ELECTROPHORETIC AND SALT FRACTIONATION OF THE SERUM PROTEINS OF NORMAL AND HYPOTHYROID RATS*

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(Received for publication, November 17, 1944)

In a previous communication (1) we reported that the sera of intact, normal rats of the Long-Evans strain, when examined electrophoretically in saline-sodium phosphate buffer at pH 7.4, contained little or none of the protein component usually designated as α-globulin. In contrast, sera taken from rats 21 days after hypophysectomy uniformly showed the presence of this component.

Earlier experiments (2) depending on salt fractionation methods had shown that the concentration of total serum globulin increased after thyroidectomy as well as after hypophysectomy and that such globulin elevation might be prevented by thyroid replacement therapy. It was therefore of interest to ascertain whether the sera of thyroidectomized rats showed the presence of α-globulin and whether this component accounted for at least a portion of the increase in total serum globulin. Likewise, it seemed probable that the sera of rats made hypothyroid (3) by thiouracil administration would show the presence of α-globulin.

The data reported in this paper, obtained from the sera of eight thyroidectomized, eleven thiouracil-fed, and eight additional untreated control rats, show that α-globulin does indeed appear in the serum of hypothyroid rats.

EXPERIMENTAL

A group of male rats of the Long-Evans strain was thyroidectomized when 3 to 4 months old. They were allowed to eat the stock diet (4) ad libitum and water was available in the cages at all times. During the postoperative period of 21 days the animals usually showed a small body weight loss, although some of them gained slightly. After this interval, the animals were anesthetized by intraperitoneal administration of sodium amytal, 10 mg. per 100 gm., and were bled by heart puncture. The blood was allowed to clot and the clear serum collected.

* Aided by a grant from the Rockefeller Foundation, administered by Dr. P. E. Smith.
Another group of male rats of similar age and weight was rendered hypothyroid by ad libitum feeding of the stock diet containing 0.2 per cent 2-thiouracil. After a 21 day period on this diet, the animals were bled as described above.

Electrophoretic analyses were made on aliquots of the serum after dilution with 2 volumes of 0.02 M sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl and dialysis of the diluted serum against large volumes of the same buffer in the usual manner. The analyses were made in a Tiselius apparatus having a tall, single sectioned cell of 2 ml. capacity (5).

Serum proteins were also determined by the sodium sulfite fractionation method of Campbell and Hanna (6).

RESULTS AND DISCUSSION

A summary of the data is presented in Table I. As may be seen, these data, electrophoretic as well as salt fractionation, confirm earlier findings (2) that thyroidectomy leads to an increase in serum globulin concentration with little change in albumin concentration. The electrophoretic analyses further show that in every thyroidectomized rat the a-globulin concentration is elevated sufficiently to permit definite detection of this component under the conditions here used. In contrast, only four of the twenty (eight in this and twelve in the previous series (1)) normal rat sera analyzed electrophoretically have shown the presence of this protein component.

Typical electrophoretic patterns obtained with sera of normal and thyroidectomized rats are reproduced in Fig. 1. In some of the patterns for normal rat serum, as illustrated in Fig. 1, A, the curve did not return completely to the base-line between the albumin and β-globulin components, indicating the presence of electrophoretically heterodisperse protein in that region. In measuring the areas under the curves for purposes of estimating protein concentration, this protein was included partially with the albumin and partially with the β-globulin fractions. The γ-globulin component is appreciably higher in the serum from the thyroidectomized rats than that from the normals.

It may also be pointed out that the sera from some of the thyroidectomized rats contained an additional component which appeared in the patterns as a shoulder on the albumin curve (Fig. 1, B). On prolonged

1 The 2-thiouracil (deracil) was kindly supplied by Dr. S. M. Hardy of the Lederle Laboratories, Inc., Pearl River, New York.

2 In the previous paper (1) of this series, it was erroneously stated that the sera were diluted with 3 volumes of buffer solution prior to dialysis. The procedure actually was identical with that indicated in the present paper; i.e., dilution with 2 volumes of buffer followed by dialysis against a large volume of the same buffer.
electrophoresis this separated into a distinct component. A similar additional component was also seen in the patterns of two of the four "normal" sera which showed the presence of α-globulin. Whenever this component appeared, it was included with the albumin in calculations of the relative concentrations of the various fractions.

