Factors Concerned with the Regulation of Fatty Acid Metabolism by Adipose Tissue

GEORGE F. CAHILL, JR., M.D.,* BERNARD LEBOEUF, M.D.† AND ALBERT E. RENOLD, M.D.‡

As EVIDENCED by the number of articles appearing in the scientific literature, both clinical and basic, much attention has recently been directed toward the metabolism of adipose tissue. This tissue was long considered an inert site of energy storage except by investigators such as Wertheimer and Shapiro,[1,2] Stetten,[3] and Hausberger.[4,5] It has recently been shown that adipose tissue releases lipid as fatty acids§, and thereby provides a major, perhaps the major metabolic fuel, as recently reviewed by Frederickson and Gordon.[6] Adipose tissue has also been shown to be acutely and exquisitely sensitive to many hormones such as insulin[4-15] and epinephrine,[1,16-18] and, in addition, its sensitivity has been shown directly or indirectly to growth hormone,[19,22] adrenocorticotropic hormone,[22,23] glucagon,[23] thyroid hormone,[24] thyroid stimulating hormone,[22,25] and prolactin.[21]

The purpose of this paper is to summarize observations made at the Baker Clinic Research Laboratories on some of the metabolic events associated with the uptake and release of fatty acids in adipose tissue and the effect of various hormones on these mechanisms. The experiments were all performed under identical conditions, as described previously,[9-11] namely, incubation of epididymal fat pads (excised from rats) for three hours at 37°C. in Krebs-Ringer bicarbonate buffer containing 0.5 mM human albumin‖ and 5 mM glucose and equilibrated with 5 per cent CO_2:95 per cent O_2. The albumin was not extracted to remove fatty acids and contained approximately 1.2 M fatty acids per M albumin.

FATTY ACID EXCHANGE

Previous studies[26] have shown that adipose tissue obtained from normally fed rats and incubated in a medium containing about 0.6 mEq. per L. fatty acids is approximately in a steady state (Fig. 1) with the concentration of fatty acids in the medium. In other words, there is no change in the concentration of fatty acid in the medium during the period of incubation. Addition of a trace amount of highly active palmitate-1-C^{14} to the albumin in the medium prior to incubation resulted in a recovery of label in tissue triglyceride at the end of the incubation (Fig. 2). Therefore, there must have been a concomitant release of unlabeled fatty acids from the tissue into the medium equal to the quantity of labeled fatty acids taken up from the medium. Other studies demonstrated that if glucose was omitted from the medium, the incorporation of labeled palmitate-1-C^{14} into tissue triglyc-

From the Departments of Medicine, Harvard Medical School and the Peter Bent Brigham Hospital and the Baker Clinic Research Laboratory, New England Deaconess Hospital, Boston, Massachusetts.

* Fellow of the Medical Foundation, Inc., Boston, Massachusetts and Associate in Medicine, Harvard Medical School; † Fellow of the Life Insurance Medical Research Fund and Research Fellow in Medicine, Harvard Medical School, present address: Scripps Clinic and Research Foundation, La Jolla, California; ‡ Director, Baker Clinic Research Laboratory and Assistant Professor of Medicine, Harvard Medical School.

Supported in part by grants from the U. S. Public Health Service, A-2460 and H-4569, Bethesda, Maryland, and the Lipotropic Research Foundation, New York, New York.

Presented at the Brook Lodge Invitational Symposium on Energy Balance, sponsored by The Upjohn Company, September 29, 1959, at Brook Lodge, Augusta, Michigan.

§ "Free" fatty acids or non-esterified (NEFA) or unesterified (UFA) fatty acids.

‖ Obtained through the courtesy of Dr. R. J. Pennell of the Protein Foundation, Inc.

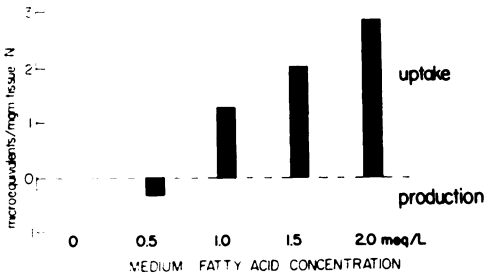

FIG. 1. Change in medium titratable fatty acids as a function of fatty acid concentration (glucose 5 mM). At approximately 0.6 mEq. per L. medium fatty acid concentration there is neither uptake into nor production of fatty acids by adipose tissue after three hours incubation under conditions described in the text. (From: Bally, P., Cahill, G. F., Jr., Leboeuf, B. and Renold, A. E. *J. Biol. Chem.*, 235: 333, 1960.[26])

