THE PROTEIN CONTENT OF THE ORGANS AND TISSUES OF THE BODY AFTER ADMINISTRATION OF THYROXINE AND DINITROPHENOL AND AFTER THYROIDECTOMY*

BY T. ADDIS, D. KARNOFSKY, W. LEW, AND L. J. POO

(From the Department of Medicine, Stanford University Medical School, San Francisco)

(Received for publication, February 10, 1938)

The total decrease in body protein induced by fasting and the increase that follows refeeding are the summations of dissimilar changes in the protein content of the various organs and tissues of the body. There are organs whose protein content remains unchanged during fasting and refeeding, there are others in which the changes are slight, and there are some that lose and then regain a large proportion of their original protein content; and not only does each organ manifest a different degree of change, but with respect to the rate of change each has its own characteristics (1). It was in the hope that some information might be gained about the relation between these peculiarities of the organs and the part, whether active or passive, played by each organ in the processes of protein anabolism and catabolism that observations were made on the protein content of the organs and tissues after the administration of thyroxine and dinitrophenol, and after removal of the thyroid gland, all of them procedures known to be followed by marked changes in the rate of metabolism.

Our original plan of observing the changes in animals that at the start were alike in body weight, age, and sex was not followed in this work because after the removal of the thyroid gland there is such a prolonged loss of body weight that an equality of all factors cannot be obtained, and so we found ourselves obliged to compare the protein content of the organs of rats whose body weights are

* This work was aided by a grant from the Rockefeller Foundation.
dissimilar at the beginning as well as at the end of the period of
subjection to the experimental conditions. It would distort the
true relations to express these quantities as gm. per unit of body
weight or of any measure of body size derived from body weight,
because that would involve the assumption that this unit remains
a constant with respect to its protein content whether the rats
are fed or fasted, or whether they are given thyroxine or have
their thyroid glands removed. That assumption of equality of
composition is denied by the fact that 100 gm. of fed rats have
more fat and less protein than 100 gm. of lean fasted rats. When
the state of nutrition as well as the body weight varies, the only
valid method is to express the quantities of organ protein in
relation to the total body protein found under each separate com-
plex of conditions. In each case the total mass of body protein
is allocated in certain proportions to the various organs. Under
all conditions these proportions are comparable, and when the
concentration of fat in the body is a variable, it is only these
proportions that have a functional as well as an arithmetical
significance.

The effect of individual variation was minimized by carrying
out the determinations on the massed bodies and organs of groups
composed, as a rule, of thirty male rats between 90 and 110 days
of age. The methods used are those recently described (2). The
significance of the differences from the control proportions
in the experimental groups can be gaged from the differences
from the average proportions found in repeated observations
under the control conditions. We have eight determinations on
fed, and on 2 day- and 7 day-fasted, control groups. The ab-
solute quantities of protein per rat, the proportions of total protein,
and the percentage deviations from the averages under each of
these three conditions are given in Table I.

Under all conditions these control groups showed a ±3 per cent
development for the heart, ±3.5 per cent for the kidney, and ±2.8
per cent for the liver protein. The greatest deviation was ±6
per cent.

Thyroxine was given subcutaneously in doses of 0.75 mg. per
100 sq.cm. of body surface every 48 hours and the protein deter-
mined on the 9th day after injections were commenced. The
thyroid gland was removed 31 to 39 days before the animals
were sacrificed. Dinitrophenol was given either by subcutaneous injection in doses of 2 mg. per 100 gm. of body weight twice a day for 7 days or by stomach tube in doses of 3 mg. per 100 gm. of body weight every day for 7 days. The controls were groups of thirty or more rats fed on the same diet, or fasted for the same length of time. The temperature of the room was regulated so that it was usually 20°, did not fall below 18°, and rarely rose above 25°.

