water added hereto, as also 2 cc. of a 10% sodium tungstate solution. Then, slowly drop 2 cc. of sulphuric acid 2/3 N, taking care to stir the fluid thoroughly. One part is filtered, of which 10 cc. are taken for titrating nitrogen, as per above described method referring to albumin, the result being then brought back to 100 cc. of plasma.

Howe used for precipitating total proteins trichloroacetic acid. However, Prof. Carneiro Filipe recommends preferably tungstic acid as a better agent of precipitation for proteins, as already demonstrated by Folin and Wu (A system of blood analysis. Journ. of Biol. Chem. 1919, vol. 38, page 81-110).

Clinical observations were effected at the Oswaldo Cruz Hospital, by special kindness of Prof. Eurico Vilela to whom we feel greatly indebted.

It is a great pleasure to express my thanks to Prof. Carneiro Filipe, under whose direction this work was done.

CONCLUSIONS

I.—Total proteins of plasma were found in decrease in hookworm disease, which diminution affects only the albumin fraction. The amount of globulin remains unchanged or in slight increase.

II.—Fibrinogen shows generally increased.

III.—Non protein nitrogen keeps within normal limits.

IV.—Changes in plasma proteins, as observed in hookworm disease, are of the same type as those found in fatty nephrosis, which fact leads us to comprise also renal disturbances, which arise in helminthic anemia, within the group of nephrosis.

Clinical observations

OBSERVATION N. 1—J. P. Regd. A. 194, 15 years, white, feminine, entered 1. 10. 928.


OBSERVATION N. 3—B. R. Regd. A. 201, 47 years, masculine, white, Portuguese, admitted 17. 10. 1928.

Weakness, lack of energy at work,

**OBSERVATION N. 4—A. M. S. Regd. A. 24; 15 years, masculine, mestizo, Brazilian. Admitted 17. 12. 1928.**


**OBSERVATION N. 5—L. R. Regd. A. 203, 11 years, white, feminine, Brazilian, residing in Cordovil. Admitted 19. 10. 1928.**


**OBSERVATION N. 6—R. R. M. Regd. 3157, 12 years, Brazilian, white, residing at rua Conti, 112.**


**OBSERVATION N. 7—R. M. R. Regd. A. 206, 22 years, masculine, white, Brazilian.**


**OBSERVATION N. 8—J. E. S. Regd. A. 269, 25 years, white, Brazilian, fisherman.**


**OBSERVATION N. 9—S. F. L. Regd. A. 421, 13 years, Brazilian, mestizo, masculine, admitted 29. 5. 1929.**


**OBSERVATION N. 10—E. P. G. Regd. A. 456, 18 years, masculine, white, labourer.**

OBSERVATION N. 11—H. C. Regd. 3802, 4 years, white, Brazilian, residing rua Thomaz Coelho 9.


OBSERVATION N. 12—W. G. Regd. A. 457, 17 years, white, Brazilian, labourer.


OBSERVATION N. 13—N. A. Regd. 3870, 11 years, white, Brazilian, residing Rua Fernando Cunha, 92.


OBSERVATION N. 14—A. S. Regd. A. 480, 24 years, masculine, white, residing in Penha.


OBSERVATION N. 15—A. A. Regd. A. 493, 13 years, white, feminine, Brazilian, residing in a brick-yard in Rua São João, 179.


OBSERVATION N. 16—E. X. Regd. 3891, white, Portuguese, gardener, residing in Penha.


OBSERVATION N. 17—B. R. Regd. A. 24 years, white, Brazilian, gardener.

Has been ill for many years. Palpitations. Lack of energy. Feeling of hammerblows in the head and very changing appetite. Co-
lourless mucosae. In the course of a month, is compelled to stop work and take rest Cardiac dilatation. In feces: hookworms' eggs. Urine: albumin negative. Hemoglobin: 38 o/o (Zahlil). Red corpuscles 2,340,000 per mm³.

OBSERVATION N. 18—V. R. R. Regd. 4028, 7 years, Brazilian, white, residing Estrada do Norte.


OBSERVATION N. 19—A. S. Regd. 513, 29 years, white, feminine, Brazilian, residing Rua Dyonisio.


Bibliography

8) HAMMARSTEN.—Lehrbuch der Physiologischen Chemie. 1924.
<table>
<thead>
<tr>
<th>N.</th>
<th>Names</th>
<th>Albumin</th>
<th>Globulin</th>
<th>Total protein</th>
<th>Non protein nitrogen</th>
<th>Fibrinogen</th>
<th>Hemoglobin (Gowers Sahli)</th>
<th>Red blood corpuscles per mm²</th>
<th>A/G Ratio</th>
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<td>1.34</td>
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