Transfer of fatty acids between triglyceride species in rat adipose tissue

C. H. HOLLENBERG*

McGill University Medical Clinic, The Montreal General Hospital, Montreal, Quebec, Canada

SUMMARY To study the rate of fatty acid transfer between triglyceride species in adipose tissue, palmitate-1-C^{14} was incorporated into adipose tissue triglyceride by incubation in buffer–glucose. Following extraction, the saturated triglycerides were precipitated in cold alcohol–acetone; this fraction contained over 40% of the incorporated label and 15% of the total tissue palmitic acid. Thus the specific activity of palmitic acid in this fraction was over 4 times that in predominantly unsaturated triglycerides. This relationship was unaltered by further incubation of tissue for 3 hr in fresh medium containing glucose–insulin only, or glucose–insulin plus unlabeled oleic acid.

Similar experiments using an in vivo incubation technique demonstrated that by two weeks after incubation the proportion of radioactivity in the saturated fraction had fallen only from 43 to 34%. This fall was apparently not due to greater fractional turnover of saturated triglycerides or to desaturation of palmitic acid, and thus was the result of slow redistribution of palmitate-C^{14} between predominantly saturated and unsaturated triglycerides. The data indicate that intermolecular rearrangement of triglyceride fatty acids in adipose tissue occurs slowly, and thus is more likely a result of hydrolysis of triglyceride and reesterification of fatty acids than of rapid, extensive transesterification.

KEY WORDS fatty acids · transesterification · triglyceride · adipose tissue · rat · free fatty acids · palmitate-1-C^{14} · diglyceride · saturated triglyceride turnover · esterification · lipolysis

MAMMALIAN ADIPOSE tissue is composed of a variety of triglyceride species differing in fatty acid composition and arrangement. Although the turnover rate of the entire tissue triglyceride pool has been studied (1, 2), little is known about the rate of transfer of fatty acids between the various component triglycerides.

The most clearly defined process which could lead to intermixing of triglyceride fatty acids involves hydrolysis of adipose tissue triglyceride by a lipase which is hormone-sensitive (3–6) and esterification of the fatty acid products to α-glycerophosphate derived from glucose metabolism (7–9). These reactions probably produce a relatively slow rearrangement of triglyceride fatty acids, since less than 2% of rat adipose tissue triglyceride is completely turned over daily (2). Esterification of fatty acids to lower glycerides would also allow acids derived from one triglyceride type to be incorporated into different species; the rate and magnitude of this reaction in fat tissue is not known. It has been suggested that adipose tissue lipid exists in a fluid state permitting inter- and intramolecular fatty acid exchange (10); this process, accelerated by tissue lipase, could lead to rapid interchange of triglyceride fatty acids.

To determine the rate of transfer of fatty acids between triglyceride species in adipose tissue, experiments were performed in which labeled palmitic acid was incorporated into adipose tissue triglyceride and the specific activity of palmitic acid in two chemically different triglyceride fractions was followed in time. Both in vitro and in vivo studies were performed.

METHODS

Male Wistar rats (175–200 g) maintained on Purina chow were used throughout the study.

In the experiments, in vitro, paired 200 mg pieces of epididymal fat were removed from animals anesthetized with sodium pentobarbital and the tissues incubated under air in 1.0 ml of medium in a Dubnoff shaker at 37°. The medium consisted of Krebs-Ringer phos-

* Markle Scholar in Medical Science.

phate buffer (pH 7.40) containing 1% human albumin pretreated to remove bound fatty acid (11), 200 mg/100 ml glucose, 10 mU/ml crystalline insulin (Lilly), and palmitic acid at a final concentration of about 1.1 mM. In experiments in which palmitic acid-1-C^{14} was used, the acid (Merck, Sharp and Dohme, Montreal, Quebec) was extracted into 0.05 N KOH in 50% ethanol, reextracted into petroleum ether after acidification of the ethanol, and, after evaporation of the petroleum ether, converted to the potassium salt by addition of a slight excess of KOH. This material was added to the required concentration of unlabeled potassium palmitate similarly purified, and the mixture heated until clear before adding it to the medium.

In the in vivo incubation studies, the system described by Stein and Stein (12) was used with only minor modifications. Under light ether anesthesia, the animals were placed on a board in a Dubnoff shaker and the exteriorized fat pads incubated in beakers containing 3.0 ml of the medium described above. The beakers were immersed in water at 37° and gently shaken throughout the experiment. After incubation the pads were either removed or replaced in the animal for later study; removal of the pads was accomplished by sectioning the tissue at its junction with the epididymis.

