injected human serum albumin-I^{131} from the plasma of mice and its appearance in the extravascular space in the skin was studied. The data demonstrate the presence of at least 2 phases in the skin in which the turnover rate for one is approximately 4 times greater than the other. The albumin in the slower moving phase is thought to be present in the space encompassed by the domain of hyaluronic acid.

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Amino Acid Utilization by Isolated Adipose Tissue of Meal-Fed Rats. (31532)

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Restricting food consumption to a single daily meal (meal-eating) induces marked adaptive changes in the laboratory rat. Liver and adipose tissue of meal-fed animals has a greater lipogenic capacity than does similar tissue from *ad libitum*-fed rats (nibblers)(1-3). The influence of meal-feeding on protein and amino acid metabolism has received little attention. It has been demonstrated that adipose tissue of meal-fed rats can oxidize and incorporate into lipid significantly more aspartate, glutamate and leucine carbon than tissue of nibbling control rats(4-6). Cohn *et al*(7) have reported that meal-fed rats excrete more ^{15}N from a dose of ^{15}N-labeled protein than do nibbling rats and, also, that the activity of hepatic arginine synthetase was enhanced by meal-feeding. Earlier studies had shown that meal-fed rats excrete more nitrogen in the urine than do nibbling controls (8). These data would suggest that protein catabolism is increased in meal-fed animals because of a limited ability to handle ingested amino acids during the short meal period. The available data do not, however, yield information concerning the metabolism of amino acids by specific tissues in meal-fed rats. Because of the important role of adipose tissue in the response to meal-feeding, the influence of meal-feeding on the ability of this tissue to utilize amino acids *in vitro* has been investigated.

Methods. Male Holtzman rats weighing approximately 200 g were housed in stainless steel cages having raised wire floors in a temperature and humidity controlled room (70° F and 50% relative humidity). The rats were divided into 2 groups, one of which was allowed access to food from 8-10 AM only (meal-eaters) and the other was fed *ad libitum*. Purina rat chow served as the diet and was ground to a powder in order to enable measurement of food consumption. Food consumption and body weight were determined weekly. The animals were maintained on these feeding regimens for 7 weeks, a period

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TABLE I. *In vitro* Utilization of L-Leucine-^{14}C or L-Lysine-^{14}C by Adipose Tissue of Meal-Eating or Nibbling Rats.

Buffer†	Meal-eater			Nibbler		
	$^{14}CO_2$	Fatty acid	Protein	$^{14}CO_2$	Fatty acid	Protein
L-leucine-^{14}C (UL)	112 ± 42*	115 ± 96	840 ± 312	53 ± 10	1.4 ± .7	551 ± 252
+ glucose	114 ± 34	266 ± 181	1014 ± 315	68 ± 15	5.5 ± 2.1	644 ± 242
+ glucose + insulin	124 ± 34	588 ± 187	1206 ± 367	75 ± 19	125.9 ± 47.3	723 ± 216
L-lysine-^{14}C (UL)	7.8 ± 1.1	2.0 ± 1.0	349 ± 102	8.7 ± 1.8	.2 ± .1	267 ± 133
+ glucose	7.7 ± 1.9	3.5 ± 1.9	354 ± 148	7.9 ± 2.2	.2 ± .1	318 ± 124
+ glucose + insulin	6.2 ± 1.9	7.4 ± 1.0	420 ± 126	6.5 ± 1.4	2.9 ± .7	387 ± 121

* Values are mμmoles of amino acid utilized/100 mg tissue/3 hr. Mean for 8 rats ± standard deviation.

† Calcium-free Krebs-Ringer bicarbonate buffer containing the following, per ml, as indicated: L-leucine, 5 μmoles; L-lysine, 5 μmoles; glucose, 5 μmoles; insulin, 0.1 unit; L-leucine-U-^{14}C, 0.167 μc; L-lysine-U-^{14}C, 0.167 μc.

previously shown to be of sufficient length to induce the meal-feeding adaptation(3). Body weight and food consumption followed the pattern described earlier(3). Meal-fed animals had a markedly reduced food consumption and lost weight during the first week; thereafter, they gained weight at about the same rate as did the control rats in spite of a reduced food intake.

