weight gain in mice. It was also shown to inhibit the increases in enzyme activities and uterine weight stimulated with estradiol dipropionate.

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Stimulation by Insulin of Protein Synthesis in Isolated Fat Cells.* (31053)

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This communication presents evidence that insulin, in physiological concentration, stimulates synthesis of protein from amino acids by individual fat cells of rat epididymal adipose tissue.

Methods. The isolated fat cell preparation is obtained by a modification of Rodbell's technique(1). Male Sprague-Dawley rats, weighing 110-210 g, were utilized. Eight animals were used in each experiment. The rats, in a fed state, were sacrificed by decapitation. Distal segments of epididymal adipose tissue were quickly excised, weighed, and added to 8-10 ml of Krebs-Ringer-bicarbonate solution containing 1 mg/ml glucose, 4% bovine albumin, and 20 mg of collagenase.† The albumin was previously purified by filtering through #42 Whatman paper followed by dialysis, utilizing Krebs-Ringer solution, for 18-24 hours. Before incubation, the pH of the medium was adjusted precisely to 7.4. Following incubation for one hour at 37°C in a Dubnoff metabolic shaker, the adipose tissue preparation was filtered through surgical gauze. The filtrate was suspended in 8-10 ml of Krebs-Ringer-bicarbonate‡ solution and centrifuged for 1-2 minutes at 600×. The infranate, containing blood vessels and connective tissue, was then aspirated. Resuspension of the preparation, centrifugation, and aspiration were repeated twice. The fat cell preparation was finally suspended in 6-8 ml of Krebs-Ringer-bicarbonate solution.‡ An amino acid mixture§ and Krebs-Ringer-bicarbonate,‡ with or without insulin, were added in 0.05 ml volumes each to plastic vials ("Analocup," Aloe Co.). The final insulin¶ concentration was 800 micro-units/ml. With constant agitation, 0.5 ml of the fat cell preparation and approximately 0.1 microcurie of radioactive Amino Acid-C^{14} (U.L.) Mixture‖ were added to each plastic vial. A 0.1 ml aliquot was taken after 5 minutes had elapsed, and the mixture was then incubated at 37°C in a Dubnoff metabolic shaker for 2 hours, when a second 0.1 ml aliquot was taken. In 2 experiments, aliquots were also taken following one hour of incubation. Disposable plastic vials, test tubes, and pipettes were utilized throughout these procedures.

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† Worthington Biochemical Corp., Freehold, N. J.

‡ Containing 1 mg/ml glucose and 4% bovine albumin.

§ Slightly modified from Borsook, J. Biol. Chem., 1957,, v229, 1059.

¶ From Eli Lilly & Co., through the kindness of Dr. Otto K. Behrens.

‖ New England Nuclear Corp., Lot No. 127-72-50a.

TABLE I. Incorporation of Radioactive Amino Acids into Protein of Individual Fat Cells—Effects of Duration of Incubation and of Insulin.

		Incorporation of amino acid into protein				
		5 Min*	60 Min		120 Min	
Exp No.	Insulin	Mean ± SEM†	Mean ± SEM	P‡	Mean ± SEM	P
1	0	6 ± 3	805 ± 40	<.01	1,531 ± 56	<.01
	800 micro-units/ml	16 ± 1	1,022 ± 27		1,914 ± 42	
2	0	3 ± 2	441 ± 13	>.01, <.02	706 ± 27	<.01
	800 micro-units/ml	1 ± 1	507 ± 13		835 ± 18	

* Duration of incubation.

† Mean ± standard error of mean of 4 determinations—expressed as counts per minute per vial.

‡ Probability of significance of difference between means of control and insulin. Computed by Student "t" test. "P" from Fisher's Tables.

The technique of Mans and Novelli(2) was employed to determine the amount of radioactive protein in the cells. Each 0.1 ml aliquot of incubation mixture was deposited on a disc of Whatman No. 3MM Chromatography Paper, 2.3 cm in diameter, mounted on a straight pin. A stream of warm air, from a commercial hair dryer, was directed over each filter paper disc until dry. The discs were then immersed in ice cold 10% TCA, containing 1% casein hydrolysate** for one hour. The discs were transferred to 5% TCA, allowed to stand for 15 minutes, then transferred to another beaker containing 5% TCA and heated to 90°C for 30 minutes. Each filter paper disc was then placed in ether: absolute alcohol (1:1 volume) for 30 minutes, washed twice in ether, air dried, and dropped into 5 ml of scintillation fluid (PPO-POPOP).†† Counting was performed with a Nuclear Chicago Scintillation Counter (Model 720). Appropriate controls, utilizing radioactive amino acid medium without cells, were employed to obtain background counts which were subtracted from all counts reported.

