ENDOCRINE REGULATION OF AMINO ACID AND PROTEIN METABOLISM DURING FASTING*

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The concept of the dynamic state of the body constituents is basic to an understanding not only of those processes which maintain the constancy of body composition, but also of those which are concerned with the phenomenon of growth. It is evident from the work of Schoenheimer[23] and his colleagues that the amount of protein in the animal body is determined by the rates of protein synthesis and breakdown which prevail. From this it follows that growth, which is associated with the accumulation of body protein, can occur only when the rates of metabolism of amino acids and proteins are so adjusted as to favor an increase in the amount of body protein. Thus accumulation of protein might result from a retardation of amino acid or protein catabolism, or by speeding up of the synthesis of protein, or conceivably, by all mechanisms operating simultaneously. It may readily be seen that the control of each of these processes by independent mechanisms would afford the animal a smooth means of regulation of growth. If the immature animal accumulates protein by retarding catabolic reactions, it is, of course, necessary to assume that in the adult these processes proceed at rates faster than during growth. On the other hand, if it is assumed that growth occurs as a result of the acceleration of protein synthesis, it must also be assumed that the rate of synthesis of protein declines as the animal matures. Expressed in a different way, these considerations imply that those agencies which regulate the rates of amino acid and protein metabolism are functions of the age of the animal, and in view of the remarkable effects of endocrine secretions on growth, it follows logically that the factors which regulate the rates of metabolism of amino acids and body proteins of the living animal are endocrine in nature.

Theoretical considerations

The interrelationships of amino acid and protein metabolism may, perhaps, best be illustrated by means of the scheme below.

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$$\text{Body proteins} \rightleftarrows \text{Amino acids} \rightarrow \text{Urea}$$
$$\uparrow$$
$$\text{Diet}$$

Since it is possible that amino acid and protein catabolism and protein synthesis are each equally likely to respond to changes in the endocrine environment, it is indeed difficult to relate the activity of a particular hormone specifically to a change in the rate of any one of these processes. A complete kinetic treatment of this problem would probably include such factors as amino acid and protein inhomogeneity, the possibility of multi-order reactions, etc. However, such an analysis presents formidable obstacles in the way of mathematical expression. Thus in order to find solutions which might reasonably describe the state of amino acids and body protein in the living animal, it has been found expedient to make assumptions and approximations which not only yield mathematical expressions of some simplicity, but which are, at the same time, susceptible to experimental test. Whether or not the assumptions so made are consistent with reality can best be determined by comparing the results which are obtained by these means with those which have been achieved by the use of other experimental approaches.

In the analysis which follows it is assumed that insofar as their utilization for synthesis and catabolism is concerned, the amino acids and body proteins constitute two separate homogeneous reservoirs of nitrogen. It is also assumed that the rate at which nitrogen moves out of a particular reservoir is proportional to the amount of nitrogen in that reservoir. In the absence of dietary nitrogen all of the amino acids of the animal are derived from body protein, and the concept of the dynamic state may be expressed in the form of the following equations:

$$\text{(1)} \qquad \mathrm{P} \underset{k_2}{\overset{k_3}{\rightleftarrows}} \mathrm{A} \xrightarrow{k_1} \text{Urinary nitrogen}$$

$$\text{(2)} \qquad dA/dt = k_3P - (k_1 + k_2)A$$

$$\text{(3)} \qquad dP/dt = k_2A - k_3P$$

where P and A are, respectively, the amounts of protein and amino acid nitrogen in the dynamic state; k_1 is the fraction of A which, per unit time, t, is catabolized and excreted; k_2 is the fraction of A which, per unit time, is transformed into P; and k_3 is the fraction of P which, per unit time, is transformed into A.

The general solutions of (2) and (3) are:

$$\text{(4)} \qquad A = me^{r_1 t} + ne^{r_2 t}$$

$$\text{(5)} \qquad P = Me^{r_1 t} + Ne^{r_2 t}$$

where m, n, M, and N are constants of integration, and r_1 and r_2 are, respectively, $\dfrac{-(\beta + k_3) + \sqrt{(\beta + k_3)^2 - 4k_1k_3}}{2}$ and $\dfrac{-(\beta + k_3) - \sqrt{(\beta + k_3)^2 - 4k_1k_3}}{2}$, where $\beta = k_1 + k_2$. The roots, r_1 and r_2, may be simplified by binomial expansion of the radicals. The elimination of quadratic terms yields, approximately, $r_1 = -(\beta + k_3) - \lambda$,

$r_2 = -\lambda$, where $\lambda = \frac{k_1 k_3}{\beta + k_3}$. It will presently be shown that β is approximately 10^{-1} per hour, whereas k_3 and λ are, respectively, approximately 10^{-2} per hour, and 10^{-3} per hour. Considering the relative magnitudes of β, k_3, and λ, it is evident that with increasing time, the exponential in $r_1 t$ decreases at a rate much faster than that in $r_2 t$, so that at the given time, t_i, (4) and (5) become

(6) $$A_i = ne^{-\lambda t_i}$$

(7) $$P_i = Ne^{-\lambda t_i}$$

where t_i is chosen sufficiently large to cause the exponential in $r_1 t_i$ to vanish, and A_i and P_i are the respective values of A and P at time, t_i. At any time, T, greater than t_i, (6) and (7) become

(8) $$A_T = A_i e^{-\lambda T}$$

(9) $$P_T = P_i e^{-\lambda T}$$

where t_i is zero with respect to T, and the subscript, T, denotes the values of A and P at time, T. Thus, after a fast of t_i hours, the loss of amino acid and protein nitrogen is represented as a process of first-order decay. The amount of amino acid and protein nitrogen lost during this decline may be seen to depend not only upon the extent of protein breakdown (k_3), but also upon the fraction of amino acids formed from protein which is catabolized and excreted (approximately equal to $k_1/(\beta + k_3)$). Thus, for example, it might be anticipated that a hormone which accelerates the catabolism of amino acids, but which does not influence protein breakdown, will nevertheless accelerate the loss of body protein. Measurements of protein loss during fasting cannot, therefore, make clear the distinction between the processes of amino acid and protein breakdown.

A quantitative relationship between A and P may now be derived. Rewriting (2) to account for the distinction between t and T yields

(10) $$dA_T/dT = k_3 P_T - \beta A_T$$

Differentiating (8) and substituting the value of dA_T/dT so obtained into (10) gives

(11) $$(\beta - \lambda) A_i = k_3 P_i$$

or since $\beta >> \lambda$, approximately

(12) $$\beta A_i = k_3 P_i.$$

Thus the foregoing treatment of the dynamic state leads to the conclusion that after fasting for t_i hours, the amount of amino acid nitrogen which enters the amino acid reservoir ($k_3 P_i$) is approximately equal to the amount of nitrogen which leaves (βA_i).