### Table I

**Variation in Proportions of Protein in Control Groups**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of rats</th>
<th>Protein per rat</th>
<th>Proportion of total protein</th>
<th>Deviation from average proportion in fed or fasted rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Heart</td>
<td>Kidney</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
</tr>
<tr>
<td>1. Fed</td>
<td>30</td>
<td>41.78</td>
<td>0.14</td>
<td>0.256</td>
</tr>
<tr>
<td>2. &quot;</td>
<td>30</td>
<td>37.04</td>
<td>0.135</td>
<td>0.252</td>
</tr>
<tr>
<td>3. &quot;</td>
<td>30</td>
<td>36.26</td>
<td>0.135</td>
<td>0.266</td>
</tr>
<tr>
<td>4. 2 day fast</td>
<td>30</td>
<td>36.04</td>
<td>0.126</td>
<td>0.222</td>
</tr>
<tr>
<td>5. 2 &quot; &quot;</td>
<td>30</td>
<td>32.92</td>
<td>0.120</td>
<td>0.225</td>
</tr>
<tr>
<td>6. 7 &quot; &quot;</td>
<td>30</td>
<td>35.45</td>
<td>0.119</td>
<td>0.204</td>
</tr>
<tr>
<td>7. 7 &quot; &quot;</td>
<td>30</td>
<td>34.13</td>
<td>0.107</td>
<td>0.202</td>
</tr>
<tr>
<td>8. 7 &quot; &quot;</td>
<td>30</td>
<td>33.62</td>
<td>0.108</td>
<td>0.187</td>
</tr>
</tbody>
</table>

In Table II the total body protein and the protein content of certain organs are given for control and experimental groups all fed *ad libitum* the diet on which they had been reared. This was a modified Steenbock diet (3) that contained 10 per cent casein, 73 per cent corn-meal, 10 per cent linseed meal, 2 per cent alfalfa, 3 per cent sardine oil, 1.5 per cent bone ash, and 0.5 per cent sodium chloride. The protein concentration was 18 per cent.

Table II shows that compared with the controls thyroxine increases and removal of the thyroid gland decreases the proportion of the protein in the heart and kidney, while dinitrophenol has no definite effect. But it is certain that after thyroxine more than the usual amount of protein was eaten and that after thyroidectomy the rats ate less. In other experiments we have
found that the protein content of the kidney and liver, but not of the heart, rises and falls with the amount of protein the rats eat and so it is possible that the observed differences from the controls in the kidney and liver protein may have been in part or wholly.

**Table II**

*Effect of Thyroxine, Thyroidectomy, and Dinitrophenol on Proportion of Total Body Protein Found in Heart, Kidneys, and Liver of Rat on Adequate Diet*

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Protein per rat (gm.)</th>
<th>Proportion of total protein</th>
<th>Relation to controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Heart</td>
<td>Kidney</td>
</tr>
<tr>
<td>Controls</td>
<td>90</td>
<td>38.36</td>
<td>0.137</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>30</td>
<td>37.14</td>
<td>0.172</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>41</td>
<td>31.84</td>
<td>0.087</td>
</tr>
<tr>
<td>Dinitrophenol</td>
<td>30</td>
<td>36.88</td>
<td>0.124</td>
</tr>
</tbody>
</table>

**Table III**

*Thyroidectomized and Control Rats after 7 Day Fast*

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Protein per rat (gm.)</th>
<th>Proportion of total protein</th>
<th>Relation to controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Heart</td>
<td>Kidney</td>
</tr>
<tr>
<td>Controls</td>
<td>90</td>
<td>34.40</td>
<td>0.111</td>
</tr>
<tr>
<td>Thyroidecto-</td>
<td>25</td>
<td>27.51</td>
<td>0.082</td>
</tr>
</tbody>
</table>

*By error the average proportion of protein in the liver of rats fasted 7 days was given in a previous paper (1) as 3.3 per cent instead of 3.03 per cent.*

determined by these inequalities in food consumption. In order to eliminate this variable, the same determinations were carried out on fasted rats. Table III gives the results on control and thyroidectomized groups fasted for 7 days.