**Table I**

**Summary of Data on Sera from Normal, Thyroidectomized, Thiouracil-fed, and Hypophysectomized Rats**

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Salt fractionation, gm. per cent</th>
<th>Electrophoretic fractionation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein</td>
<td>Albumin</td>
</tr>
<tr>
<td>20, normal</td>
<td>6.04</td>
<td>3.86</td>
</tr>
<tr>
<td></td>
<td>±0.25†</td>
<td>±0.23</td>
</tr>
<tr>
<td>8, thyroidectomized</td>
<td>6.70</td>
<td>3.50</td>
</tr>
<tr>
<td></td>
<td>±0.65</td>
<td>±0.32</td>
</tr>
<tr>
<td>11, thiouracil-fed</td>
<td>7.03</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>±0.49</td>
<td>±0.25</td>
</tr>
<tr>
<td>6, hypophysectomized</td>
<td>5.76</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>±0.23</td>
<td>±0.21</td>
</tr>
</tbody>
</table>

* Expressed in arbitrary units derived from the descending patterns.
† Standard deviation, $\sqrt{\Sigma (x - \bar{x})^2/(N - 1)}$.
‡ Only four of the twenty specimens showed the presence of α-globulin, the average of these four values being 26 ± 8.

![Fig. 1. Electrophoresis patterns of serum from (A) normal and (B) thyroidectomized rats. Buffer, 0.02 M sodium phosphate + 0.15 M NaCl at pH 7.4. Serum diluted with 2 parts of buffer.](http://www.jbc.org/)

Thiouracil feeding, as was expected, also produces an increase in serum globulin level. The figures derived by both the salt fractionation and electrophoretic methods agree in showing that the increase in globulin level during thiouracil feeding is not as great as that resulting from thyroidectomy. Likewise both methods agree in showing that during the thiouracil feeding there is a significant increase in albumin concentration, whereas
after thyroidectomy the albumin concentration remains stationary or decreases slightly. The reason for this difference in behavior of thyroidectomized and thiouracil-treated rats is unknown to us at the present time.

The fact that in the thiouracil-treated rats the concentrations of both the albumin and globulin components are above normal might be interpreted as being a result of hemoconcentration. Although such a concept agrees with the reported relation of blood volume to thyroid activity in man (for review of literature see (7)), the few hematocrit readings we have made do not support this possibility. In every case, the hematocrit values were found to be normal or below normal, indicating a normal or perhaps slightly increased plasma volume. If the plasma volume is indeed actually decreased, one must interpret our hematocrit values as indicating a coincident and proportionally greater decrease in total cell volume.

Similarly, the increased serum globulin concentration found after thyroidectomy may be interpreted as being due to hemoconcentration. Such a conclusion, however, is even more difficult than in the case of thiouracil-fed rats, for after thyroidectomy not only does the hematocrit reading fall, but the albumin concentration is consistently lower than in normal rats (2). Therefore, if the postthyroidectomy increase in serum globulin is due to a decrease in plasma volume, it must be assumed that there is a coincident, and proportionally greater, decrease in total red cell volume as well as in total circulating albumin. In this respect it is interesting to analyze the blood volume data of Gibson and Harris (7), who studied seventeen cases of severe human hyperthyroidism which showed substantial reduction of basal metabolic rate following treatment. In five of these seventeen cases, the decrease in basal metabolic rate was accompanied by an increase (1.8 to 11.5 per cent, average 4.8 per cent) in plasma volume. In the remaining twelve cases, the decrease in basal metabolic rate was accompanied by a decrease in plasma volume of 2.2 to 29.5 per cent (average 12.0 per cent). Fifteen of the seventeen cases showed a cell volume decrease, the average change being 13.8 per cent of the original cell volume. The data of Gibson and Harris therefore agree with the subnormal hematocrit values we have found. The increased serum globulin concentration may be due to hemoconcentration but this is not yet proved, particularly in the light of the data of Gibson and Harris, which show an increase in plasma volume with decreased basal metabolic rate in one-third of their human subjects.

The data of Table I as well as those previously reported (1) show that the electrophoretic and salt fractionation methods agree in defining the direction of the changes in concentration of serum albumin and globulin. It may be noted, however, that the ratio of albumin to globulin as determined by electrophoresis is consistently higher than the ratio obtained by the salt fractionation method. It is recognized that the albumin to
globulin ratio as determined by electrophoresis is dependent on both the protein concentration and the concentration and nature of the buffer ions used. It has been shown, however, that the sodium phosphate-saline buffer and the protein concentration employed in these experiments yield a relation which reduces almost to a minimum the error obtained with buffers of lower ionic strength and protein of higher concentration (8).

Moreover, the possibility that a part of the electrophoretic albumin is precipitated along with the globulin by the high salt concentration used for the chemical separation has previously been suggested (1). To investigate this possibility a sample of pooled rat serum was fractionated into "albumin" and "globulin" fractions by addition to 19 volumes of 21 per cent sodium sulfite. The "albumin" filtrate was concentrated by pressure dialysis and then dialyzed against large volumes of the pH 7.4 sodium phosphate buffer described above.

The precipitated globulin was washed with a small volume of fresh solvent, centrifuged down to form a compact pellet, and the supernatant decanted. The precipitate was then dissolved in water and an aliquot of the solution dialyzed against large volumes of the pH 7.4 buffer. The remainder of the "globulin" solution was reprecipitated by adding it to 19 volumes of 21 per cent sodium sulfite and, after repetition of the washing procedure, another aliquot was removed and dialyzed. The remainder was precipitated for the third time and this precipitate dissolved and dialyzed. The four resulting solutions, namely that of "albumin" and of "globulins" precipitated one, two, and three times respectively, were analyzed electrophoretically.