The rate of nitrogen excretion is evidently

(13) $$dN/dt = k_1 A$$

where dN/dt is the rate of excretion of the nitrogen produced by the catabolism of A. From the relations expressed in (8) and (13) it may be anticipated that after a preliminary fast of t_i hours, the rate of excretion of urinary nitrogen will begin to decline exponentially at the rate, λ. That this conclusion has a basis in fact is indicated by the common observation that after the first day, the nitrogen output of fasting rats falls so slowly that it is difficult to detect day-to-day differences. With λ of the order of 10^{-3} per hour, the expected daily fall in the rate of nitrogen excretion is approximately 2%. This is a difference which under ordinary conditions is difficult to demonstrate, since the number of animals required to provide data of statistical significance is unusually large.

A method for the measurement of k_1, k_2, and k_3 would, of course, provide the data necessary to relate the activity of a particular hormone specifically to the rate processes of amino acid and protein metabolism. Approximate values of k_1, k_2, and k_3P_4 can be calculated from data obtained by the use of isotopic nitrogen (N^{15}). In addition, the effect of a given hormone on the value of k_3 alone can be estimated by taking into account the changes in the amount of body protein which follow alterations in the endocrine environment. It is therefore possible to describe in quantitative terms the effects of various hormones on each of the rate processes.

When glycine containing an excess of N^{15} is administered to fasting rats, the excretion of isotopic nitrogen as urinary urea is as represented in the graph in Figure 1. Here the fraction of the administered dose which is excreted is plotted against the time. It may be seen that after the first 48 hours, the rate of excretion of N^{15} becomes exceedingly slow. The portion of the curve to the left of the broken line has been shown by Rittenberg[16] to fit the empirical equation

(14) $$x = p(1 - e^{-qt})$$

where x is the fraction of the administered isotopic nitrogen excreted in t hours, and q and p are undetermined constants. The portion of the curve to the right of the broken line may be shown approximately to fit an expression of the same general form as (14). Whereas the value of q calculated from (14) is approximately 10^{-1} per hour, the corresponding value of the rate constant which determines the slope of the flat portion of the curve is approximately 10^{-3} per hour. It is clear that the steep portion of the curve is determined by the rate of catabolism of the administered amino acid, and by the rate of its utilization for the synthesis of protein. The flat portion of the curve must represent the excretion of N^{15} derived from the breakdown of labelled body protein.

Recently Sprinson and Rittenberg[28] have shown that (14) is equivalent to

(15) $$x = \frac{U}{U+S}\left(1 - e^{\frac{-(U+S)t}{A}}\right)$$

where x is as defined above, U is the amount of urinary nitrogen excreted per unit time, t, S is the amount of amino acid nitrogen which, per unit time, is incorporated into protein, and A is the amount of nitrogen in the amino acid reservoir. It is consistent with the assumptions mentioned in the mathematical analysis of the dynamic state that (15) is also equivalent to

(16) $$E = \frac{k_1 a}{\beta}(1 - e^{-\beta t})$$

where k_1 and β are as previously defined, E is the amount of N^{15} excreted in t hours, and a is the dose of administered N^{15}. It should be noted that neither k_3 nor P appear in any of the isotope equations. This omission is justified on the grounds that the rate at which isotopic nitrogen is contributed to the amino acid reservoir by the breakdown of labelled protein is small as compared with the rates of utilization of the administered amino acid.

Equation (16) is used to calculate k_1 and k_2 as follows: In this equation the exponential in $-\beta t$ may be made negligibly small by assigning a large value to t. For example, when $t = 48$ hours, and $\beta = 0.10$ per hour, $e^{-4.8} = 0.008$, which for practical purposes may be considered zero. If the total amount of N^{15} excreted during this time is E_t,

(16) is reduced approximately to

(17) $$E_t = k_1 a/\beta$$

and since a is known, k_1/β is readily calculated. Substitution of (17) into (16) yields

(18) $$E = E_t(1 - e^{-\beta t})$$

which when converted into the logarithmic form is

(19) $$\beta = \frac{2.3}{t} \log \frac{E_t}{E_t - E}$$

Thus by measuring the amounts of isotopic nitrogen excreted at times, t, and t_t, (17) and (18) or (19) may be solved. The values of k_1/β and β provide the information necessary to calculate k_1 and k_2. Equation (15) may be used to calculate the value of A by measuring, in addition to k_1, the rate of nitrogen excretion.

The use of the foregoing method is not restricted to the fasting state. When such a condition is imposed, however, the relationship expressd in (12) may be used to calculate the rate of protein breakdown (k_3P_t). The analysis of the dynamic state during fasting suggests that after one or two days, the amount of nitrogen which leaves the amino acid reservoir (βA_t) is approximately equal to the amount which enters (k_3P_t). If, after such a fast, a small dose of an isotopic amino acid (containing a mg. of N^{15}) is administered, the system may be considered to remain undisturbed, i.e., the amino acid reservoir is not significantly expanded by the amount of nitrogen given.* It may readily be shown, using the relations already expressed, that for the conditions outlined above, the rate of protein breakdown may be calculated from the formula

(20) $$k_3P_t = 100a/C_tT_t$$

where C_t is the isotope concentration (atom per cent excess) of the urinary urea which has been collected for T_t hours, and T_t is the time corresponding to t_t in obtaining Equation (17).

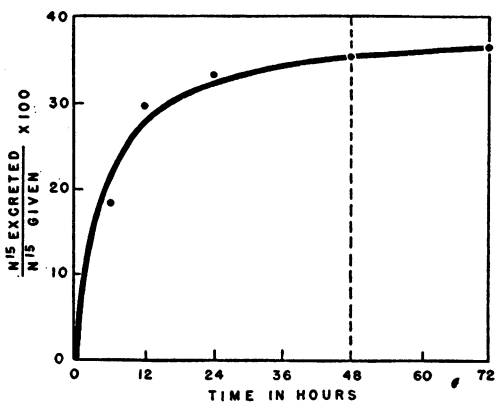

FIG. 1. Urinary excretion of N^{15} after the administration of glycine to normal fasting rats.

It has been a common observation in this laboratory that the nitrogen output of fasting rats falls exceedingly slowly from the second to as long as the fifth day of fasting. For this reason it has been the practice in carrying out the experiments described in this report to administer isotopic nitrogen (as N^{15} glycine) to rats after a preliminary fast of 24 hours. By this procedure the requirement demanded by Equations (6) and (7) is nearly quantitatively fulfilled. The curve in Figure 1 shows that the excretion of N^{15} is an exceedingly rapid process during the first 24 hours. For this reason it is important that collections of urine are started as soon after the admin-

* In the experiments described in this report, the amount of nitrogen in the isotopic glycine administered was equivalent to 2% of the amino acid nitrogen content of the animals.

istration of the labelled amino acid as is practicable. It is evident that accurate calculations can be made only when the differences between E and E_4 are significant. Although it would be desirable to obtain frequent samples of urine, experience in this laboratory indicates that collections of urine 10 and 48 hours after the injection of isotopic glycine are most compatible with practicability. Samples obtained at some reasonable interval after 10 hours already contain amounts of isotopic nitrogen which preclude use in evaluating Equations (18) or (19). Collections made at some time before 10 hours are likely to suffer from inaccuracy.