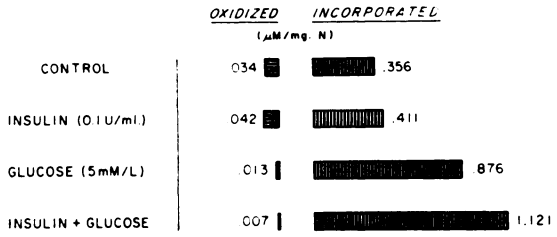

FIG. 2. Comparison of insulin and glucose on palmitate-1-C^{14} metabolism in adipose tissue. (From: Bally, P., Cahill, G. F., Jr., Leboeuf, B. and Renold, A. E. *J. Biol. Chem.*, 235: 333, 1960.[26])

eride was markedly reduced. In addition, the tissue was no longer in a steady state with the medium, but released more fatty acids than those incorporated, and caused an elevation in the concentration of fatty acid in the medium. These experiments suggest that glucose accelerated the process of esterification. This will be discussed later in this report.

EPINEPHRINE

With knowledge of the marked sensitivity of adipose tissue to epinephrine and norepinephrine, as reflected by fatty acid mobilization,[17] experiments were performed with glucose-C^{14} as substrate. It was expected that the mode of action of epinephrine might be elicited by a decreased rate of fatty acid esterification, thus disrupting the steady state in favor of the release of fatty acid. Surprisingly, the incorporation of glucose-C^{14} into glyceride-glycerol was increased five or six times by

TABLE I

Relative Metabolism of Glucose (5 mM) and Glycerol (5 mM) by Adipose Tissue *in vitro**

	CO_2	Glyceride-Glycerol	Fatty Acid	Glycogen
Glucose-U-C^{14}	2.540	2.660	0.810	0.090
Glycerol-1,3-C^{14}	0.126	0.371	0.018	0.003
Glycerol/Glucose	5%	14%	2%	3%

* Values expressed as micromoles substrate carbon per mg. tissue nitrogen per three hours.

epinephrine,[18] thus making untenable the hypothesis that epinephrine might inhibit the process of esterification. It was apparent, therefore, that epinephrine primarily accelerated the breakdown of triglyceride to fatty acids. Concomitantly, if there was an increased rate of glyceride-glycerol synthesis from glucose, a large portion of glycerol already present in tissue triglyceride prior to epinephrine stimulation and liberated by lipolysis was not re-utilized for the process of esterification. Determination of formaldehydogenic material in the medium after periodate oxidation with correction for reducing material present in the medium, showed that epinephrine did cause the release of a substance[27] which was subsequently identified as glycerol by paper chromatography.[28]

GLYCERIDE-GLYCEROL SYNTHESIS

Studies on adipose tissue by Shapiro, Chowers and Rose[29] suggested that "activated" glycerol (as reviewed by Wertheimer in this issue) in the form of glycerolphosphate, is required as the glycerol donor in the process of esterification and is similar to the precursor in the formation of triglyceride in liver[30] or intestinal mucosa.[31] Table I compares the incorporations of glucose and glycerol carbon into components of adipose tissue. It is evident that glycerol, compared with glucose, is a poor precursor of glyceride-glycerol, and is not well incorporated into CO_2, fatty acids and glycogen. Incubation of adipose tissue in the presence of epinephrine decreases the incorporation of labeled glycerol carbon into glyceride-

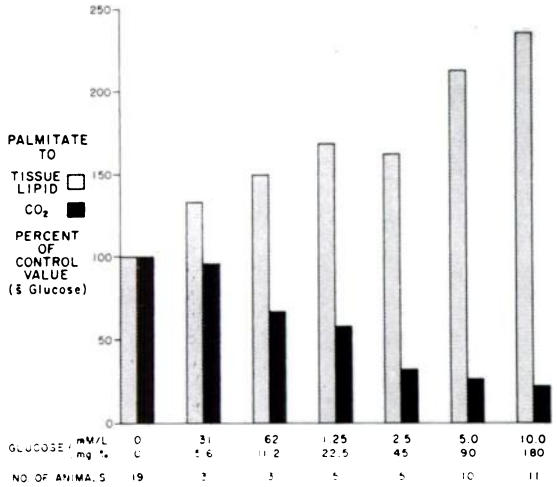

FIG. 3. Effect of glucose upon incorporation of palmitate-1-C^{14} into tissue lipids and upon its oxidation to CO_2 by rat adipose tissue. (From: Bally, P., Cahill, G. F., Jr., Leboeuf, B. and Renold, A. E. *J. Biol. Chem.*, 235: 333, 1960.[26])

glycerol by 33 per cent, probably due to a greater dilution of glycerol-C^{14} by unlabeled glycerol generated in the tissue.[28]

