Evidence has accumulated which indicates that hormones play an important rôle in protein metabolism. For example, according to the current concept growth hormone stimulates protein anabolism (1) and adrenal cortical extract increases glucose neogenesis at the expense of protein (2). Various investigators have tried to relate changes in the free amino acid of the blood to the endocrine regulation of protein metabolism. The reports of previous workers have not been in complete agreement, probably because of differences in analytical methods employed, variations in endocrine extract, dosage, the route of administration, and time relationships.

According to the majority of papers, insulin causes a lowering of the amino acid level (3–5), and Greene’s claim (6) that insulin had no effect has not been supported. Okada (7) reported that epinephrine does not change the amino acid concentration of rabbit blood. Luck and coworkers, however, state that epinephrine alone is as effective as insulin in producing hypoaminoacidemia (8, 9), while still another group of investigators found an increase (10). A drop in the plasma amino acid level was shown to occur with hypophysectomy and with thyroidectomy (11), but Okada (7), who could not obtain any change when epinephrine was given, also claims that neither the removal of the thyroid gland nor hyperthyroidism influences the amino nitrogen content of blood. Investigations of the action of thiouracil treatment, adrenalectomy, estrogen administration, and prolonged adrenocortical steroid injections have been neglected.

The present project was started to clarify earlier work on the influence of endocrines on amino acid metabolism and was extended to include not only plasma, but also liver, kidney, and skeletal muscle. The gasometric ninhydrin reaction (12) was chosen in preference to the colorimetric procedure (13), the gasometric nitrous acid method (14), and the formaldehyde titration (15) because of its specificity for free amino acids.

**Experimental**

The animals were anesthetized with nembutal. Blood was drawn from the heart, heparin being used as anticoagulant. 2 ml. of plasma were...
treated with 10 ml. of 1 per cent picric acid and analyzed for amino acids
by the ninhydrin-carbon dioxide method of Hamilton and Van Slyke (12).
About 1 gm. of tissue, previously frozen in dry ice and acetone, was ground
with powdered glass and then extracted with two 10 ml. portions of 1 per
cent picric acid. After centrifugation and filtration, 5 ml. of the filtrate
were analyzed in the same manner as the plasma filtrates. If necessary,
the tissue can be stored after lyophilization to a dry powder. The powder
is extracted in a Waring blender and the analysis is the same as for frozen
tissue; however, the weight of the water removed must be recorded.

Hypothyroidism—This condition was caused by adding 0.2 per cent
thiouracil (deracil, Lederle Laboratories, Inc.) to the diet for 6 weeks.
At sacrifice the rats were examined for enlargement of the thyroid.

Thyroidectomy—A period of 6 weeks elapsed after the operation.

Hyperthyroidism—This condition was produced by feeding 1 per cent
thyroid powder (Armour Laboratories) in the diet for 3 weeks. Only
animals that lost weight during the treatment were used.

Adrenalectomy—During the postoperative period, 1 per cent sodium
chloride was administered in order to sustain life and prevent hemocon-
centration. 10 days after the operation the food was removed, the salt
water was replaced by ordinary water, and, as soon as adrenal crisis set
in (muscular weakness, etc.), the animal was sacrificed.

Lipo-Adrenal Cortex Extract—Three injections of 2 ml. of lipo-adrenal
cortex1 (80 rat units) at 2 day intervals were administered subcutaneously.

Epinephrine—1.5 mg. per kilo of body weight were given subcutaneously
to rats fasted 12 hours. The animals were sacrificed after 1 hour.

Insulin—20 units per kilo of body weight were injected subcutaneously
into rats fasted 12 hours. The animals were sacrificed after 3 hours.

Hormone Treatment—Estrogen, 500 rat units (progynon-B),2 was admin-
istered subcutaneously each 3 days for 4 weeks.

Hypophysectomy—The animals were hypophysectomized when 40 days
old and sacrificed 18 days later. Only animals that showed a definite loss
in weight were used.

Non-Protein Diet—The diet consisted of 54.7 per cent sucrose, 25 per
cent starch, 15 per cent Crisco, 5.3 per cent salts, and vitamins. The
rats on this regimen were fed for 8 weeks, when they had lost about one-
third of their weight.

In all experiments, normal male rats weighing between 100 and 200 gm.
were used. They were fasted 12 hours before sacrifice.

The data obtained are given in Table I.

1 Generously donated by The Upjohn Company through the kindness of Dr.
D. J. Ingle.
2 Generously donated by the Schering Corporation through the kindness of
Dr. W. H. Stoner, Medical Director.
DISCUSSION

Analysis of amino acid levels is made possible by the constancy of the normal concentration in blood and tissues. Each tissue has its own characteristic value, which is about 3 to 9 times that of blood. It is not known how the amino acids are concentrated by the tissues. Our values for rat liver are slightly lower and the values for skeletal muscle are less than half of those given by Luck (17). The amino acid levels obtained on dogs by Hamilton (18), using the ninhydrin reaction, show great variations. Hence, they cannot readily be compared, but they are lower than those found by Van Slyke (19) with the nitrous acid method. The highest amino acid concentrations were observed by us in the kidney and the lowest in the skeletal muscle (Tables I and II). The most constant concentration was found in blood plasmas.