The values of E_4, E, and k_3P_4 have been calculated from the isotope concentration of the urinary total nitrogen rather than from the isotope concentration of the urea. However, the isotope concentration of the urea, as well as the amount of urea nitrogen, excreted by several animals in each group was measured in all experiments reported. The data obtained show that under whatever conditions the experiments were performed, the isotope concentration of the urea bore an almost constant relationship to that of the total nitrogen. The amount of N^{15} excreted as urea accounted for 90 to 100% of the isotope in the urinary total nitrogen.

Experimental

Male rats of the Sprague-Dawley strain, 13-15 weeks of age, were used. Adrenalectomy and thyro-parathyroidectomy were performed by the usual procedures. Hypophysectomized Sprague-Dawley rats were obtained from a commercial laboratory.* Diabetes was induced by the intravenous injection of alloxan in a dose of 40 mg. per kilogram of body weight. The fasting blood sugar and the extent of weight loss were the criteria used to establish the presence of diabetes.

Adrenalectomized animals were provided with drinking bottles containing 1% sodium chloride solution, and the thyro-parathyroidectomized rats with an aqueous solution containing 1.8% calcium lactate and 0.2% calcium chloride. All animals were fed Purina Fox Chow pellets preliminary to the experimental period.

Experiments were performed on adrenalectomized rats on the 6th postoperative day, on thyro-parathyroidectomized animals on the 30th postoperative day, and on hypophysectomized rats on the 28th postoperative day. Diabetic rats were used 14 days after the administration of alloxan.

The injection of isotopic glycine and the collection of urine were carried out in a dehumidified room maintained at approximately 25° C. All animals were fasted for 24 hours before the administration of N^{15} glycine and, unless otherwise stated, for 48 hours after the injection of glycine. One ml. of a 1% solution of isotopic glycine (32.2 atom per cent excess) was administered per 100 gm. of body weight by intraperitoneal injection. In order to assure the collection of adequate volumes of urine, 0.85% sodium chloride solution was injected intraperitoneally at 2-hour intervals during the first 10 hours of each experimental day. One ml. of this solution was administered per 100 gm. of body weight. Adrenalectomized rats received, in addition to saline, 0.25 mg. of desoxycorticosterone acetate† by subcutaneous injection on the morning of each day of fasting.

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* Hormone Assay Laboratories, Chicago, Illinois.

† Percorten, Ciba. Supplied through the courtesy of Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Inc.

Results

Nitrogen metabolism of fasting normal and adrenalectomized rats. In Tables 1 and 2 are recorded the data obtained when the rate of excretion of N^{15} was measured after the administration of isotopic glycine to fasting normal and adrenalectomized rats. In agreement with the reports of others[5, 8] it was found that the nitrogen output of fasting adrenalectomized rats is less than that of normal animals. It may be seen that this defect is

TABLE 1

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF NORMAL RATS

*Weight**	E_{10}	E_{48}	C_{48}	*Urinary* N†	k_1	k_2	k_3P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
300	0.410	0.669	0.223	2.08	3.48	6.01	5.70
340	0.412	0.685	0.199	2.08	3.04	6.16	6.40
330	0.379	0.613	0.195	2.00	2.94	6.68	6.52
310	0.352	0.610	0.209	1.97	2.77	5.83	6.09
316	0.396	0.654	0.199	2.16	3.11	6.19	6.40
328	0.500	0.745	0.200	2.37	4.13	6.97	6.36
Mean				**2.11**	**3.25**	**6.31**	**6.25**
S. E.‡				0.06	0.20	0.18	0.12

* Dose, $a = \frac{\text{weight (to nearest dekagram)} \times 0.61}{100}$

‡ Standard error $= \sqrt{\frac{1}{n(n-1)} \sum_{i=1}^{n} (x_i - \bar{x})^2}$

† Calculated from 48-hour nitrogen output.

not related to any detectable abnormality in the catabolism of amino acids. However, the amount of amino acid nitrogen formed from body protein is significantly depressed by adrenalectomy ($P < 1\%$), while at the same time the fraction of amino acid nitrogen used for the synthesis of protein (k_2) is greater than normal ($P < 2\%$).

Comparison of the body compositions of normal and adrenalectomized rats has shown that the protein content of the latter is proportionately greater than normal.[27] The slower rate of protein breakdown associated with a deficiency of adrenal hormones must therefore be ascribed to a decrease in the magnitude of the rate constant, k_3, denoting that the process

of conversion of body protein to amino acids is inhibited. This effect is sufficient almost quantitatively to account for the fall in the output of urinary nitrogen. To a lesser extent the acceleration of protein synthesis is also responsible for the lower rate of excretion of nitrogen.

Other investigators have reported that adrenalectomy may be responsible for a defect in the deamination of amino acids,[20, 21] or that the adrenal steroids specifically influence the rate of protein breakdown.[4] The stimulation of the process of protein formation, as observed in the present study,

TABLE 2

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF ADRENALECTOMIZED RATS

Weight	E_{10}	E_{18}	C_{18}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
315	0.452	0.642	0.243	1.78	4.02	8.02	5.35
320	0.353	0.549	0.211	1.74	2.89	7.41	6.15
379	0.541	0.782	0.232	1.85	3.96	7.79	5.48
338	0.431	0.695	0.237	1.81	3.24	6.41	5.36
367	0.431	0.676	0.216	1.78	3.07	7.07	5.89
350	0.490	0.755	0.247	1.82	3.70	6.76	5.15
Mean				**1.80**	**3.48**	**7.24**	**5.56**
S. E.				0.02	0.20	0.25	0.15

is consistent with the observation of White, *et al.*,[26] who administered N^{15} glycine to fasting normal and adrenalectomized mice and found that the net uptake of N^{15} into the proteins of the internal organs, serum proteins, lymphatic tissue, and muscle was increased by adrenalectomy.

Insofar as the metabolism of nitrogen is concerned, removal of the adrenal glands appears chiefly to influence protein metabolism; the rate of incorporation of amino acids into protein increases, and the rate of protein breakdown falls.

Effects of cold on the nitrogen metabolism of fasting normal rats. It is well known that adrenal hypertrophy follows exposure to cold.[24] In order to study the changes in the metabolism of nitrogen which might be induced by overactivity of the adrenal glands, normal rats, each occupying a separate cage, were placed in a cold room at 4° C. Food and water were provided for 9 days. On the 10th day, while still in the cold room, the

animals were subjected to a fast of 24 hours, after which N^{15} glycine was administered. Fasting was continued, and the rate of excretion of N^{15} was measured. Of the 12 rats subjected to this procedure, 2 survived for more than 36 hours after the injection of isotopic glycine. The data obtained in this experiment are recorded in Table 3.