For the liver the −12 per cent in Table II becomes +7 per
cent in Table III. We take this as an indication that thyroidec-
tomy as such has no appreciable effect on total liver protein.
The reduction in liver protein in the fed thyroidectomized group
may be ascribed to lessened food intake and the possible increase
compared with the controls after the 7 day fast to a lower rate of
depletion of the liver in the thyroidectomized group. In the
kidney there is a 19 per cent decrease in protein but in the heart
reduction is not so great as in the fed rats. In interpreting these
results it will be noted that the fasted controls have an 11 per cent
smaller proportion of heart protein and 32 per cent less liver
protein than the fed controls. But when fed and fasted thyroidecto-
tomized rats are compared the heart of the fasted rat has 11 per
cent more protein, while the liver loses only 18 per cent instead of
32 per cent. A 7 day fast thus has a different effect on thyroidecto-
tomized rats than on normal rats. If we assume that the decrease
in heart protein in the fasted controls is due to a diminution in
the work of the heart as the metabolic rate falls, then the difference
in the effect on the heart in the fasted thyroidectomized group
suggests that fasting does not further depress the state of lowered
metabolism already induced by removal of the thyroid gland.

The effect of thyroxine and of dinitrophenol on groups fasted for
2 and for 7 days is given in Table IV. In these experiments the
protein of the alimentary tract and of the drawn blood was not
determined and so the proportions refer to the total protein
determined, not to the total body protein.

The general result shown in Table IV is the pronounced increase
induced by thyroxine as contrasted with the negligible changes
caused by dinitrophenol. Since in both groups the general me-
tabolism was greatly increased, we have to look for a reason for
this difference to some divergence in the mechanism of action of
the two substances. There was a general similarity in the be-
behavior of the rats given thyroxine and dinitrophenol. Unlike
the controls they did not huddle together but lay separately,
only on their backs with their limbs fully extended. But there
was a difference in their appearance. At the height of the dini-
троphenol effect the bright red eyes turned purple as the oxygen
unsaturation of the blood increased. It has been shown that the
increased oxygen required following dinitrophenol administration
is obtained by the removal of much more than the usual proportion
of oxygen from the blood, with little or no increase in cardiac work (4), whereas with thyroxine the additional oxygen is supplied by an increase in the rate of flow of blood. This is a divergence in mechanism that might lead to increased work by the heart after thyroxine, and no increase after dinitrophenol, and so it seems reasonable to look on the increased protein in the heart following thyroxine as a result of work hypertrophy and to regard the unchanged protein of the heart following dinitrophenol as an expression of a relatively unaltered demand for work. It is simply an extension of this hypothesis to ascribe the atrophy of the heart after removal of the thyroid gland to a decrease in heart work corresponding to a diminished rate of blood flow consequent to the decrease in the oxygen requirements of the body.

It has been shown that thyroxine leads to an increase in nitrogen excretion by the kidney, while there is no evidence of such a marked increase in protein catabolism after dinitrophenol (5). It is known that thyroidectomy leads to a decrease in nitrogen excretion (6). As with the heart, so also in the case of the kidney

<table>
<thead>
<tr>
<th>Table IV</th>
<th>Thyroxine- and Dinitrophenol-Treated Rats and Control Rats after Fasting 2 and 7 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein per rat</td>
</tr>
<tr>
<td></td>
<td>No. of rats</td>
</tr>
<tr>
<td>2 day fast</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>60</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>30</td>
</tr>
<tr>
<td>Dinitrophenol</td>
<td>30</td>
</tr>
<tr>
<td>7 day fast</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>90</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>20</td>
</tr>
<tr>
<td>Dinitrophenol</td>
<td>30</td>
</tr>
</tbody>
</table>
it is therefore possible to suppose that the changes we find in the kidney protein are results of changes in the amount of work imposed upon it, and we have additional support for this hypothesis on the ground that the curve of increase of protein against time after thyroxine has the same general form as that observed when the work of the kidney is increased by feeding protein after a fast (1). Smith and MacKay (7) found a linear relation between the increase in heart weight and the increase in O2 consumption induced by thyroxine and they interpret this as an indication that the cardiac hypertrophy produced by thyroxine is a simple work hypertrophy. MacKay, Smith, and Closs (8) found a similar relation between increase in kidney weight and oxygen consumption after thyroxine, but they could not interpret this as indicative of work hypertrophy because the increase in kidney weight was considerably greater than that which MacKay and MacKay (9) found to be accounted for by virtue of the increased protein intake—the only known factor that would increase the work of the kidney. They consequently regard the major part of the renal enlargement as not at present susceptible of explanation. We agree with them that new data are needed but we are not yet willing to give up the hypothesis of work hypertrophy, simply because the increase in protein intake does not seem to be enough. The increase in purine excretion and the creatinuria that follow thyroxine administration (10) as well as changes in the concentrations of urinary constituents in the blood and urine may well increase the osmotic work of the kidney beyond the levels that might be predicted from consideration of the protein intake alone. The question might be approached directly and we are at present trying to devise a technique for the measurement of the work of the kidney and may later be able to give evidence for or against the work hypothesis.

