Lipogenesis by Adipose Tissue, Dietary Effects. (23373)

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The effects of dietary factors upon lipogenic activity of adipose tissue have been reported by several investigators(1-4). Tuerkischer and Wertheimer(1) reported that fat deposition in adipose tissue of fasted rats is apparent one day after the beginning of recovery feeding. Good correlation was found between glycogen deposition and fat accumulation in adipose tissue. Shapiro and Wertheimer(2) demonstrated that adipose tissue incubated *in vitro* in serum enriched with deuterium oxide was capable of introducing stably-bound deuterium into its fatty acids. The rate of introduction was greater in the adipose tissue of rats on a diet accelerating fat synthesis in the body. In investigations of dietary effects on utilization of radioglucose by adipose tissue *in vitro* Hausberger and Milstein(3) found that fasting or feeding a high fat diet abolished lipogenesis and greatly diminished glucose oxidation. The "block" in fatty acid synthesis by adipose tissue from fasted animals was studied by Rose and Shapiro(4) and led them to the conclusion that the pathway of glucose metabolism is changed during fasting to one which does not facilitate fat synthesis. The present report supplements previous knowledge of dietary effects upon lipogenic activity of adipose tissue and, in addition, provides information concerning lipid synthesis as a function of time after recovery feeding and tissue composition.

Procedures. Minced rat (Sprague-Dawley) epididymal adipose tissue plus 3 ml normal dog serum and 100 μl labelled acetate solution (2.38 μm acetate) was incubated for 3 hours at 37.5°C in a 10 ml flask containing a center well tightly stoppered with a rubber serum stopper. Following incubation, 0.4 ml Hyamine(5) solution was injected into the center well, followed by 0.5 ml 6N acetic acid into the main compartment of the flask. After equilibration at 37.5°C for 1½ hours the Hyamine solution was quantitatively collected for counting. The contents of the flask were then saponified, made acidic, extracted with petroleum ether and the lipid extract chromatographed by the procedure described by Popjak and Tietz(6) to rid it of contaminating traces of C^{14}-acetate. All samples were counted in a liquid scintillation counter.† Carbon dioxide samples were counted in a system consisting of Hyamine solution (combined with CO_2) and 0.3% diphenyl-oxazole (DPO)‡ in toluene(5). All other samples were counted in a system consisting of 2% water, 25% absolute ethanol, 73% toluene (containing 3 g per liter DPO).

Analysis of the nitrogen content of adipose tissue was carried out by micro-Kjeldahl steam distillation into a boric acid-mixed indicator solution and subsequent titration with standard HCl. A mixture of H_2SO_4, $CuSO_4$, K_2SO_4 and Se was used for digestion. Lipid content was estimated gravimetrically on a petroleum ether extract of tissue digested with 2N H_2SO_4. Reducing substances were determined by a modification of the Folin method (7) on a lipid-free digest of the adipose tissue. Quantitative recovery of glucose added to adipose tissue was obtained with the method. The results are expressed in terms of glucose equivalent.

Results. A group of 9 normal rats on Rockland rat diet were divided into 2 groups. Three animals were sacrificed and served as the source of adipose tissue for control values. The remainder were fasted with water *ad libitum* to a loss of approximately 20% of their initial weights, which required about 48 hours. The animals were then refed on a high carbohydrate diet§ *ad libitum*. Rats were sacrificed at intervals and adipose tissues obtained. The tissue was analyzed for nitrogen, glucose, lipid content and rate of conversion of 1-C^{14} ace-

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† Tri-Carb Counter, Model 314, Packard Instrument Co., LaGrange, Ill.

‡ Pilot Chemicals, Inc., Waltham, Mass.

TABLE I. Lipid Synthesis as a Function of Time after Refeeding and Tissue Composition. Normal rats

Animal	Condition	% wt loss (before refeeding)	Tissue composition % nitrogen	% lipid	% glucose	% of total acetate incorporated per 100 mg tissue CO_2	Lipid
1	Normal (before fasting)	—	.27	87.3	.10	1.46	9.39
2			.30	88.0	.32	—	—
3			.29	82.4	.08	2.10	12.38
		Avg	.29	85.9	.17	1.78	10.89
4	0 hr after refeeding	20.4	.45	69.3	.14	1.59	1.26
5	6 " "	20.3	.36	81.4	.09	1.98	1.00
						1.35 (avg 1.67)	1.01 (avg 1.00)
6	12 " "	21.4	.38	77.2	.13	1.97	5.69
							5.78 (avg 5.74)
7	18 " "	19.0	.42	76.6	.19	2.96	8.76
						2.32 (avg 2.64)	8.01 (avg 8.38)
8	24 " "	20.6	.51	68.1	.44	2.61	12.01
						3.36 (avg 2.99)	11.45 (avg 11.73)
9	48 " "	23.1	.89	59.1	1.03	3.48	17.70
						2.91 (avg 3.20)	15.79 (avg 16.75)

tate into CO_2 and long chain fatty acids *in vitro*. Analyses were usually done in duplicate or triplicate. The data obtained are given in Table I. In most cases duplicate studies were carried on in the *in vitro* metabolism of radiocarbon-labelled acetate for each animal studied. To show the reproducibility, these individual results are presented in the table with average values.