These results indicate that the catabolism of amino acids and body protein of normal fasting animals is considerably accelerated by severe cold, while the utilization of amino acids for protein synthesis declines. Consequent to these changes, the output of urinary nitrogen is strikingly increased.

TABLE 3

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF NORMAL RATS SUBJECTED TO COLD

Weight	E_{10}	E_{36}	C_{36}	*Urinary* N*	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
300	0.797	1.112	0.166	6.20	7.66	5.00	10.2
312	0.915	1.290	0.165	6.95	8.48	3.92	10.3

* Calculated from 36-hour nitrogen output.

At autopsy of 9 of the 12 animals the average weight of the adrenal glands was 73.6 ± 2.1 mg., as compared with 37.3 ± 3.5 mg. for 9 controls. The adrenal weights of the 2 surviving rats were 81.3 mg. and 65.3 mg. Involution of the thymus, spleen, and lymph nodes of the animals exposed to cold was notable.

Effects of thyro-parathyroidectomy. Data on the effects of thyro-parathyroidectomy on the nitrogen metabolism of fasting rats are summarized in Table 4. Comparison of these observations with those in Table 1 indicates that the catabolism of amino acids is significantly accelerated in the absence of the thyroid gland ($P < 1\%$), and that the rate of protein breakdown is less than normal ($P < 1\%$). In order to avoid the assumption that the body compositions of normal and thyro-parathyroidectomized rats are identical, the rates of protein breakdown are better compared when they are calculated for the whole animal, rather than per 100 gm. of body weight. When this is done it is found that the hypothyroid rats converted an average of 13.5 mg. of protein nitrogen to amino acid nitrogen, each hour, as compared with 20.1 mg. for the normal group, a decrease of 33%.

Addis, *et al.*,[1] who compared the protein contents of normal and thyroidectomized rats found that the latter contained only 17% less protein than normal. Thus it follows that in the absence of the thyroid hormone, the mechanisms which bring about the breakdown of body protein are operating at a lower level than normal.

Although the rate of protein breakdown is diminished by thyroparathyroidectomy, the concomitant increase of amino acid catabolism appears to be sufficient to cause a slight increase over normal in the rate

TABLE 4

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF THYRO-PARATHYROIDECTOMIZED RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
264	0.562	0.801	0.229	2.76	6.10	5.99	5.54
272	0.565	0.876	0.300	2.24	5.50	4.85	4.23
285	0.705	0.955	0.312	2.24	7.24	6.15	4.06
275	0.554	0.820	0.261	2.38	5.40	5.85	4.86
294	0.492	0.780	0.277	2.00	4.39	5.57	4.58
270	0.571	0.797	0.222	2.77	6.10	6.49	5.70
Mean				**2.40**	**5.79**	**5.82**	**4.83**
S. E.				0.13	0.39	0.23	0.23

of excretion of urinary nitrogen ($P < 5\%$). That the urea output of hypothyroid rats is not less than normal has also been reported by Persike,[15] and confirmed by Rupp, *et al.*[17] The latter also showed that the administration of small doses of thyroxin to thyroidectomized rats brought about the retention of nitrogen.

It will be noted that the rate of incorporation of amino acids into protein is not significantly different from normal.

Effects of adrenalectomy on the nitrogen metabolism of thyro-parathyroidectomized rats. The acceleration of amino acid catabolism exhibited by normal animals subjected to cold suggests that hyperactivity of the adrenal cortex may be responsible for the effects observed. Since thyro-parathyroidectomized animals also catabolize amino acids at a high level, the influence of adrenalectomy on the nitrogen metabolism of these animals was investigated. The data obtained from this study are shown in

Table 5. Removal of the adrenal glands did not bring about a decline in the value of k_1 in thyro-parathyroidectomized rats. It is also evident that adrenalectomy appears to be responsible for stimulation of protein synthesizing reactions whether performed on normal or hypothyroid rats. ($P < 1\%$).

Although amino acid catabolism is accelerated, the nitrogen output is significantly lower in the absence of both the adrenal and thyroid glands than after extirpation of either gland alone. The decrease in the excretion

TABLE 5

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF THYRO-PARATHYROIDECTOMIZED RATS AFTER ADRENALECTOMY

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
285	0.605	0.806	0.438	1.34	6.35	7.55	2.91
292	0.693	0.901	0.402	1.67	7.50	7.20	3.16
279	0.652	0.863	0.474	1.36	7.15	6.95	2.68
279	0.638	0.855	0.401	1.59	6.85	6.85	3.16
280	0.562	0.752	0.583	0.96	6.07	7.63	2.18
297	0.615	0.862	0.385	1.57	5.89	6.61	3.29
Mean				**1.41**	**6.63**	**7.13**	**2.90**
S. E.				0.11	0.26	0.16	0.17

of nitrogen may readily be accounted for by the conspicuous fall in the rate of conversion of body protein to amino acids.

Effects of hypophysectomy and of the administration of adrenocorticotrophic hormone to hypophysectomized rats. Evidence has been presented above to show that a high rate of amino acid catabolism is related to adrenal hyperactivity. The adrenal steroids also seem to influence the metabolism of protein by increasing the rate of protein breakdown and decreasing the utilization of amino acids for protein synthesis. In order to obtain information relating the adrenal glands more specifically to these processes, a study was made of the effects of adrenocorticotrophic hormone (ACTH) on the nitrogen metabolism of fasting hypophysectomized rats. Two-tenths (0.2) ml. of a 0.5% solution of ACTH* in isotonic saline was administered by intraperitoneal injection to hypophysectomized rats 4

* Kindly made available by Armour and Co., Chicago, Illinois.

times daily for 7 days. Each rat thus received 4 mg. of the hormone each day. On the 6th day of treatment, isotopic glycine was administered, and the rate of excretion of N^{15} was measured. The results obtained with the

TABLE 6

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF HYPOPHYSECTOMIZED RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_2P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
159	0.336	0.446	0.321	1.82	6.40	7.60	3.96
159	0.332	0.437	0.297	1.92	6.35	7.85	3.83
163	0.310	0.446	0.258	2.20	5.45	6.45	4.93
164	0.326	0.470	0.258	2.31	5.73	6.07	4.93
156	0.274	0.386	0.293	1.76	4.92	7.48	4.34
158	0.291	0.433	0.256	2.23	4.95	6.15	4.97
Mean				**2.04**	**5.63**	**6.93**	**4.50**
S. E.				0.10	0.27	0.33	0.21

TABLE 7

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF HYPOPHYSECTOMIZED RATS RECEIVING ACTH

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_2P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
153	0.328	0.490	0.233	2.86	5.93	5.17	5.44
151	0.329	0.517	0.225	3.18	5.72	4.38	5.65
148	0.394	0.571	0.212	3.80	7.34	4.36	6.00
152	0.325	0.473	0.221	2.94	6.02	5.58	5.75
142	0.292	0.449	0.204	3.23	5.55	4.95	6.22
132	0.269	0.422	0.247	2.71	5.42	4.68	5.14
Mean				**3.12**	**6.00**	**4.85**	**5.70**
S. E.				0.05	0.28	0.19	0.05

untreated and treated hypophysectomized rats are given in Tables 6 and 7. These data show that the catabolism of amino acids in fasting hypophysectomized rats is significantly greater than normal ($P < 1\%$), and

that this process was not measurably influenced by ACTH. On the other hand, the rate of protein catabolism, which is depressed in the absence of the pituitary gland, is notably increased ($P < 1\%$), while the value of k_2 is significantly diminished by the treatment ($P < 1\%$). It is interesting that the value of k_2 tends to be greater than normal in hypophysectomized rats ($P < 5\%$).