These results also suggest that the increased rate of re-esterification, as evidenced by accelerated glyceride-glycerol synthesis from glucose, is a secondary phenomenon. Further evidence for this conclusion was obtained by comparing glyceride-glycerol synthesis from glucose-C^{14} in tissues incubated at low (0.6 mEq. per L.) and high (3.5 mEq. per L.) medium fatty acid concentrations.[18] Incubation in the medium containing the high concentration of fatty acids resulted in increased glyceride-glycerol synthesis and the other associated changes in carbohydrate metabolism noted with epinephrine stimulation, namely, increased oxidation of carbon-6 of glucose and increased glucose utilization. The degree of these changes following the addition of fatty acid to the medium was less than those found in the presence of high concentrations of epinephrine, probably due to inability to elevate intracellular fatty acids to comparable levels.

CONTROLS OF RELEASE

The data therefore suggests that two factors control the rate of exchange of fatty acids between tissue triglyceride and medium, one being the esterification process, which is dependent on glucose for the synthesis of necessary glycerolphosphate, and the other the rate of lipolysis, which is sensitive to epinephrine. Other experiments have shown that growth hormone and adrenocorticotropic hormone preparations similarly increase the rate of lipolysis, thereby accelerating fatty acid release, increasing glucose uptake, glyceride-glycerol synthesis and glucose oxidation, particularly that of glucose-carbon–6.[32] In other words, these hormones produce a pattern of metabolism similar to that observed after epinephrine stimulation.

TABLE II

Glyceride-Glycerol Synthesis from Glucose-1–C^{14} and Glucose-6–C^{14} in Adipose Tissue *in vitro**

Glucose Concentrations μM	Insulin	Total Recovered Label†		Glyceride-Glycerol		Per Cent of Total Recovered Label in Glycerol	
		C-1	C-6	C-1	C-6	C-1	C-6
1.25	0	0.61	0.62	0.19	0.29	31	47
5	0	1.23	1.19	0.22	0.51	18	43
20	0	2.23	1.64	0.39	0.58	17	35
80	0	4.08	3.05	0.43	0.64	11	21
1.25	+	4.80	2.70	0.37	0.54	8	19
5	+	10.47	4.54	0.69	0.68	7	15
20	+	16.49	8.06	0.78	0.71	5	9
80	+	23.69	14.61	0.73	1.06	3	7

* Insulin 0.1 unit per ml. Values in micromoles glucose carbon per gm. adipose tissue per three hours. Values from JEANRENAUD, B. and RENOLD, A. E.[12]

† Sum of label in CO_2, glyceride-glycerol, fatty acids and glycogen.

TABLE III

Metabolism of Glucose-1-C^{14} and Glucose-6-C^{14} by Adipose tissue *in vitro* in Different Metabolic States*

	No. of Animals	CO_2		Glyceride-Glycerol		Fatty Acid		Glycogen	
		C-1	C-6	C-1	C-6	C-1	C-6	C-1	C-6
Fed + Insulin (0.1 unit/ml.)†	7	1.850 ± 0.218	0.225 ± 0.022	0.409 ± 0.033	0.584 ± 0.036	0.783 ± 0.138	1.482 ± 0.457	0.245 ± 0.037	0.264 ± 0.094
Fed†	38	0.353 ± 0.017	0.130 ± 0.005	0.323 ± 0.018	0.381 ± 0.012	0.056 ± 0.006	0.113 ± 0.016	0.013 ± 0.001	0.014 ± 0.001
Fasted twenty-four hours	3	0.153 ± 0.029	0.128 ± 0.017	0.237 ± 0.025	0.271 ± 0.019	0 015 ± 0.004	0.025 ± 0.005	0.008 ± 0.001	0.007 ± 0.001
Fasted seventy-two hours	3	0.090 ± 0.023	0.048 ± 0.007	0.166 ± 0.032	0.150 ± 0.020	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.004 ± 0.001
Alloxan-diabetic‡	4	0.026 ± 0.009		0.042 ± 0.020		0 ± 0		0.004 ± 0.001	

* Values in micromoles per mg. tissue nitrogen per three hours. Glucose 5 mM.
† Values from CAHILL, G. F., JR., LEBOEUF, B. and RENOLD, A. E.[11]
‡ Values for glucose-U-C^{14}.