### Table I

**Free Amino Acid Levels in Blood and Tissues**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma</th>
<th>Liver</th>
<th>Kidney</th>
<th>Skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per 100 ml.</td>
<td>mg. per 100 gm.</td>
<td>mg. per 100 gm.</td>
<td>mg. per 100 gm.</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7) 6.4 ± 0.1</td>
<td>(7) 32.1 ± 1.7</td>
<td>(7) 44.0 ± 1.7</td>
<td>(7) 19.2 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Thiouracil</td>
<td>(5) 5.2 ± 0.2</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>(7) 5.0 ± 0.3</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>(4) 9.0 ± 0.6</td>
<td>&gt;0.01</td>
<td>&gt;0.05</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>(6) 5.2 ± 0.2</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Adrenal cortex</td>
<td>(5) 7.9 ± 0.6</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>(4) 4.5 ± 0.4</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Insulin</td>
<td>(5) 4.8 ± 0.6</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Estrogen</td>
<td>(6) 5.2 ± 0.4</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>(6) 5.7 ± 0.2</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Non-protein diet</td>
<td>(4) 10.8 ± 0.6</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.01</td>
</tr>
</tbody>
</table>

The figures in parentheses represent the number of animals. The values represent the arithmetic mean plus or minus the standard error of the mean.

* Probability (if P values are 0.05 and less the result is significant); Fisher (16).
Every hormone so far tested affects the amino acids of blood and tissues. The metabolism of carbohydrate, protein, and fat is interrelated. The action of hormones on the amino acids is probably indirect and due to primary changes of other metabolites. In addition there may be tropic effects between different endocrines. Insulin, epinephrine, estrogen, hypophysectomy, thiouracil treatment, and thyroidectomy, decrease the blood amino acid concentration; thyroxine, lipo-adrenal cortex extract, and also protein starvation increase it. In the kidney, insulin, epinephrine, estrogen, and hypophysectomy produce a significant fall. Thyroxine, lipo-adrenal cortex extract, and protein starvation elevate the liver amino acid level. Thyroxine and lipo-adrenal cortex extract also bring about an increase of the free amino acids in muscle. Adrenalectomy results in hypoaminoacidemia but simultaneously causes a rise in the free amino acid level of muscle.

**TABLE II**

*Free Amino Acid Levels for Few Normal Tissues*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Amino nitrogen per 100 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>(4) 43.1 ± 1.8</td>
</tr>
<tr>
<td>Testis</td>
<td>(2) 26.7 ± 0.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>(2) 36.0 ± 2.7</td>
</tr>
<tr>
<td>Thoracic lymph</td>
<td>6.7*</td>
</tr>
</tbody>
</table>

The figures in parentheses represent the number of animals.
The values represent the arithmetic mean plus or minus the standard error of the mean.

* Amino nitrogen per 100 ml. of lymph obtained by pooling.

It is known that muscular wasting occurs in adrenal insufficiency which may be overcome by epinephrine injections (20).

The changes in amino acids are most definite in the blood and are less marked in the tissues. A lowered level will show up more readily in kidney than in liver, while an increased level can be detected better in liver than in kidney.

Interpretation of the amino acid changes should be delayed until more direct evidence on their metabolism is at hand. A decreased amino acid level could mean (1) decreased proteolysis of tissue proteins, (2) increased utilization of amino acids for protein synthesis, glycogenesis, lipogenesis, and energy production, or (3) increased elimination.

In animals on a non-protein diet, the plasma amino acid level is approximately doubled, the liver level is raised considerably, while the muscle and kidney values increase only slightly. This is in contrast to the findings of Goettsch et al. (21) who state: "It was rather a surprise to find that the
value of plasma amino acid during fasting was maintained at a normal level in spite of the severe degree of muscle wasting which occurred as hypoproteinaemia progressed." Endocrine disturbances often result in weight changes, which in turn might affect the amino acid levels.

**SUMMARY**

Insulin, epinephrine, estrogen, hypophysectomy, thiouracil treatment, and thyroidectomy decrease the blood amino acid concentration; thyroxine, lipo-adrenal cortex extract, and also protein starvation increase it.

Adrenalectomy results in hypoaminoacidemia but simultaneously causes a rise in the free amino acid level of muscle.

In the kidney, insulin, epinephrine, estrogen, and hypophysectomy produce a significant fall. Thyroxine, lipo-adrenal cortex extract, and protein starvation elevate the liver amino acid level. Thyroxine and lipo-adrenal cortex extract also bring about an increase of the free amino acids in muscle.

The amino acid concentration is highest in kidney and brain and decreases in other tissues in the following order, spleen, liver, testis, muscle.

**BIBLIOGRAPHY**

ENDOCRINE REGULATION OF AMINO ACID LEVELS IN BLOOD AND TISSUES
Felix Friedberg and David M. Greenberg

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