Meal-eating rats were sacrificed immediately following their daily meals; nibbling animals were fasted for 22 hours and refed for 2 hours on the day of sacrifice. The rats were killed by decapitation; the epididymal adipose tissue was rapidly excised and pieces weighing approximately 200 mg were taken from the thin peripheral portion of the pad for incubation. The tissue was quickly weighed on a rapid weighing torsion balance and transferred to an Erlenmeyer flask containing 3.0 ml of the appropriate buffer. Tissues were incubated in calcium-free Krebs-Ringer bicarbonate buffer(9), pH 7.4, and containing 5 μmoles per ml of L-leucine or L-lysine and 0.5 μc/3 ml of L-leucine-U-^{14}C or L-lysine-U-^{14}C. Glucose and insulin were added at levels of 5 μmoles and 0.1 unit per ml, respectively, as indicated in the Table. Incubations were carried out in a shaking water bath (90 strokes/min) for 3 hours. Treatment of the tissue following incubation, collection of CO_2, isolation of the fatty acids and counting were carried out as previously described(6). Protein was isolated from the dry defatted adipose tissue essentially as described by Herrera and Renold(10). The tissue and 30 mg of carrier bovine serum albumin were homogenized in 3 ml of 10% TCA. After centrifugation, the supernatant was discarded and the precipitate was resuspended in 3 ml of 10% TCA and heated at 90°C for 10 minutes to remove nucleic acids. The sample was again centrifuged, the supernatant discarded and the precipitate was washed 2 more times with 90°C, 10% TCA. In order to remove lipid contaminants, the precipitate was washed once with 3 ml of hot 95% ethanol and twice with ethanol:ether (1:1). The precipitate was dried and dissolved in 2 ml of 1 N KOH and an aliquot used for radioactivity measurements by liquid scintillation. The data were analyzed statistically by analysis of variance and intercell comparisons were made using the "t" test.

Results. The results of the experiment are presented in Table I, and a summary of the statistical evaluation is shown in Table II. The data in Table I demonstrate the influence of meal-feeding on the ability of isolated adipose tissue to utilize the two amino acids studied, namely, leucine and lysine. Tissue from meal-fed rats oxidized significantly greater quantities of leucine but not of lysine than tissue from nibbling rats. The incorporation by adipose tissue of both lysine and leucine into fatty acids was enhanced by meal-feeding, as was the incorporation of leucine-U-^{14}C into protein. Although more lysine was incorporated into protein by adipose tissue of meal-fed animals than by tissue of nibbling rats, this difference was not statistically significant. The addition of glucose to the incubation medium increased the incorporation of leucine into fatty acids and protein in tissue

TABLE II. Evaluation of the Data by "t" Test.

Comparison	Meal-eater			Nibbler		
	CO_2	FA	Prot	CO_2	FA	Prot
Leucine *vs* leucine + glucose	ns*	<.005	<.025	<.005	<.001	<.050
" *vs* leucine + glucose + insulin	ns	<.001	<.005	<.005	<.001	<.010
Leucine + glucose *vs* leucine + glucose + insulin	ns	<.001	<.005	ns	<.001	ns
Lysine *vs* lysine + glucose	ns	<.025	ns	ns	ns	ns
" *vs* lysine + glucose + insulin	ns	<.001	ns	<.001	<.001	<.025
Lysine + glucose *vs* lysine + glucose + insulin	ns	<.001	<.050	ns	<.001	ns

* Probability of significance; ns = not significant.

of both meal-fed and nibbling rats. The incorporation of leucine was increased further when insulin was added to the incubation medium along with glucose. When L-lysine-^{14}C was used, the differences due to glucose and insulin were in the same direction as noted with leucine; however, they did not attain significance.