Patterns of the unfractionated serum and the "albumin" filtrate are reproduced in Fig. 2. An appreciable amount of globulin has remained unprecipitated (Fig. 2, B). Also the "globulin" precipitate contained a considerable quantity of electrophoretic albumin, as is indicated by the patterns of Fig. 3. A comparable result was obtained with another sample of pooled rat serum treated in a similar manner.

The fact that the attempt to separate the electrophoretic albumin from the precipitated "globulins" by repeated sodium sulfite precipitations did not succeed suggests that this albumin differs from the main bulk of the serum albumin. Supporting evidence is afforded by its mobility \( u = -4.0 \times 10^{-3} \text{ sq. cm. per volt per second} \) which is definitely less than that \( (u = -4.6 \times 10^{-3} \text{ sq. cm. per volt per second}) \) of the albumin not precipitated by sodium sulfite. These results suggested to us the possibility that

3 The mobilities of all the globulin fractions were increased by each successive precipitation and the third precipitate gave a distorted pattern with little or no protein having the mobility of \( \gamma \)-globulin. The mobility of the fastest component (presumably albumin) did not change appreciably with reprecipitation.
the fraction having the lower mobility might be the high carbohydrate-containing fraction isolated by McMeekin (9). A carbohydrate analysis was, therefore, attempted on the small amount of material at our disposal. The analyses were made by a quantitative modification of the Molisch reaction depending on comparison of the color developed by the unknown against the color developed by glucose standards. Because the color produced by the proteins was not identical with that of the glucose standard, the absolute values for carbohydrate content cannot be stated with accuracy. The analysis did definitely show, however, that the albumin fraction precipitated by the sodium sulfite contained about 2.5 times as much carbohydrate as does the albumin which is not precipitated by sodium sulfite.

![Figure 2](image-url)  
**Fig. 2.** (A) Pooled unfractionated serum of normal rats. (B) 20 per cent sodium sulfite filtrate of the same serum. Buffer, as in Fig. 1.

![Figure 3](image-url)  
**Fig. 3.** 20 per cent sodium sulfite precipitate from normal rat serum after (A) first precipitation, (B) reprecipitation, and (C) second reprecipitation. Buffer, as in Fig. 1.

The actual figures obtained, 4.3 and 1.8 per cent carbohydrate, respectively, for the precipitated and non-precipitated fractions, indicate that the precipitated fraction is indeed similar to the albumin fraction (5.5 per cent carbohydrate) isolated by McMeekin (9) from horse serum.

It was also of interest to see whether the electrophoretic albumin in the precipitate was similar to the α-globulin fraction described by Longsworth (10) and found in sera from normal and hypophysectomized rats (1) when the analyses were made in sodium diethylbarbiturate buffer at pH 8.6. Whole rat serum and the filtrate fraction were analyzed in this buffer. As may be seen in Fig. 4, the ratio of the two components was unchanged after precipitation and removal of the precipitate. The mobilities of all of the

*We are greatly indebted to Miss Marion Blanchard for these analyses.*
fractions were, however, appreciably increased by the sodium sulfite. It is evident, therefore, that the second component in the patterns obtained at pH 8.6 is not selectively precipitated with the globulins by 20 per cent sodium sulfite, and it is probable that this is a third albumin fraction (the first being the fastest moving component in the patterns obtained at pH 8.6 and the second being in the sodium sulfite precipitate), since by the criteria of salt fractionation and electrophoresis at lower pH it is albumin. At present it is impossible to say, however, whether the fractions are native or are produced by the procedures employed.

An attempt was made to use ultracentrifugal data as a criterion for establishing whether the various substances were albumins or globulins, but unfortunately all fractions, even though they were clear solutions, yielded sedimentation constants which indicated aggregation. Comparison of serum protein fractions obtained by other chemical means known not to affect the physical properties of the protein appreciably is in progress and will be reported later.

The authors are indebted to Miss Helen Sikorski and Miss Dorothy Wangerin for technical assistance.

**SUMMARY**

The protein component usually designated as α-globulin was found in definite quantities in only four of twenty sera of normal rats. In contrast, this component was found in the serum of every hypothyroid rat examined, whether the hypothyroidism was due to thyroidectomy or to thiouracil feeding.

The globulins precipitated from rat serum by 20 per cent sodium sulfite contained a component having an electrophoretic mobility of serum albumin. This "albumin" was not separated from the globulins by repeated reprecipitation with sodium sulfite.

![Fig. 4](http://www.jbc.org/) Pooled unfractionated serum of (A) normal rats. (B) 20 per cent sodium sulfite filtrate. Buffer, sodium diethylbarbiturate at pH 8.6.
BIBLIOGRAPHY

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