acid are administered simultaneously to the I strain mouse, fatty acid oxidation is depressed. It is interesting to note that in the A strain mouse carbohydrate stimulates the oxidation of tripalmitin, a result difficult to interpret in light of the reports of a diminished fat oxidation when fat and carbohydrate are fed simultaneously(12,13).

This study points to two important differences in the lipid metabolism of the A and I strain animals. The A strain mouse is able to channel more ingested fat into depots (Table II) and upon fasting may liberate larger quantities of FFA into the plasma. The larger size of the fat stores in the A strain(4) may partly account for both the higher concentration of plasma FFA and, in turn, the greater hepatic lipid content of the fasted animal(5). Carbohydrate is probably not available in sufficient quantities to meet the requirements of the tissues of the fasting I strain mouse, forcing a greater reliance on fatty acid oxidation in this strain.

Summary. Differences in the metabolism of 2 highly inbred strains of mice were studied. There was a higher level of FFA in the plasma of the A/Fn strain mouse as compared to the I/Fn, although measurements of FFA release *in vitro* revealed no differences in the rate of mobilization per unit weight of adipose tissue. Ketone body production by liver slices was greater in the I, and in both strains was inversely related to the hepatic glycogen content. Tripalmitin-C^{14} oxidation to $C^{14}O_2$ *in vivo* was more marked in the I than in the A, while fat retention was greater in the A. The simultaneous feeding of a 25% glucose-50% starch solution markedly depressed CO_2 output from tripalmitin in the I strain and caused an enhanced output in the A.

1. Fenton, P. F., Dowling, M. T., Mershon, J. S., J. Nat. Cancer Inst., 1954, v15, 429.
2. Marsh, J. M., Fenton, P. F., Endocrinology, 1959, v65, 916.
3. Lyon, J. B., Jr., Fenton, P. F., Am. J. Physiol., 1956, v187, 415.
4. Fenton, P. F., Am. J. Clin. Nutr., 1960, v8, 748.
5. Fenton, P. F., Dowling, M. T., J. Nutr., 1953, v49, 319.
6. Dole, V. P., Meinertz, H., J. Biol. Chem., 1960, v235, 2595.
7. Lyon, J. B., Jr., Bloom, W. L., Can. J. Biochem. Physiol., 1958, v36, 1047.
8. Fales, F. W., J. Biol. Chem., 1951, v193, 113.
9. Gora, E. K., Hickey, F. C., Anal. Chem., 1954, v26, 1158.
10. Wieland, O., Weiss, L., Eger-Neufeldt, I., Adv. Enzyme Reg., 1965, v2, 85.
11. Lyon, J. B., Jr., Porter, J., Biochim. Biophys. Acta, 1962, v58, 248.
12. Lewis, K. F., Allen, A., Weinhouse, S., Arch. Biochem. Biophys., 1959, v85, 499.
13. Lassow, W. J., Chaikoff, I. L., ibid., 1955, v23, 23.

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Urinary Excretion of Ingested α-Aminoisobutyric Acid-N^{15} in Dogs Treated with Growth Hormone or Corticotropin.* (30775)

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In previous experiments on dogs(1), we found that excretion of N^{15} ingested as glycine, alanine, or ammonia was reduced by growth hormone (STH) and increased by corticotropin (ACTH). That STH increases incorporation of nitrogen from all of the above sources into tissue proteins was demonstrated in experiments on rats(2-4), but the possibility that this hormone also alters the rate of N^{15} excretion by influencing transport of utilizable substances was not excluded. Moreover, it would be difficult to prove directly that increased output of N^{15} in experiments with ACTH was due entirely to accentuated protein breakdown or gluconeogenesis. We have, therefore, studied effects of both

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TABLE I. Rate of Appearance in Urine of N^{15} Ingested as Glycine, Alanine and α-Aminoisobutyric Acid (AIB).

Source of N^{15}	Dog No.	% of 2-day excretion of N^{15} (hr after ingestion) 0-6	6-12	12-24	24-48
Glycine	57	21	38	24	17
"	64	29	34	20	16
Alanine	57	24	41	23	12
"	64	24	38	19	19
AIB	76	29	30	24	17
"	77	26	32	25	17

hormones on excretion of N^{15} from an unutilizable source, α-aminoisobutyric acid (AIB), in dogs.

The transport characteristics of AIB have been extensively investigated, both *in vivo* and in a variety of *in vitro* preparations(5-9). Its behavior is considered to resemble closely that of glycine. Since it is not utilized, any change in rate of excretion during hormone treatment would have to result from something other than chemical transformation of the molecule. For these reasons, effects of STH and ACTH on excretion of N^{15} from AIB are particularly relevant in connection with our previous findings.

Materials and methods. AIB-N^{15}, containing 33.2 atoms per cent excess N^{15}, was synthesized from acetone, sodium cyanide and ammonium chloride-N^{15} by a method similar to that of Zelinsky and Stadnikow(10). Identity and purity were established by parallel paper chromatography with an authentic sample in 2 solvent systems, and by elementary analysis. Using 14.3 as the atomic weight of nitrogen with the stated excess of N^{15}, calculated percentages were: C, 46.46; H, 8.77; N, 13.83. Found: C, 46.41; H, 8.74; N, 13.77.