TABLE IV

Metabolism of Glucose-1-C^{14} and Glucose-6-C^{14} by Adipose Tissue *in Vitro* in the Presence and Absence of Oxygen*

	Glucose Label	CO_2	Glyceride-Glycerol	Glycogen	Fatty Acids
O_2	C-1	1.064	0.435	0.017	0.238
	C-6	0.312	0.556	0.018	0.625
N_2	C-1	0.049	0.114	0.006	0.004
	C-6	0.028	0.082	0.007	0.009

* Krebs-Ringer-bicarbonate buffer gassed with 95 per cent O_2:5 per cent CO_2 or 95 per cent N_2:5 per cent CO_2 for ten minutes with the tissue present prior to the addition of labeled substrate. Glucose 5 mM. Values in micromoles substrate carbon per 350 mg. tissue per three hours. Mean of four tissues.

CONTROL OF ESTERIFICATION

Earlier in this report, glucose was described as accelerating the incorporation of palmitate-1-C^{14} into triglyceride. Other experiments have shown that adipose tissue from normally fed animals, is sensitive to glucose concentrations of 0.312 mM (5.6 mg. per cent) (Fig. 3). Thus a small amount of glucose is able to supply a significant amount of glycerolphosphate to effectively alter the fatty acid-triglyceride interchange in favor of esterification.

In Table II are tabulated the recoveries of glucose-C^{14} in total tissue components, in glyceride-glycerol and their per cent relationship. With low concentrations of glucose, in the absence of insulin, one-third or more of the glucose is recovered in glyceride-glycerol. As glucose uptake is increased by increasing concentrations of glucose, or to a greater degree, by addition of insulin to the medium, glyceride-glycerol synthesis becomes proportionately less important as a route for glucose metabolism.

In Table III are compared the metabolism of glucose-1-C^{14} and glucose-6-C^{14} in adipose tissue from fed rats, twenty-four and seventy-two hour fasted rats as well as the metabolism of glucose-U-C^{14} in adipose tissue from rats with alloxan-diabetes. As the metabolism of glucose becomes progressively less due to the fasted or diabetic state, a greater proportion is recovered in glyceride-glycerol. Likewise, incubation of adipose tissue in the absence of oxygen (Table IV) markedly reduces the total recovery of labeled glucose, with the least reduction in the synthesis of glyceride-glycerol.

GLYCERIDE-GLYCEROL *IN VIVO*

To determine whether the high rate of glyceride-glycerol turnover relative to the rate of