With respect to the meaning of the 15 per cent increase in the proportion of the protein in the liver of rats given thyroxine after a 7 day fast, the grounds for any explanation are even less secure; but it may be hoped that determinations of liver protein in varying metabolic states will be useful in trying to define the part played by the liver in the processes of protein metabolism.

The comparisons of the effects of thyroxine and of dinitrophenol given in Tables II and IV seem to us to be important because they
eliminate the hypothesis that hypertrophy or atrophy of the heart, kidney, and liver can be a function of changes in their own internal rate of metabolism. But this result is not given directly. The figures only show that after thyroxine there is hypertrophy and that with dinitrophenol there is no definite change. Both substances increase the total metabolism of the body. But though we know that thyroxine increased the metabolic rate of the internal organs, it was necessary to be sure that in our experiments dinitrophenol also increased organ as well as total oxygen consumption. Dr. W. Dock was therefore good enough to measure the oxygen requirement of the abdominal viscera by his method for determining organ metabolism by exclusion (11). He found that under the conditions and with the dosage of dinitrophenol we used there was an even greater increase in organ metabolism than in the body as a whole. This makes it certain that after dinitrophenol the kidney and liver use more $O_2$, yet grow no larger, and so we cannot say that the contrast between the effect on organ size of the removal of the thyroid gland and of thyroxine administration is due to the opposite effects of these two procedures on the oxygen requirement of the organs. The size of these organs seems to be determined by the amount of work they have to do, not by the amount of oxygen they use.

We have only a few scattered observations on other organs and tissues. In thyroidectomized fed rats the protein of the testicles and adrenal glands was almost identical with that of the controls but in the seminal vesicles the protein decreased by 18 per cent and in the prostate gland by 24 per cent.

The effect on the blood is in consonance with the view that all the differences we find, other than those in the seminal vesicles and prostate, are responses to change in function. All of the blood that could be obtained from 7 day-fasted rats was collected and the protein of the serum and clot was determined. After thyroxine the serum protein was 2 per cent less than in the controls but the clot had increased 12 per cent. On the other hand, after removal of the thyroid gland the changes were in the reverse direction. The clot protein decreased by 27 per cent, while the serum protein increased the proportion of the total body protein allotted to it by 20 per cent.
SUMMARY

1. The proportion of the total body protein found in the heart, kidneys, and liver was determined in control rats and in groups of rats whose metabolic rate had been increased by the administration of thyroxine and of dinitrophenol. The protein content was increased by thyroxine but no definite change was found after dinitrophenol.

2. In thyroidectomized rats the proportion of total body protein in the heart and kidney was less than in the controls.

BIBLIOGRAPHY

THE PROTEIN CONTENT OF THE ORGANS AND TISSUES OF THE BODY AFTER ADMINISTRATION OF THYROXINE AND DINITROPHENOL AND AFTER THYROIDECTOMY


Access the most updated version of this article at http://www.jbc.org/content/124/1/33.citation

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/124/1/33.citation.full.html#ref-list-1