Fasting increases the nitrogen content of adipose tissue, probably in part by decreasing the lipid content. Upon refeeding the nitrogen content of the tissue increases even further. The reducing substances show no essential change on fasting or during the first 18 hours of refeeding but at 24 hours a sharp increase is apparent which continues during the next 24 hours. The results are in good agreement with those of Tuerkischer and Wertheimer(1) on fasted and refed rats. The depression of fatty acid synthesis *in vitro* from acetate following fasting confirms previous reports(3,4). Reduced lipogenic activity persists for 6 hours after refeeding but then fatty acid synthesis rises rapidly to the high level of incorporation of 16.8% of the labelled acetate added. Under the conditions of these experiments a considerably greater conversion of acetate to fatty acid was obtained than Hausberger *et al.*(9) report with glucose. Fasting and refeeding exert a much smaller effect on the rate of conversion of acetate to $C^{14}O_2$. It is interesting to note that increased fatty acid synthesis begins before an appreciable rise in tissue reducing substances occurs, apparently glycogen content does not rise first as Tuerkischer and Wertheimer(1) inferred. The lipid content of the tissue declines during the refeeding period in spite of an increased rate of synthesis of fatty acids. The mobilization of fat evidently is proceeding at a rate which exceeds that of synthesis. Because of the high fat content of adipose tissue it was not possible to determine the extent to which net fat synthesis occurred.

Feller(10) studied incorporation of C^{14}-1-acetate into fatty acids with normal mouse adipose tissue in an *in vitro* system consisting of Krebs-bicarbonate buffer supplemented with succinate and glucose. On a fat-free basis he found an even greater per cent acetate incorporation per 100 mg tissue in adipose tissue than in liver (2.51% as compared to 1.24%). Under more favorable circumstances (fasted and refed rat adipose tissue incubated in serum) the present study has demonstrated levels of incorporation of acetate into lipid as great as 40% per 100 mg of fat-free adipose tissue (Table I).

§ Osborne-Mendel salt mixtures(8) 3.83%; wheat germ oil 0.29%; cod liver oil 0.29%; cellulose 9.55%; corn oil 0.77%; sucrose 67%; casein 14.35%; dried brewer's yeast 3.91%.

During this work the lipogenic activity of adipose tissue from B-complex deficient rats has also been studied. The per cent of total acetate incorporated into lipid per 100 mg tissue in the deficient rats averaged 1.59% (CO_2 formation 1.53%). One week following supplementation of the diet with B-complex vitamins, lipogenic activity of adipose tissue from these rats was found to average 19.08% (CO_2 formation 0.81%). Tuerkischer and Wertheimer(1) have found that addition of dried yeast to the diet of B-complex deficient rats will cause deposition of adipose tissue glycogen.

Summary. 1. The incorporation of C^{14}-acetate into lipid and carbon dioxide by rat adipose tissue (*in vitro*) was investigated. This investigation concerned the effect of the nutritional status upon the lipogenic activity of adipose tissue. 2. Fasting was found to produce an increase in nitrogen concentration and a decrease in lipid concentration and lipogenic activity. 3. In normal rats lipogenic activity of the tissue was found to increase during recovery feeding following fasting. The increased activity was paralleled by increased nitrogen and glucose content of the tissue and by decreased lipid content. 4. Lipogenic activity of adipose tissue of rats could be made relatively constant by depletion of fat stores followed by recovery feeding.

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Received July 2, 1957. P.S.E.B.M., 1957, v95.

Effect of Pituitary Growth Hormone on Transplantable Mouse Tumors. (23374)

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In a recent study of the effect of growth hormone on a mammary adenocarcinoma transplantable in DBA/1 mice, Mirand(1) and Mirand and Hoffman(2) reported that tumor volume was significantly increased when the host mouse was given hormone beginning at the time of inoculation. Reid's (3) review indicates that mouse tumor response to growth hormone was not a consistent finding. We examined this problem further with more quantitative experiments on 4 mouse tumors using purified growth hormone which we assayed for its effect on hypopituitary dwarf mice(4,5).

Materials and methods. The effect of growth hormone was studied on 4 transplantable tumors. 1. The DBA/1 mammary adenocarcinoma which originated spontaneously in the DBA/1 mouse, 2. the Marsh Albino (MA) mammary adenocarcinoma which originated spontaneously in the MA mouse. 3. and 4.: Tumors 3 and 4 were fibrosarcomas induced in the DBA/1 and MA mouse strains respectively by desoxycorticosterone acetate in sesame oil(6). These 4 tumors grew in castrated mice indicating their lack of hormonal dependence on the host. All tumors were grown subcutaneously in male mice having an average age of 3 months at the time of inoculation. Cell suspensions of the tumors were made by the method of Reinhard, Goltz and Warner(7).