Under the conditions employed in these experiments, the nitrogen output of hypophysectomized rats does not appear to be different from normal.

TABLE 8

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF HYPOPHYSECTOMIZED RATS RECEIVING GROWTH HORMONE

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_2P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
172	0.222	0.361	0.301	1.45	3.34	6.21	4.22
154	0.251	0.346	0.349	1.34	4.91	7.99	3.64
163	0.281	0.368	0.337	1.39	5.45	8.95	3.76
158	0.181	0.268	0.276	1.28	3.02	8.18	4.60
180	0.197	0.312	0.254	1.42	2.95	7.03	5.01
158	0.151	0.253	0.247	1.30	2.36	6.72	5.14
Mean				**1.36**	**3.67**	**7.51**	**4.40**
S. E.				0.03	0.44	0.42	0.26

The explanation of this observation in terms of the rates of nitrogen metabolism is similar to that offered in the case of the thyro-parathyroidectomized animals, i.e., the acceleration of amino acid catabolism compensates for the fall in protein catabolism. The rise in the output of nitrogen following treatment with ACTH appears to be a consequence of a more rapid rate of protein breakdown and of a decline in the rate of incorporation of amino acids into protein.

Effects of growth hormone on the nitrogen metabolism of hypophysectomized rats. It is well known that the administration of anterior pituitary extracts or growth hormone either to normal or hypophysectomized rats is followed by a decrease in the output of urinary nitrogen. In order to relate this effect to specific processes involved in the metabolism of

* Supplied through the courtesy of Dr. A. E. Wilhelmi, Department of Physiological Chemistry, Yale University School of Medicine. The growth hormone was estimated to be 95% homogeneous by electrophoresis.

amino acids and body protein, growth hormone was administered to hypophysectomized rats, and a study made of the rate of excretion of N^{15} after the injection of isotopic glycine. Two-tenths ml. of a 0.5% solution of crystalline growth hormone* in isotonic saline was administered by intraperitoneal injection to hypophysectomized rats 3 times daily for 3 days. Each rat thus received 3 mg. of the hormone each day. On the second day of treatment isotopic glycine was injected, and the rate of excretion of N^{15} was measured in the usual manner.

TABLE 9

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF THYRO-PARATHYROIDECTOMIZED RATS RECEIVING GROWTH HORMONE

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
255	0.392	0.560	0.269	1.77	4.25	7.75	4.93
260	0.188	0.339	0.217	1.31	1.73	6.35	6.12
240	0.392	0.549	0.287	1.73	4.69	7.81	3.49
250	0.359	0.506	0.265	1.66	4.18	8.42	5.00
260	0.342	0.522	0.247	1.76	3.50	7.10	5.36
260	0.363	0.561	0.289	1.62	3.69	6.71	4.59
Mean				**1.64**	**3.67**	**7.36**	**4.91**
S. E.				0.07	0.42	0.31	0.36

Comparison of the data in Tables 6 and 8 show that growth hormone reduced the value of k_1 to a normal level ($P < 1\%$). A similar effect of growth hormone on the catabolism of amino acids has been reported by Russell and Cappiello,[19] who observed that the accumulation of urea in the blood of nephrectomized rats receiving a mixture of amino acids is depressed by growth hormone.

It will be noted that growth hormone did not appear to influence the rate of protein breakdown or the rate of utilization of amino acids for the synthesis of protein.

Effect of growth hormone on the nitrogen metabolism of thyro-parathyroidectomized rats. The data of Table 5 would seem to exclude the possibility that the acceleration of amino acid catabolism in hypothyroidism is due to hyperactivity of the adrenal cortex. Thus it may be that either the synthesis or secretion of growth hormone is depressed in the absence of the

thyroid gland, or that the peripheral activity of growth hormone depends upon thyroxin for its expression. To distinguish between these mechanisms, growth hormone* was administered to thyro-parathyroidectomized rats. The dosage used, and the schedule of injections were as described earlier. The data of Table 9 show that growth hormone does indeed repress the catabolism of amino acids in the absence of the thyroid gland ($P < 1\%$). On the other hand, the data indicate that the treatment did not evoke a change in the rate of protein breakdown. It should be noted, however, that the rate of incorporation of amino acids into protein was significantly increased by the hormone ($P < 1\%$).

It has already been shown that removal of the adrenal glands brings about a profound fall in the rate of protein breakdown of thyro-parathyroidectomized rats. The failure of growth hormone to produce a similar effect in hypothyroid animals is therefore consistent with the hypothesis that the effects of growth hormone on the metabolism of protein are not due to a direct repression of the activity of the adrenal cortex or to the peripheral inhibition of the protein catabolic effects of the adrenal steroids.

Effect of growth hormone on the nitrogen metabolism of adrenalectomized rats. The experiments described earlier in this report show that although growth hormone inhibits the catabolism of amino acids in the absence of the hypophysis or thyroid gland, no significant changes in the metabolism of protein were detected in the absence of the pituitary gland. On the other hand, it has been shown that growth hormone does accelerate the synthesis of protein in thyro-parathyroidectomized animals. Since the activity of the adrenal cortex is low in hypophysectomized animals, the possibility must be considered that growth hormone does not influence protein synthesis in the absence of the adrenal cortex. In order to test this hypothesis, growth hormone, in the dosage already mentioned, was administered to adrenalectomized rats. The data in Table 9 show that growth hormone did not influence protein metabolism in these animals. On the other hand it may be seen that a significant fall in the rate of catabolism of amino acids occurred ($P < 1\%$), suggesting that the effect of growth hormone on amino acid catabolism is independent of the adrenal cortex.

It has previously been reported that the nitrogen output of adrenalectomized animals is lowered by the administration of anterior pituitary extracts.[9] It may be noted in Table 10 that the nitrogen output of growth hormone-treated adrenalectomized rats is also less than that observed in

* Supplied by Armour and Co. through the kindness of Dr. Irby Bunding.

untreated controls ($P < 1\%$). This may be accounted for entirely in the depression of amino acid catabolism resulting from the treatment.