Two normal adult mongrel bitches served as experimental animals. Details of diet and care have been described(1). The growth hormone was of bovine origin, and was supplied by the Endocrine Study Section, National Institutes of Health. Five mg doses, dissolved in 1 ml of normal saline, were given subcutaneously for 4 successive days at 9:00 A. M. The corticotropin preparation, Armour's Acthar Gel, was given subcutaneously in 2 daily doses of 10 U each at 9:00 A. M. and 3:00 P. M. for 3 successive days. A single dose of AIB-N^{15}, either 413 mg containing 19.96 mg excess N^{15} or 290 mg containing 14.01 mg excess N^{15}, was fed with the daily ration on the third day of treatment with growth hormone and on the second day of corticotropin treatment, the periods of maximal nitrogen storage or loss. Urine was collected under toluene 6, 12, 24, and 48 hours after the labeled compound had been fed. Each collection period was terminated by catheterizing and washing the bladder.

In one experiment, enrichment with N^{15} was determined on urinary urea isolated as the dixanthylurea derivative, and on ammonia isolated by aeration. In all experiments N^{15} enrichment was determined in the total urinary nitrogen. Analytical procedures have been described(1).

Results and discussion. All of the excess N^{15} found in the urine was presumably excreted as AIB-N^{15}, since no excess above normal abundance of the isotope could be detected in urea or ammonia nitrogen. Assuming that any N^{15} separated from the administered compound would be excreted as urea or ammonia, any amount greater than 2% of that ingested would have been detected. This confirms the nonutilization reported by others employing AIB-C^{14}(5).

In Table I results of the previously reported control experiments with N^{15}-labeled glycine and alanine(1) have been compared with the present control experiments with AIB-N^{15}. The total amount of N^{15} excreted in 2 days is taken as 100% in each case. It is apparent that N^{15} from the 3 sources was excreted, and therefore absorbed, at rather similar rates. This is not to imply that concentrations of the 3 amino acids were similar in all, or in any, tissues at a given time. Of course, 90% or more of the N^{15} excreted after the ingestion of alanine and glycine had been metabolized to urea and ammonia, while AIB was excreted unchanged. The per cent of *ingested* N^{15} excreted in 48 hours was in the case of glycine 46.2, alanine 57.8, and AIB 83.7. AIB is apparently excreted more rapidly by the dog than by the

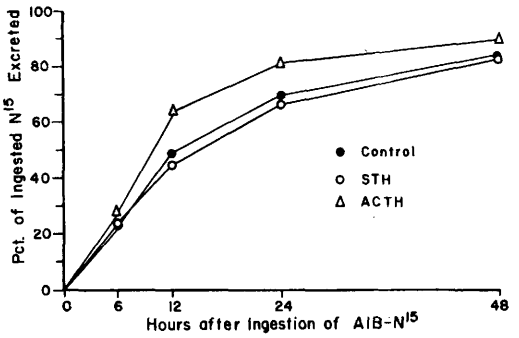

FIG. 1. Cumulative excretion of ingested AIB-N^{15} during control, growth hormone (STH) and corticotropin (ACTH) experiments. Each point is the average of results obtained in 2 dogs.

rat. However, part of the more rapid excretion may have been due to the high-protein diet, 36.2% casein, used in the present study, since protein content of the diet has been shown to influence profoundly the rate of urinary excretion of AIB by the rat(11).

The cumulative excretion of AIB-N^{15} during the control experiments and during treatment with growth hormone and corticotropin is shown in Fig. 1. It is evident that there was only a slight retardation in excretion of the isotope during growth hormone treatment, even though maximal nitrogen storage was occurring. This storage during the 48-hour period of observation was 9.32 g in one dog and 3.11 g in the other. In the previous experiments growth hormone treatment reduced excretion of N^{15} fed as glycine and alanine by 53.7% and 21.1% respectively(1). It seems reasonable to assume that this effect was due primarily to increased utilization of the ingested compounds rather than to changes in other factors such as transport. Indeed, one might speculate that changes in transport of amino acids play only a minor role in the mechanism of growth hormone action, in view of the demonstration of increased amino acid incorporation into protein (*i.e.*, increased utilization) in cell-free systems derived from animals treated with growth hormone(12).

As indicated in Fig. 1, corticotropin treatment definitely increased the rate of urinary excretion of AIB-N^{15}. Nitrogen loss during the 48-hour period was 9.55 g in one dog and 5.77 g in the other. In previous experiments corticotropin increased excretion of N^{15} ingested as glycine and alanine by 20.8% and 42.3% respectively(1). The increase in output of AIB-N^{15} is of considerable interest, since it cannot be due to changes in protein metabolism or gluconeogenesis; it must be due to changes in absorption, distribution, or excretion of AIB. An effect of corticotropin on any of these processes could also alter the time curve of urea production or excretion in the case of metabolizable compounds. Corticotropin is known to mobilize amino acids to the liver, and transient increases in blood amino acid levels(13), as well as considerable increases in urinary excretion of amino acids (14), have been observed during treatment with this hormone. In the present experiments it is possible that the increased excretion of AIB during corticotropin treatment was due to decreased penetration of the amino acid into tissues other than liver, resulting in higher blood levels, and consequently more rapid urinary excretion. Whether this is the case, or whether some other mechanism such as a specific effect on the kidney is involved, must await further study.

