Changes in protein levels after thyroidectomy or in hypothyroidism have been reported by several authors. For these studies, different procedures have been used, such as: salting out, free electrophoresis (Levin & Leatham, 1942; Moore, Levin & Smelser, 1945; Lewis & McCullagh, 1944) and zone electrophoresis (Lamberg & Gräsbek, 1955; Boas, 1955).

In a previous report from this laboratory, a significant hypoproteinemia was observed in thyroidectomized rats compared with normal controls (Abreu et al., 1956). This was also found in thyroidectomized dogs (Podhraszky, 1942 a, b).

In the previous experiments (Abreu et al., 1956), total serum protein-bound carbohydrates (determined as galactose-mannose) was lowered in the operated animals, this being interpreted as a consequence of the hypoproteinemia. On the other hand, Boas (1955), reported increased plasma hexosamine concentrations after thyroidectomy and these changes were explained as being due to an increase in the amino sugar content of the β- and γ-globulin fractions. It was also found that there was a significant increase in the relative amount of the γ-globulin and a relative reduction of the albumin and α-globulin fractions of rat plasma. However, total plasma protein and the absolute values of the different protein fractions were not reported.

The present work deals with the paper electrophoretic determination of the relative and absolute levels of serum proteins in thyroidectomized rats.

MATERIALS AND METHODS

Male albino rats of the Wistar strain weighing 50–80 gm. were thyroidectomized under ethyl urethane anesthesia. These animals (8 rats) and a group of normal controls (11

*) Working with grants from The National Research Council of Brazil.
rats) were maintained on a standard laboratory diet. After 45 days, blood samples were obtained by heart puncture under light ether anesthesia. The blood was allowed to clot and the clear serum separated. Gross post-mortem examination showed no regeneration of thyroid tissue at the site of operation.

Total serum proteins were determined by the biuret method of Weichselbaum (1946) calibrated by duplicate Kjeldahl protein nitrogen determinations.

A horizontal paper strip electrophoresis apparatus* was used for all separations. The runs were made using a veronal buffer at pH 8.6, ionic strength 0.05, and a temperature of 10° C.

The apparatus was loaded with Whatman No. 1 filter paper strips (1.5 × 12 inches) and four strips were run simultaneously. After equilibration of buffer levels, the serum (0.01 ml.) was added as a thin streak (about 0.5 inch long) across the width of the strips, by means of a calibrated micropipette. A potential gradient of 25 volts per strip was used which should give a current of 0.5 milliamperes per strip, in our experimental conditions. The electrophoretic separations proceeded for 16 hours.

After the electrophoretic separations, the paper carrier was removed and set quickly in an oven at 105–110° C for about 30 minutes. The strips were then placed in a frame and stained with bromophenol blue. The technique used was essentially the same as described by Jencks, Jetton & Durrum (1955) with minor modifications.

After the staining period the strips were removed from the dye bath, washed with 3 changes of 2 per cent acetic acid and then immersed in a 2 per cent sodium acetate solution containing 10 per cent of acetic acid. The strips were allowed to dry at 110° C and mounted for scanning. The Photovolt Densitometer Mod. 425** with a stage for scanning and a semi-automatic plotting of density curves was used. After drawing the curve the areas were determined by planimetry and the amount of each fraction calculated.

RESULTS AND DISCUSSION

The paper electrophoretic diagrams of serum proteins obtained for normal and thyroidectomized rats are shown in Fig. 1. As can be seen the two curves are almost identical.

Table 1 shows the relative (per cent) average protein values found for normal and thyroidectomized animals. Their standard deviations and the statistical evaluation of the differences were obtained according to Student's t test. It can be clearly seen that the relative composition of serum protein fractions in both groups of animals was practically the same.

In Table 2, the results obtained were expressed in absolute values (gm. per 100 ml.) and the differences statistically evaluated. It appears that 45 days after thyroidectomy all serum protein fractions decreased. However, this decrease was slight and not statistically significant for the α- and γ-globulins. On the other hand, the lower concentration of the albumin fraction in operated

**) Photovolt Corporation, New York, N. Y.
**Fig. 1.**

Paper electrophoretic diagrams of serum proteins from normal and thyroidectomized rats (veronal buffer pH 8.6, ionic strength 0.05).