The data in Tables 9 and 10 indicate that the synthesis of protein is accelerated by growth hormone only in the presence of the adrenal cortex. It has already been suggested that growth hormone does not influence either amino acid or protein metabolism by repressing the activity of the adrenal cortex. Nevertheless, it would appear that adrenal steroids must be present in order that growth hormone may increase the rate of utilization of

TABLE 10

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF ADRENALECTOMIZED RATS RECEIVING GROWTH HORMONE

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
310	0.296	0.490	0.198	1.70	2.40	6.86	6.56
315	0.358	0.522	0.238	1.48	3.10	8.50	5.46
285	0.286	0.482	0.226	1.59	2.46	6.54	5.74
270	0.258	0.462	0.243	1.50	2.30	5.87	5.35
293	0.275	0.481	0.267	1.31	2.30	6.17	4.86
Mean				**1.52**	**2.51**	**6.79**	**5.59**
S. E.				0.06	0.15	0.46	0.28

amino acids for the synthesis of protein. It has further been suggested that growth hormone does not influence protein metabolism by the peripheral inhibition of the protein catabolic effect of the adrenal steroids. It would therefore seem clear that growth hormone must influence protein synthesis by peripheral inhibition of that effect of the adrenal hormones which specifically inhibits protein synthesis.

Nitrogen metabolism in alloxan-diabetes. Previous investigators have suggested that the nitrogen-sparing action of anterior pituitary extracts is exerted through effects on the islets of the pancreas.[14] Since insulin has been shown to inhibit the accumulation of amino nitrogen in the blood of eviscerated rats,[7, 18] it might be anticipated that in diabetes the rate of incorporation of amino acids into protein is slower than normal. In Table 11 are recorded the data obtained when the rate of excretion of N^{15} was studied after the administration of N^{15} glycine to alloxan-diabetic rats. These data show that in diabetes the catabolism of amino acids and body

protein is significantly accelerated. Attention should also be directed at the value of the rate constant, k_2, which is lower than any value previously observed. It will be noted that the depression of protein synthesis and the acceleration of protein breakdown are similar to those effects resulting from the administration of ACTH to hypophysectomized rats. Thus it is difficult in this experiment to distinguish between effects which on the one hand might be due to the absence of insulin, and on the other, to the presence of a high concentration of adrenal steroids. For this reason N^{15} glycine was

TABLE 11

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF ALLOXAN-DIABETIC RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
190	0.507	0.824	0.139	6.50	6.80	2.74	9.14
195	0.586	0.953	0.145	7.03	7.43	2.09	8.95
233	0.516	0.874	0.156	5.11	5.56	3.38	8.34
252	0.581	0.900	0.167	4.55	6.10	4.20	7.77
Mean				**5.80**	**6.47**	**3.10**	**8.55**
S. E.				0.58	0.41	0.45	0.31

administered to diabetic animals from which the adrenal glands had been extirpated. It may be seen in Table 12 that after removal of the adrenal glands from diabetic animals, the rapid breakdown of amino acids and body protein ceases, and, indeed, is reduced to the same levels as previously described for adrenalectomized-non-diabetic animals. However, the rate of utilization of amino acids for the synthesis of protein remains depressed, indicating that it is the absence of insulin, rather than adrenal hyperactivity, which is directly responsible for the low rate of protein synthesis. This suggests that the adrenal steroids inhibit the utilization of amino acids in anabolic reactions by bringing about the inactivation of insulin, or by otherwise nullifying the peripheral effects of insulin. This view is supported by Ingle's[10] observation that in diabetic rats, tolerance to insulin is markedly increased by the administration of 17-hydroxy-11-dehydrocorticosterone (Kendall's Compound E).

Influence of Compound E on the nitrogen metabolism of adrenalectomized and adrenalectomized-thyro-parathyroidectomized rats. Data have been

presented which indicate that both the adrenal cortex and the thyroid gland influence the rate of conversion of body protein to amino acids. It seemed of interest to determine to what degree the hormones of the adrenal cortex influence the breakdown of body protein in the absence of the thyroid gland. For this purpose a suspension of Compound E in peanut oil was prepared in a concentration of 8 mg. per ml. Two mg. of Compound E (0.25 ml. of the suspension) were injected subcutaneously into adrenalectomized and adrenalectomized-thyro-parathyroidectomized rats two hours before the

TABLE 12

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF ALLOXAN-DIABETIC RATS AFTER ADRENALECTOMY

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
240	0.380	0.689	0.266	2.84	3.77	4.23	4.78
240	0.378	0.698	0.235	2.63	3.72	4.08	5.52
210	0.176	0.500	0.237	2.09	1.67	2.61	5.48
240	0.341	0.656	0.246	2.32	3.29	4.06	5.16
Mean				**2.47**	**3.11**	**3.75**	**5.24**
S. E.				0.17	0.49	0.38	0.17

administration of isotopic glycine, and again 10 hours after the injection of isotope. These doses were again administered on the following day. The data in Tables 13 and 14 show that Compound E accelerates the catabolism of amino acids in adrenalectomized rats ($P < 1\%$), and also significantly raises the level of amino acid catabolism in adrenalectomized-thyro-parathyroidectomized rats ($P < 1\%$). It is evident that the treatment also caused a marked increase in the rate of protein breakdown in the adrenalectomized animals ($P < 1\%$), and almost doubled the rate of protein breakdown in the adrenalectomized-thyro-parathyroidectomized rats. Although the rate of protein catabolism induced by the treatment tends to be less marked in the doubly operated group, the hormone tended to increase the rate of protein catabolism in these animals above that observed in untreated thyro-parathyroidectomized rats ($P < 5\%$). It will also be noted that in both groups of animals the values of k_2 are significantly less than in the untreated controls ($P < 1\%$, in each instance). It is of considerable

TABLE 13

EFFECT OF COMPOUND E ON THE NITROGEN METABOLISM OF ADRENALECTOMIZED RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
288	0.548	0.819	0.168	3.52	5.12	5.92	7.56
297	0.554	0.820	0.165	3.48	5.04	6.21	7.70
340	0.678	0.975	0.212	2.82	5.59	6.31	6.00
350	0.716	1.172	0.172	4.06	5.17	4.25	7.38
302	0.651	0.990	0.194	3.52	5.79	4.91	6.55
338	0.502	0.896	0.212	2.61	3.56	4.66	6.00
Mean				**3.34**	**5.05**	**5.38**	**6.87**
S. E.				0.22	0.32	0.36	0.32

TABLE 14

EFFECT OF COMPOUND E ON THE NITROGEN METABOLISM OF ADRENALECTOMIZED-THYRO-PARATHYROIDECTOMIZED RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
265	0.835	1.11	0.249	3.50	9.39	4.56	5.10
240	0.726	1.05	0.213	4.29	8.40	3.29	5.96
240	0.721	1.01	0.228	3.84	8.62	3.88	5.58
245	0.710	1.08	0.215	4.26	7.59	3.16	5.91
Mean				**3.97**	**8.50**	**3.72**	**5.64**
S. E.				0.19	0.31	0.33	0.20

interest that this effect is significantly more pronounced in the hypothyroid animals ($P < 1\%$).