Discussion. The data presented suggest quite strongly that protein synthesis in adipose tissue is enhanced by meal-eating. A highly significant increase in leucine-^{14}C incorporation into protein was observed in tissue of meal-eating rats and, although the incorporation of lysine-^{14}C into protein by tissue of meal-fed animals was not statistically different from that by tissue from nibblers, the differences were in the same direction as observed for L-leucine incorporation. These findings are consistent with the observations of Benjamin and Gellhorn(11-13) which suggest that regulation of metabolism in adipose tissue of fasted-refed animals is mediated by control of enzyme synthesis at the level of DNA-directed synthesis of RNA. From these studies, it might be anticipated that in meal-fed animals RNA synthesis and, consequently, the synthesis of enzyme protein would be enhanced. This possibility is presently being studied and may account for the enhancement of amino acid incorporation into protein observed in this study.

The observation that amino acid carbon is incorporated into fatty acids to a greater extent by adipose tissue of meal-fed rats is in accord with previous studies(4-6). The stimulation in fatty acid synthesis induced by glucose or glucose and insulin probably reflects enhanced metabolic activity resulting from glucose availability. Goodman(14) has shown that insulin stimulates lipogenesis from leucine-^{14}C in adipose tissue by stimulating the uptake of leucine and by increasing the availability of glucose. The stimulation of protein synthesis by insulin, however, seems to be independent of its effect on glucose and amino acid entry into the adipose tissue cell (15). The data in the present report suggest that the effects of insulin are not altered by meal-feeding since similar relative differences were induced by glucose and insulin in tissue of meal-fed and nibbling rats.

Summary. Meal-feeding (limiting access to food to 2 hours per day) in the rat stimulated the utilization of leucine- and lysine-^{14}C by isolated adipose tissue. Epididymal adipose tissue from meal-fed rats incorporated significantly more amino acid carbon into protein and fatty acids than did similar tissue from nibbling (*ad libitum*-fed) animals. Greater quantities of amino acids were also oxidized by adipose tissue of meal-fed rats. The incorporation of leucine- and lysine-^{14}C into protein and fatty acids was stimulated by addition of glucose to the incubation medium, and insulin addition resulted in a further stimulation. The possible regulatory mechanisms involved are discussed in light of available knowledge concerning adipose tissue metabolism.

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Effects of "Net Acid" Excretion on Acid-Base Status in Dogs Following 2-Amino-2-Hydroxymethyl-1,3-Propanediol (THAM) Administration.* (31533)

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It is generally recognized that an important function of the kidneys is to contribute to the maintenance of pH homeostasis by excreting titratable acid (TA) and ammonium ion. According to current concepts, the principal effect of TA excretion is to promote the generation of bicarbonate from hydrated CO_2, and since bicarbonate excretion necessarily nullifies this effect, it has been usual, as pointed out by Elkinton, *et al*(1), to calculate "net acid" excretion in terms of urinary bicarbonate as well as of TA and ammonium ion from the expression, "net acid" = TA + NH_4^+ − HCO_3^-. A widely accepted interpretation of the effects of ammonium ion excretion is that although ammonium ion is not measurable by titration with base to blood pH, it nevertheless carries an excess proton and may therefore be regarded as equivalent to urinary buffers in which the excess protons are directly measurable by titration and are therefore accounted for as TA. If this interpretation were extrapolated to other protonated, and similarly not-titratable urinary products, it would appear that the latter, as well as ammonium ion, should be taken into account in the "net acid" expression. Thus, Nahas *et al*(2), in calculating "hydrogen ion" excretion following 2-amino-2-hydroxymethyl-1,3-propanediol (THAM) infusion in dogs, employed the expression, $UV_{H^+} = UV_{NH_4^+} + UV_{TA} + UV_{RNH_3^+} - UV_{HCO_3^-}$, in which RNH_3^+ designates protonated THAM, and regarded earlier calculations reported from the present authors' laboratory(3) as erroneous for not similarly considering RNH_3^+ as a component of "acid" excretion.

On the other hand, it is well known that ammonium ion is readily metabolizable, leading to production of hydrogen ion and urea from the net reaction, $2\ NH_4^+ + CO_2 \rightarrow 2\ H^+ + (NH_2)_2CO + H_2O$, and it follows that the effects of ammonium ion excretion might alternatively be interpreted as owing to elimination of a metabolic precursor of hydrogen ion rather than of immediately available hydrogen ion. If this alternative interpretation were the correct one, then excretion of RNH_3^+, since it is not metabolizable, could not be equivalent to ammonium

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