**Table 1.**

Serum protein fractions in normal and thyroidectomized rats (relative values).

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Normal (11 rats)*</th>
<th>Thyroidectomized (8 rats)*</th>
<th>P***)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>31.1 ± 2.3</td>
<td>31.6 ± 3.3</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>α1-globulin</td>
<td>16.9 ± 2.4</td>
<td>17.6 ± 3.2</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>α2-globulin</td>
<td>9.7 ± 1.1</td>
<td>8.9 ± 2.5</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>β-globulin</td>
<td>24.9 ± 1.1</td>
<td>24.8 ± 1.7</td>
<td>&gt; 0.5</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>17.5 ± 2.5</td>
<td>17.1 ± 3.6</td>
<td>&gt; 0.5</td>
</tr>
</tbody>
</table>

*) Mean ± standard deviation [\(\sqrt{\sum(x - \bar{x})^2/(n - 1)}\)].

***) Statistical significance of differences between normal and thyroidectomized groups.
Table 2.
Serum protein fractions in normal and thyroidectomized rats (absolute values, gm./100 ml.).

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Normal (11 rats)*</th>
<th>Thyroidectomized (8 rats)*</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>1.91 ± 0.15</td>
<td>1.65 ± 0.13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>α₁-globulin</td>
<td>1.03 ± 0.14</td>
<td>0.92 ± 0.19</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>α₂-globulin</td>
<td>0.60 ± 0.08</td>
<td>0.48 ± 0.16</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>β-globulin</td>
<td>1.53 ± 0.12</td>
<td>1.31 ± 0.21</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>1.08 ± 0.20</td>
<td>0.91 ± 0.26</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>Total protein</td>
<td>6.16 ± 0.40</td>
<td>5.26 ± 0.69</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*) Mean ± standard deviation [$\sqrt[2]{\sum(x-x)^2/(n-1)}$].

**) Statistical significance of differences between normal and thyroidectomized groups.

animals was highly significant and the fall in the β-globulin was also significant.

The highly significant decrease in total serum proteins following thyroidectomy can be almost entirely accounted for by the decrease in concentrations of the albumin and β-globulin fractions.

The low levels of serum albumin obtained in thyroidectomized rats are in agreement with the results found in human hypothyroidism (Lewis & McCulloch, 1944; Lamberg & Gråsbeck, 1955) and with the data of Moore et al. (1945) using salt fractionation methods in thyroidectomized rats. Boas (1955) also obtained a low relative albumin level but in our data only the absolute values were significantly different from the normal controls.

The α₁- and α₂-globulin fractions were not statistically reduced in our thyroidectomized animals although some changes have been observed by other investigators (Boas, 1955).

In the data of Moore et al. (1945), it can be seen that the β-globulin fraction was slightly reduced after thyroidectomy while in our series of experiments this component was found to be statistically decreased. Boas (1955) found unaltered relative amounts of this fraction in the plasma of thyroidectomized rats.

It is interesting to recall that Boas (1955) carried out the electrophoretic separations with plasma. In this case, the fibrinogen was not separated from the β-globulin with which it is associated (Deutsch & Goodloe, 1946). This makes the study of the electrophoretic behaviour of β-globulin difficult and possibly also affects the estimation of γ-globulin in the plasma of thyroidectomized animals. This could account for the differences found in our and the above mentioned data for these fractions.
We were unable to find any relative or absolute increase in the globulins in our experimental conditions although several investigators (Levin & Leathem, 1942; Moore et al., 1945; Boas, 1955) obtained a high serum globulin, and particularly of the \( \gamma \)-globulin concentrations.

The causes of the changes in serum protein patterns in thyroid dysfunction (Lamberg & Gräsbeck, 1955) are at the present time difficult to explain without further research though the fact that thyroidectomized rats do not show any \( \alpha \)-globulin changes, as observed in human hypothyroidism, could be attributed to species differences as already pointed out by Boas (1955).

**SUMMARY**

Paper electrophoresis of serum proteins in thyroidectomized rats showed a significant decrease in the albumin and \( \beta \)-globulin, considering the absolute values of these fractions. A significant fall in the total protein content of the serum in thyroidectomized animals was also noted. However, no marked differences could be found considering the relative amount (per cent) of each fraction. The significance of these results is discussed.

**REFERENCES**