Discussion

The results of the experiments described in this report provide additional evidence to support the view that the metabolism of amino acids and body proteins is significantly influenced by secretions of the endocrine glands.

Endocrine regulation of amino acid catabolism. The data which have

been presented show that in fasting rats the catabolism of amino acids is accelerated by adrenal cortical steroids and is depressed by the pituitary growth hormone. Since the depression of the catabolism of amino acids by growth hormone is observed in adrenalectomized animals, it may be concluded that in the intact animal this effect is not produced by repression of the activity of the adrenal cortex.

The acceleration of amino acid breakdown which follows thyro-parathyroidectomy would also seem to be independent of the function of the adrenal cortex. This suggests that in hypothyroidism the rapidity of breakdown of amino acids is due to a release of the catabolizing system from the inhibitory influence of growth hormone. The observation that in hypophysectomized rats the catabolism of amino acids proceeds at approximately the same rate as in thyro-parathyroidectomized animals is consistent with this view. The marked effect of Compound E in accelerating the catabolism of amino acids of adrenalectomized-thyro-parathyroidectomized rats therefore favors the concept that in the intact animal the cortical steroids do not exert a direct effect on the activity of growth hormone. It would follow from the above that ACTH should accelerate the catabolism of amino acids when administered to hypophysectomized rats. This was not observed in the experiments reported here. However, it should be pointed out that the effect of the adrenal cortex on the catabolism of amino acids does not appear to become manifest until the level of cortical activity is greater than normal. It is therefore possible that under the conditions of the experiment referred to above, the required activity of the adrenal cortex was not obtained.

The question might well be raised as to the nature of the mechanism which, in the absence of the thyroid gland, leads to a decline in the activity or amount of growth hormone. The data in Table 9 would suggest that the peripheral activity of growth hormone does not depend upon the presence of thyroxin, for a significant depression of amino acid breakdown occurred when growth hormone was administered to thyro-parathyroidectomized rats. This indicates that the secretion or synthesis of growth hormone is strongly influenced by the activity of the thyroid gland. The fact that growth hormone depressed the level of amino acid catabolism in hypophysectomized animals is also consistent with this view. It has been reported,[6] however, that the treatment of hypophysectomized rats with anterior pituitary extracts and thyroxin results in a more rapid rate of growth than when either of these preparations is administered separately, suggesting that thyroxin potentiates the activity of growth hormone.

Whether or not the growth-promoting effects achieved by the combination of these hormones is entirely related to the repression of amino acid catabolism cannot be stated at this time.

The data obtained from diabetic animals would seem to exclude the possibility that the inhibition of amino acid catabolism induced by growth hormone is exerted by an effect on the pancreas. Although the catabolism of amino acids is considerably accelerated in fasting diabetic animals, the data in Table 12 show that after adrenalectomy, the level of amino acid catabolism is restored to normal. This interpretation is consistent with the observations of Russell and Cappiello,[19] who showed that in nephrectomized rats, insulin does not reproduce the effect of growth hormone in depressing the rate of formation of urea from administered amino acids. It has long been suggested[18] that the alleviation of diabetes induced by adrenalectomy might in part be explained as being due to a depression of amino acid and protein catabolism, with a concomitant fall in the rate of gluconeogenesis. The results of the experiments reported in the present communication provide additional evidence to support this view.

Experiments (unpublished) recently performed in this laboratory with isotopic nitrogen show that in fasting rats the conversion of ammonia to urea is far too rapid to offer the possibility that the hormonal regulation of amino acid catabolism is concerned with the operation of the Krebs-Henseleit cycle. Moreover, when ammonium citrate, labelled with N^{15}, was administered to fasting hypophysectomized rats, the rate of formation of isotopic urea was not lowered by growth hormone. Thus it may be that in the fasting intact animal the rate of urea formation is determined by the rate of deamination of amino acids. It is on this process that the influence of growth hormone and the adrenal steroids would seem to be exerted.

It follows from the foregoing considerations that during fasting the intact animal possesses two independent means for the hormonal regulation of amino acid catabolism. During growth the retention of nitrogen resulting from a diminution in the catabolism of amino acids might conceivably be brought about either by the secretion of "extra" growth hormone, or by the suppression of adrenal cortical activity. The first of these two possible mechanisms is more consistent with the evidence presented in this report, for in the adult animal the catabolism of amino acids does not appear to be depressed by adrenalectomy. This may also be interpreted to indicate that in the absence of stress the adrenal cortex has but little quantitative influence on the catabolism of amino acids.

Hormonal regulation of protein breakdown. The experiments presented

in this communication indicate that the rate of conversion of body protein to amino acids is increased by the adrenal cortical steroids (e.g. Compound E), and is depressed in the absence of the adrenal, thyroid, or pituitary glands. Removal of both adrenal and thyroid glands brings a decline in the rate of protein breakdown which is greater than when either of these organs is removed separately. This suggests either that the thyroid and adrenal hormones operate in synergy to mobilize amino acids from body protein, or that the proteins susceptible to Compound E-induced breakdown are qualitatively different from those whose destruction is induced by the activity of the thyroid gland.

White and Dougherty,[25] who studied the effects of endocrine secretions on the loss of protein during fasting, have suggested that the mobilization of nitrogen from the carcasses of fasting mice is under thyroid control, whereas the breakdown of the proteins of lymphatic tissue is directly regulated by the adrenal hormones. They have also suggested that by indirect action, i.e., via the thyroid gland, the adrenal steroids may also bring about the breakdown of carcass protein. They suggest further that the adrenal and thyroid hormones may also exert a synergistic effect on protein catabolism.

It will be recalled (Tables 13 and 14) that the administration of Compound E to adrenalectomized-thyro-parathyroidectomized rats causes a striking acceleration of protein breakdown. It can be calculated from the amount of nitrogen excreted by these animals that in two days, protein-containing tissue* equivalent to 8% of the body weight must have been destroyed. This would seem to be somewhat in excess of that which might be estimated to arise from non-carcass sources and suggests that the proteins of the rat carcass are indeed sensitive to the catabolic effects of the cortical hormones whether or not the thyroid gland is present.

An estimation of the amount of body protein which is readily available for conversion to amino acids provides additional information relative to the hormonal regulation of protein catabolism.

The amount of body protein which is susceptible to breakdown during fasting is defined in Equation (11). In the logarithmic form this expression is

$$\lambda = \frac{2.3}{T} \log \frac{P_i}{P} \qquad (21)\dagger$$

* Assumed to contain 15% protein.