In each experiment, the cell preparation was tested by a modification of Rodbell's technique(1) for measuring evolution of $C^{14}O_2$ from uniformly labelled C^{14} glucose. Significant stimulation of $C^{14}O_2$ production by 800 micro-units/ml of insulin was observed in every experiment.

Results and discussion. Table I demonstrates that incorporation of radioactive amino acid into isolated fat cell protein is increased in proportion to duration of incubation. Tables I and II demonstrate that the rate of amino acid incorporation into protein is further increased, significantly, by 800 micro-units/ml of insulin. Preliminary experiments utilizing this technique suggest that insulin also increases synthesis of protein from individual amino acids, and that a lower concentration of insulin, 50 micro-units/ml, significantly increases fat cell protein synthesis.

Mans and Novelli(2) have emphasized that their technique, employing a filter paper assay with highly efficient washing and extraction procedures, minimizes contamination of the isolated protein with radioactive nonprotein materials. By combining this procedure with Rodbell's method(1) of obtaining isolated fat cells, a system is evolved permitting study of rates of protein synthesis in the individual isolated fat cell. Superior fat cell preparations are obtained from younger rats, weighing less than 220 g. The albumin purification step also is very important. Some preparations of untreated bovine albumin are highly toxic to the fat cells, and no protein synthesis occurs.

Experiments utilizing intact epididymal adipose tissue have indicated significant stimulation of amino acid synthesis into protein by insulin(3-7). Relatively high concentrations of insulin, 10,000-100,000 micro-units/ml were employed, usually. However, Christophe and Wodon(7) have used a considerably lower concentration of insulin, 250 micro-

** Calbiochem.

†† PPO-(2,5-Dephenyloxozole)-5 g/l POPOP-(1,4-bis-2-[5-phenyloxasolyl])-benzene-400 mg/l. Calbiochem.

TABLE II. Effect of Insulin upon Incorporation of Amino Acids into Protein by Isolated Adipose Tissue Cells.

	Cell protein (CPM)*				
	Control	800 μunits/ml insulin	Mean increase of cell protein in response to insulin		
Exp No.	Mean ± SEM†	Mean ± SEM	CPM	%‡	"P"§
1	1,531 ± 56	1,914 ± 42	383	25	<.01
2	706 ± 27	835 ± 18	129	18	<.01
3	957 ± 33	1,139 ± 27	182	19	<.01
4	3,428 ± 63	4,169 ± 53	741	22	<.01
5	2,210 ± 95	2,722 ± 37	512	23	<.01
6	1,443 ± 34	1,631 ± 17	188	13	<.01
7	1,526 ± 52	1,800 ± 25	274	18	<.01

* Counts per minute per vial.
† Mean ± standard error of mean of 4 determinations following 2-hr incubation.
‡ % Increase of insulin (mean CPM) compared with control (mean CPM).
§ Probability of significance of difference between means of control and insulin computed by Student "t" test. "P" from Fisher's Tables.

units/ml. Krahl(4) emphasizes that insulin has a specific stimulatory effect on adipose tissue protein synthesis that cannot be simulated by other substances which increase glucose uptake or lipid synthesis in fat.

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Entry of L-Phenylalanine-C^{14} into Brain and Cerebrospinal Fluid.* (31054)

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Previous studies from this laboratory have indicated that elevated plasma levels of L-phenylalanine inhibit the entry of 5-hydroxytryptophan-C^{14} into cerebrospinal fluid (CSF) of dogs(1). In order to assess the significance of the entry of labelled amino acid into CSF, it was felt necessary to obtain data correlating radioactivity of brain and CSF.

In the present experiments, L-phenylalanine-C^{14} was administered to anesthetized dogs and levels of radioactivity in CSF were compared with simultaneously determined levels in the trichloracetic acid (TCA) insoluble and TCA soluble fractions of several anatomical regions of the brain. The results form the basis of the present report.

Methods. Operative procedures. Eight purebred beagle dogs of either sex were utilized in these experiments. The animals were fasted overnight prior to the experiment. They were anesthetized with 30 mg/kg of intravenous pentobarbital. Respirations utilizing room air were maintained with a mechanical respirator attached to an oral cuffed endotracheal tube. Three μc/kg, L-phenylalanine-UL-C^{14} (sp act 190 mC/

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