Summary. The rate of urinary excretion of ingested α-aminoisobutyric acid-N^{15} (AIB) was determined in dogs, in control experiments and during treatment with growth hormone or corticotropin. Growth hormone had only a slight effect, while corticotropin increased the rate of excretion somewhat, particularly during the second 6-hour period after ingestion. Apparently changes in absorption, distribution, and excretion play only a minor role in growth hormone action, while corticotropin directly affects one or more of these processes in addition to its effects on protein metabolism and gluconeogenesis. None of the N^{15} ingested as AIB appeared in urinary urea or ammonia.

1. Gaebler, O. H., Glovinsky, R., Lees, H., Kurrie, D., Choitz, H. C., Endocrinology, 1959, v65, 283.

2. Lees, H., Gaebler, O. H., Arch. Biochem. Biophys., 1959, v84, 188.

3. Vitti, T. G., Gaebler, O. H., ibid., 1963, v101, 292.

4. Vitti, T. G., Vukmirovich, R., Gaebler, O. H., ibid., 1964, v106, 475.

5. Noall, M. W., Riggs, T. R., Walker, L. M.,

Christensen, H. N., Science, 1957, v126, 1002.
6. Christensen, H. N., Perspectives, Biol. Med., 1959, v2, 228.
7. Riggs, T. R., Walker, L. M., J. Biol. Chem., 1960, v235, 3603.
8. Eichhorn, J., Scully, E., Halkerston, I. D. K., Hechter, O., Proc. Soc. Exp. Biol. and Med., 1961, v106, 153.
9. Christensen, H. N., Aspen, A. J., Rice, E. G., J. Biol. Chem., 1956, v220, 287.
10. Zelinsky, N., Stadnikow, G., Berichte, 1906, v39, 1726.
11. Nimni, M. E., O'Day, P., Bavetta, L. A., J. Nutrition, 1963, v80, 91.
12. Korner, A., Biochem. J., 1959, v73, 61.
13. Sheffner, A. L., Bergeim, O., Arch. Biochem. Biophys., 1954, v49, 327.
14. Ronzoni, E., Roberts, E., Frankel, S., Ramasarma, G. B., Proc. Soc. Exp. Biol. and Med., 1953, v82, 496.

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Amino Acids in Blood Plasma of Young and Aged Adults.* (30776)

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Concentration of amino acids in blood, urine and a variety of other biological materials has been examined by a number of investigators. However, little attention has been given to aged individuals in this regard (1,2,3). An interest in the protein metabolism of elderly human subjects has permitted the collection of data to fill the void.

Early in the investigation it was necessary to decide whether or not clinically detectable senile brain damage would have a major effect on plasma amino acid levels. Since there was no basis for the decision, the subjects employed were divided into 2 groups, those who showed brain damage and a second group which appeared normal in this respect. The data collected have permitted a statistical comparison between the 2 groups of aged individuals and between each of these and the control group of healthy young adults.

Materials and methods. The subjects were patients at the Jewish Home for the Aged, Miami, Fla., selected for this study by Dr. Charles Beber, its Medical Director. Eleven normal and 16 brain damaged geriatric patients (ages 79-92) were used. Thirteen healthy adults (ages 22-40) comprised the control group and were either laboratory and hospital staff or selected control subjects.

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The medical basis for distinguishing between the normal and brain damaged geriatric groups was the face-hand test, neurological and mental status testing. A negative or questionable result in the face-hand test was considered normal, while a positive test was interpreted as that of a senile individual. A normal neurological and mental status test indicated a normal individual, and a brain damaged individual with no evidence of a stroke or gross neurological disorders, was indicated by abnormal results on these tests.

A fasting sample of venous blood was drawn before breakfast from each subject. Each 15-20 ml sample was delivered into a 30 ml centrifuge tube containing one drop Sequester-Sol, Cambridge Chemical Products, Inc., Dearborn, Mich. (dipotassium ethylenediaminetetraacetate) per 5 ml blood. After mixing, the blood was centrifuged and the plasma removed, measured, and stored at −5°C.

Five ml plasma were diluted with 20 ml triple distilled water and ultrafiltered at 375 psi nitrogen pressure for 5 hours through an Ara-Flo 65 unit (Applied Research Associates), with a one ml wash of triple distilled water at the end of the run. The volumetric recovery was 90-95%; the filtrate was then flash evaporated to dryness and brought back to the original (5 ml) plasma volume with pH 2.2 sodium citrate buffer. Duplicate fil-