† Although as derived, the use of this equation is limited to the period of starvation following a preliminary fast of one or two days, theoretical considerations indicate that little error is introduced into the calculations which follow when this condition is not met.

Since P is the amount of protein remaining in the animal after a fast of T days, (21) may also be written

$$\lambda = \frac{2.3}{T} \log \frac{P_i}{P_i - y} \tag{22}$$

where y is the amount of protein lost during the fast. Rewriting (22) as follows

$$\frac{k_1 k_3}{\beta + k_3} = \frac{2.3}{T} \log \frac{k_3 P_i / k_3}{k_3 P_i / k_3 - y} \tag{23}$$

yields an equation which may be solved for k_3 if the values of k_1, k_2, $k_3 P_i$, and y are known.

Addis, Poo, and Lew[2] have reported that in 7 days the average amount of protein nitrogen lost by normal fasting rats is 296 mg. per 100 gm. of body weight. With y thus equal to 296 mg., $k_1 = 0.0325$ per hour, $k_2 = 0.0631$ per hour, and $k_3 Pi = 6.25$ mg. per 100 gm. per hour, (Table 1); the value of k_3 obtained by evaluation of these data in (23) is 4.9×10^{-3} per hour. The values of λ and Pi are thus 1.6×10^{-3} per hour, and 1.28 gm. per 100 gm. respectively. The total amount of body protein in the rats employed by Addis, *et al.*, was equivalent to 2.74 gm. of nitrogen per 100 gm. of body weight. Thus 1.28/2.74, or approximately 47% of the total protein of the fasting rat is estimated to be susceptible to breakdown at the rate, k_3. The remaining 53%, it must be assumed, yields its amino acids at a much slower rate. On this basis the degree of lability of a tissue, measured in terms of its protein content, would be proportional to the amount of protein in that tissue in the more labile state. Thus the difference in apparent labilities of the total protein contents of the various tissues and organs would largely disappear. For example, from the data of White and Dougherty, it can be calculated that if 50% of the carcass protein of the mouse is susceptible to rapid breakdown, 25% of this labile protein would be lost during a fast of 48 hours. Assuming that 100% of the protein of lymphatic tissues is similarly labile, 31% would be lost from these animals in the same period. Thus the point may be made that measurements of protein loss during fasting would not seem to provide evidence sufficiently definitive to show that the protein content of an individual organ is specifically influenced by a hormone. It is more consistent with present evidence that the effect of hormones which induce the acceleration of protein catabolism express their activities in a non-specific manner. It may also be tentatively assumed that the thyroid hormone potentiates the protein-catabolic effects of the adrenal cortical steroids.

Hormonal regulation of protein synthesis. The results of the experiments presented in this report show that the biochemical reactions leading to the formation of protein are accelerated in adrenalectomized and hypo-

physectomized rats, and, when the adrenal glands are intact, in animals treated with growth hormone. On the other hand, the synthesis of body protein is diminished in those animals in which the adrenal cortex is hyperactive, or, when the adrenal glands are removed, in diabetic animals. It is clear from these observations and from the work of others that insulin is required in order for the synthesis of protein to take place at normal or accelerated rates. It would therefore appear that the acceleration of the conversion of amino acids to protein in adrenalectomized and hypophysectomized rats is to be attributed to an increase in the effective concentration of insulin, a view which is consistent with the fact that adrenalectomized and hypophysectomized animals are hypersensitive to insulin.

The fact that ACTH brings about a depression of protein formation in hypophysectomized rats indicates that insofar as the metabolism of protein is concerned, insulin, rather than growth hormone, is the target at which the activity of the adrenal cortical hormones is directed. Recent data (unpublished) obtained in this laboratory show that in hypophysectomized rats the depression of protein synthesis resulting from the administration of ACTH is partially reversed by growth hormone. On the other hand it was found that growth hormone did not inhibit the protein-catabolic effects of ACTH. Earlier in this report it was pointed out that growth hormone does not repress the breakdown of protein in hypophysectomized and in thyroparathyroidectomized rats. Similar effects (unpublished) have recently been observed when growth hormone was administered to normal animals. Although the synthesis of protein was increased by the treatment, no depression of protein catabolism was observed. The available evidence is therefore consistent with the hypothesis that growth hormone accelerates the synthesis of protein by inhibiting the adrenal-induced inactivation of insulin, or by inhibiting those effects of the adrenal cortical steroids which oppose the peripheral effects of insulin.

The data in Table 14 show that in hypothyroidism, Compound E induces a greater diminution in the rate of conversion of amino acids to protein than when the thyroid gland is present. This may be interpreted as indicating that in the absence of thyroxin the anabolic effects of insulin are more readily influenced by adrenal cortical activity, suggesting that the thyroid gland is concerned with the regulation of the secretion or synthesis of growth hormone. It may be recalled that a similar suggestion was offered to explain the effects of hypothyroidism on the catabolism of amino acids. Although it might be anticipated that the rate of conversion of amino acids

to protein would be less than normal in hypothyroidism, the data in Table 4 do not support this expectation. However, these data would be consistent with the foregoing hypothesis if it can be demonstrated that the activity of the adrenal cortex is depressed in the absence of the thyroid gland.

The biochemical reactions which are so modified by insulin as to bring about a faster rate of utilization of amino acids for the synthesis of protein have not been identified. Charalampous and Hegsted[3] have recently reported that the acetylation of p-aminobenzoic acid is depressed in diabetic rats and suggest that a primary deficiency of adenosine triphosphate (ATP) is responsible for this effect. This hypothesis is supported by the observation of Kaplan and Greenberg[11] that an increase in the uptake of P^{32} into liver ATP can be demonstrated after the administration of insulin. If the formation of peptide bonds occurs in a manner analogous to the acetylation of amines, other evidence[12] would seem to indicate that the depression of protein synthesis observed in diabetic rats may also be assumed to be due to a deficiency in ATP. In any event the relationship of insulin to energy-yielding processes would seem to support the hypothesis that in the living animal, the rate of utilization of amino acids for the synthesis of protein is determined by the rates of those reactions which supply the energy required for the formation of peptide bonds. It would seem to be consistent with this that the major effects of those hormones which influence the synthesis of protein are exerted on those reactions which yield the energy required for the synthesis of peptide bonds.

It is evident that the rate of protein formation can be limited not only by the rate at which energy is provided, but also by such important and variable elements as the availability of amino acids, the concentrations of energy-yielding substrates, the amounts and activities of the enzymes which direct syntheses, etc. In addition, there are factors which must determine the qualitative properties of protein structures, i.e., which direct the synthesis of a particular "kind" of protein. The results of the present study do not at all exclude the possibility that hormones may indeed specifically influence any one or all of these factors. They do demonstrate, however, that the endocrine secretions strongly influence the rate of utilization of amino acids for the formation of protein through their influence on energy-yielding reactions.

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