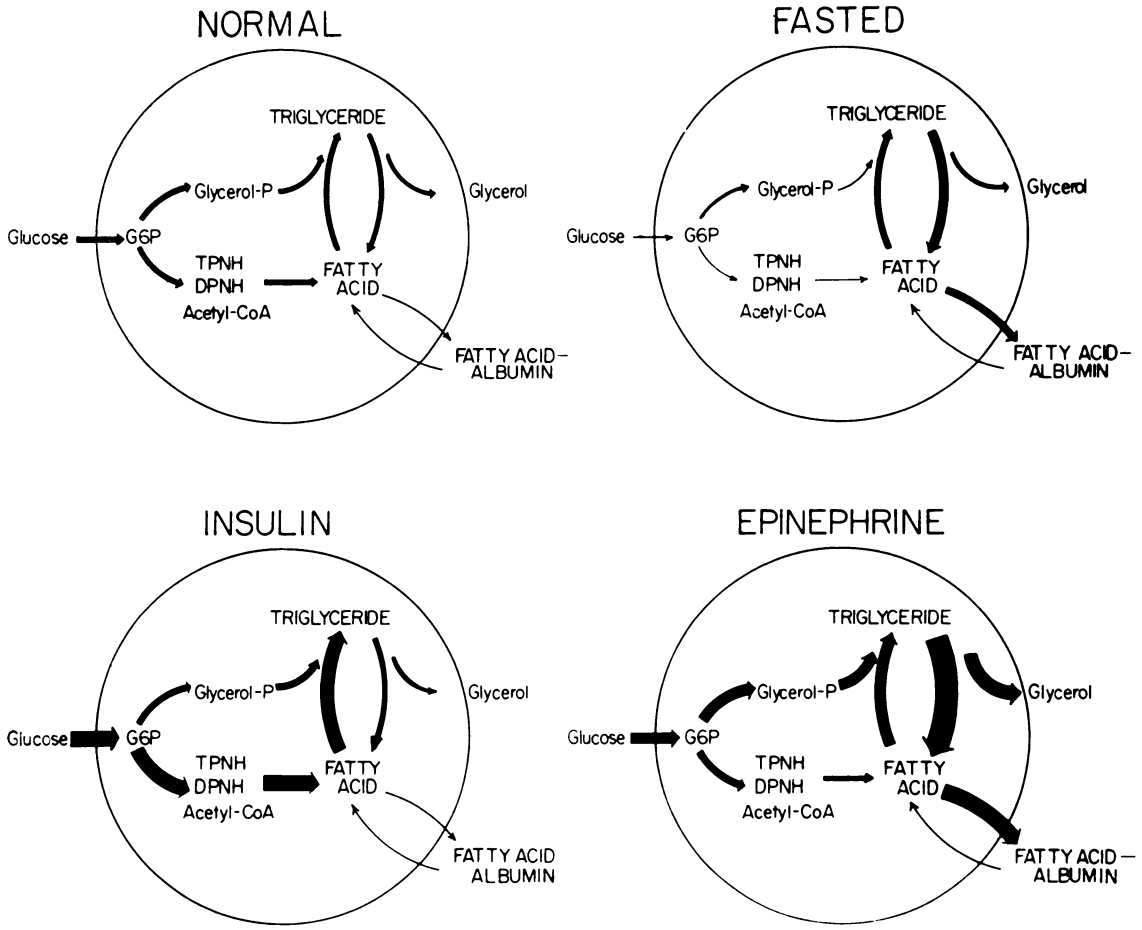

FIG. 4. Adipose tissue. A speculative summary of the concepts described in the text. In the normal rat glucose uptake is sufficient for the formation of glycerol-phosphate needed to esterify the fatty acids formed by lipolysis. In the fasted rat (or diabetic) glucose availability is decreased, consequently lipolysis outweighs esterification, fatty acids accumulate inside the cell and are released into the medium. With insulin, glucose uptake is much greater than that amount needed to esterify the fatty acids formed by lipolysis. The surplus glucose is converted to fatty acids and these also are esterified and deposited as triglyceride. After epinephrine, there is markedly accelerated rate of lipolysis, glucose uptake is increased as a response to the high concentration of fatty acids inside the cell, but the cell apparently does not re-esterify the major quantity of these fatty acids and they are released into the medium.

fatty acid synthesis was an *in vitro* artifact, uniformly labeled glucose-C^{14} was administered to rats which were killed one hour later. Table v summarizes data from these experiments and illustrates that twice as much glucose carbon may be recovered in glyceride-glycerol compared to the amount recovered in glyceride fatty acids. Since glycerol contains 3 carbons and the fatty acids in triglyceride an estimated 51 carbons, glyceride-glycerol is synthesized twenty to thirty times more rapidly than that amount needed to esterify the newly formed fatty acids.

COMMENTS

As glucose uptake is decreased in adipose tissue, whether by fasting, diabetes or anoxia, a greater proportion of glucose is recovered in glyceride-glycerol, suggesting that this pathway assumes first priority. Extrapolating from these observations to the total organism, a regulatory mechanism can be visualized whereby a decreased glucose uptake in adipose tissue allows the rate of lipolysis to exceed the rate of esterification due to lack of adequate glycerolphosphate formation. The smaller the glucose uptake, the greater the release of fatty

TABLE V

In Vivo Glyceride-Glycerol Synthesis from Glucose-U-C^{14}*

Rat	CPM/ 850 mg. Total Lipid	CPM/ mM Glycerol	CPM/ 3mM Fatty Acid	Recovery† of Counts (%)
A	6,000	4,770	2,190	116
B	10,500	3,990	5,130	88
C	5,400	3,930	1,730	105

* 125 gm. male rats injected intravenously with 55 mg. glucose containing 8 microcuries. Rats killed one hour later and epididymal fat excised and extracted.

† (CPM/μM glycerol + CPM/3μM fatty acid) ÷ CPM/850 mg. total lipid.

acids, in spite of the fact that the greatest proportion of the glucose is being converted to glycerolphosphate. As the glucose uptake is increased, whether by insulin or by increasing glucose concentrations, glycerol-phosphate synthesis increases until the rates of esterification and lipolysis are in a steady state, thereby inhibiting fatty acid release from the tissue. The surplus glucose is then metabolized by the adipose tissue for lipogenesis. These mechanisms are schematically illustrated in Figure 4.

Due to the exquisite sensitivity of this tissue to insulin, as measured by glucose metabolism, as perhaps occurs in mild or non-ketotic diabetes, only a small amount of insulin is sufficient to allow adequate glucose uptake, glycerolphosphate formation and triglyceride synthesis and thereby preventing the massive mobilization of fatty acids which would result eventually in ketoacidosis. On the other hand since the control of fatty acid release by the metabolism of glucose is presumably affected by chronic metabolic changes, the acute need for mobilization must be governed by increased lipolysis as suggested by the epinephrine studies *in vitro*[27] and discussed in this report.

SUMMARY

The rate of fatty acid release by adipose tissue is dependent on the relative rates of fatty acid esterification and lipolysis. Glucose is necessary for the esterification process, probably due to glycerolphosphate formation. Epinephrine and other hormones which accelerate fatty acid release appear to affect the rate of lipolysis. As glucose uptake is diminished, glyceride-glycerol formation becomes the major metabolic product of glucose utilization. As glucose uptake is increased, after glyceride-glycerol synthesis becomes sufficient to esterify the fatty acids, the surplus metabolized glucose is utilized for lipogenesis.

REFERENCES

1. SHAPIRO, B. and WERTHEIMER, E. Phosphorolysis and synthesis of glycogen in animal tissues. *Biochem. J.*, 37: 397, 1943.
2. WERTHEIMER, E. and SHAPIRO, B. The physiology of adipose tissue. *Physiol. Rev.*, 38: 451, 1948.
3. STETTEN, D., JR. and BOXER, G. E. Studies in carbohydrate metabolism. III. Metabolic defects in alloxan diabetes. *J. Biol. Chem.*, 156: 271, 1944.
4. HAUSBERGER, F. X., MII STEIN, S. W. and RUTMAN, R. J. The influence of insulin in glucose utilization of adipose and hepatic tissues *in vitro*. *J. Biol. Chem.*, 208: 431, 1954.
5. HAUSBERGER, F. X. Action of insulin and cortisone on adipose tissue. *Diabetes*, 7: 211, 1958.
6. FREDERICKSON, D. S. and GORDON, R. S. Transport of fatty acids. *Physiol. Rev.*, 38: 585, 1958.
7. RENOLD, A. E., MARBLE, A. and FAWCETT, D. W. Action of insulin on deposition of glycogen and storage of fat in adipose tissue. *Endocrinology*, 46: 55, 1950.
8. MILSTEIN, S. W. Oxidation of specifically labeled glucose by rat adipose tissue. *Proc. Soc. Exper. Biol. & Med.*, 92: 632, 1956.
9. WINEGRAD, A. I. and RENOLD, A. E. Studies on rat adipose tissue *in vitro*. I. Effects of insulin on the metabolism of glucose, pyruvate and acetate. *J. Biol. Chem.*, 233: 267, 1958.
10. WINEGRAD, A. I. and RENOLD, A. E. Studies on rat adipose tissue *in vitro*. II. Effects of insulin on the metabolism of specifically labeled glucose. *J. Biol. Chem.*, 233: 273, 1958.
11. CAHILL, G. F., JR., LEBOEUF, B. and RENOLD, A. E. Studies on rat adipose tissue *in vitro*. III. Synthesis of glycogen and glyceride-glycerol. *J. Biol. Chem.*, 234: 2540, 1959.
12. JEANRENAUD, B. and RENOLD, A. E. Studies on adipose tissue *in vitro*. IV. Metabolic patterns produced in rat adipose tissue by varying insulin and glucose concentrations independently from each other. *J. Biol. Chem.*, 234: 3082, 1959.
13. BALL, E. G., MARTIN, D. B. and COOPER, O. Studies on the metabolism of adipose tissue. I. The effect of insulin on glucose utilization as measured by the manometric determination of carbon dioxide output. *J. Biol. Chem.*, 234: 774, 1959.

14. HAGEN, J. M., BALL, E. G. and COOPER, O. Studies on the metabolism of adipose tissue. II. The effect of changes in the ionic composition of the medium upon the response to insulin. *J. Biol. Chem.*, 234: 781, 1959.
15. MARTIN, D. B., RENOLD, A. E. and DAGENAIS, Y. M. An assay for insulin-like activity using rat adipose tissue. *Lancet*, 2: 76, 1958.
16. GORDON, R. S. and CHERKES, A. Production of unesterified fatty acids from isolated rat adipose tissue incubated *in vitro*. *Proc. Soc. Exper. Biol. & Med.*, 97: 150, 1958.
17. WHITE, J. E. and ENGEL, F. L. A lipolytic action of epinephrine and norepinephrine on rat adipose tissue *in vitro*. *Proc. Soc. Exper. Biol. & Med.*, 99: 375, 1958.
18. CAHILL, G. F., JR., LEBOEUF, B., and FLINN, R. B. Studies on adipose tissue *in vitro*. VI. Effect of epinephrine on glucose metabolism. *J. Biol. Chem.*, 235: 1246, 1960.
19. RABEN, M. S. and HOLLENBERG, C. H. Effect of growth hormone on plasma fatty acids. *J. Clin. Invest.*, 38: 484, 1959.
20. ENGEL, H. P., HALLMAN, L., SIEGEL, S. and BERGENSTAL, D. M. Effect of growth hormone on plasma unesterified fatty acid levels of hypophysectomized rats. *Proc. Soc. Exper. Biol. & Med.*, 98: 753, 1958.
21. WINEGRAD, A. I., SHAW, W. N., LUKENS, F. D. W., STADIE, W. C. and RENOLD, A. E. Effects of growth hormone *in vitro* on the metabolism of glucose in rat adipose tissue. *J. Biol. Chem.*, 234: 1922, 1959.
22. WHITE, J. E. and ENGEL, F. L. Lipolytic action of corticotrophin on rat adipose tissue *in vitro*. *J. Clin. Invest.*, 37: 1556, 1958.
23. VAUGHAN, M., STEINBERG, D. and SHAFRIR, E. The effect of epinephrine, glucagon and adrenocorticotropic hormone (ACTH) on phosphorylase activity in adipose tissue. *J. Clin. Invest.*, 38: 1051, 1959. (Abstract.)
24. RICH, C., BIERMAN, E. L. and SCHWARTZ, I. L. Plasma nonesterified fatty acids in hyperthyroid states. *J. Clin. Invest.*, 38: 275, 1959.
25. FREINKEL, N. Personal communication.
26. BALLY, P., CAHILL, G. F., JR., LEBOEUF, B. and RENOLD, A. E. Studies on rat adipose tissue *in vitro*. V. Metabolism of palmitate-1-C^{14}. *J. Biol. Chem.*, 235: 333, 1960.
27. CAHILL, G. F., JR., JEANRENAUD, B., LEBOEUF, B. and RENOLD, A. E. Effects of insulin on adipose tissue. *Ann. New York Acad. Sci.*, 82: 403, 1959.
28. LEBOEUF, B., FLINN, R. B., and CAHILL, G. F., JR. Effect of epinephrine on glucose uptake and glycerol release by adipose tissue *in vitro* *Proc. Soc. Exper. Biol. & Med.*, 102: 527, 1959.
29. SHAPIRO, B., CHOWERS, I. and ROSE, G. Fatty acid uptake and esterification in adipose tissue. *Biochim. et biophys. acta*, 23: 115, 1957.
30. KORNBERG, A. and PRICER, W. E., JR. Enzymatic esterification of α-glycerophosphate by long chain fatty acids. *J. Biol. Chem.*, 204: 345, 1953.
31. BUELL, G. C. and REISER, R. Glyceride glycerol precursors in the intestinal mucosa. *J. Biol. Chem.*, 234: 217, 1959.
32. LEBOEUF, B., and CAHILL, G. F., JR. Studies on rat adipose tissue *in vitro*. VIII. Effect of preparations of pituitary adrenocorticotropic and growth hormones on glucose metabolism. *J. Biol. Chem.*, in press.