All tissues were extracted by the isopropanol–heptane–water system described by Dole (13); in the in vitro experiments the extracts contained 93% of the radioactivity lost from the medium during incubation. Aliquots of the extracts were applied to silicic acid columns for separation of triglycerides and free acids from lower glycerides (14); column recoveries were complete. Separate aliquots were used for isolation of free and glyceride acids (15) and the glyceride fractions saved for separation of predominantly saturated triglycerides from more unsaturated species by the method of Reiser et al. (16) as modified by Rodbell (17). In this procedure 100–200 mg of lipid was dissolved in hot ethanol–acetone 85:15 and the mixture allowed to stand at 4° overnight. The precipitate containing the saturated triglycerides was collected on a sintered glass filter and washed with cold ethanol–acetone, and the wash was added to the filtrate (unsaturated fraction). The precipitate (saturated fraction) was then dissolved by repeated washing with ethyl ether and collected separately. In preliminary experiments recovery of pure tripalmitin in the insoluble fraction exceeded 92%, while total recovery of label in all experiments averaged 97%. Lower glycerides in the extracts were recovered in the unsaturated fraction and an appropriate correction was made for this contribution in each instance.

Aliquots of all column, free fatty acid, and saturated and unsaturated fractions were taken for radioactive counting; portions of the saturated and unsaturated fractions were also used for measurement of triglyceride content (18) and for analysis of fatty acid composition by gas–liquid chromatography (19).

Counting was done in a Packard Tri-Carb liquid scintillation counter; the dried lipid extracts were dissolved in toluene or dioxane containing 0.6 g/100 ml 2,5-diphenyloxazole and 0.02 g/100 ml *p*-bis[2-(5-phenyloxazolyl)]-1-benzene.

TABLE 1 COMPOSITION OF SATURATED AND UNSATURATED TRIGLYCERIDE FRACTIONS

	Saturated Triglyceride		Unsaturated Triglyceride	
	Right Pad	Left Pad	Right Pad	Left Pad
Fatty Acid Composition, mole %				
14:0	3.8	3.9	2.7	2.7
16:0	62.6 ± 0.3*	62.3	27.2 ± 0.3	27.0
18:0	10.6	10.5	4.4	4.3
16:1	3.0	3.1	9.3	9.4
18:1	14.2 ± 0.2	14.3	33.7 ± 0.2	33.5
18:2	5.8	6.0	22.8	23.3
% of Total tissue triglyceride	6.1 ± 0.4	5.8 ± 0.3	93.9	94.2
% of Total tissue palmitic acid	13.0 ± 0.8	12.5 ± 0.6	87.0	87.5

Paired pads from 18 animals were analyzed. Each fatty acid is represented by two numbers, the first indicating chain length, the second, the number of double bonds. The percentage of the total tissue palmitic acid in the saturated and unsaturated triglyceride fractions was calculated from the percentage of the total triglyceride and percentage of palmitic acid in each fraction.

* Mean ± SE

RESULTS

IN VITRO EXPERIMENTS

Composition of Saturated and Unsaturated Triglyceride Fractions

The composition of the saturated and unsaturated triglyceride fractions separated by precipitation of the saturated species in cold alcohol–acetone is shown in Table 1. The saturated fraction comprised about 6% of the total triglyceride, was composed of 77% saturated acids, and contained about 13% of the palmitic acid of the total tissue triglyceride. When right and left pads were compared the results were identical and no change was induced by incubation of the tissue in buffer for 4 hr. Since over 20% of the acids in the alcohol–acetone insoluble fraction were unsaturated, this fraction contained not only all the fully saturated triglycerides but also a portion of the mixed triglycerides of the tissue, probably in the form of disaturated components (17).

TABLE 2 DISTRIBUTION OF TISSUE RADIOACTIVITY

Incubation Procedure	% of Tissue Radioactivity FFA	LG	TG
45–60 min with palmitate-1-C^{14} (6)*	2	15 ± 2†	83 ± 3
45 min with palmitate-1-C^{14}; 45 min with unlabeled palmitate (16)*	1	8 ± 1	91 ± 1

The concentrations of labeled and unlabeled palmitate varied from 1.0 to 1.3 meq/liter. FFA, free fatty acid. LG, lower glyceride. TG, triglyceride.
* Number of experiments.
† Mean ± SE

TABLE 3 DISTRIBUTION OF TRIGLYCERIDE RADIOACTIVITY

	Saturated Triglyceride Right Pad	Saturated Triglyceride Left Pad	Unsaturated Triglyceride Right Pad	Unsaturated Triglyceride Left Pad
% of Total triglyceride radioactivity	47* (43–60)	50 (47–52)	53	50
% of Total tissue palmitic acid	16 (13–20)	14 (10–18)	84	86
		Right Pad	Left Pad	
Specific activity palmitic acid	Saturated TG / Unsaturated TG	4.4 (3.0–5.7)	6.4 (4.7–8.0)	

Paired pads from 3 animals were incubated for 45 min each with palmitate-1-C^{14}, then with radioactive palmitate, and extracted.
* Mean and range.

Distribution of Tissue Radioactivity

Table 2 indicates the distribution of radioactivity between free fatty acids, lower glycerides, and triglycerides after incubating adipose tissue in two incubation systems. Incubation of the tissues for 45–60 min in a medium containing palmitate-1-C^{14} resulted in recovery of 15% of the label in lower glycerides. When the tissues were transferred at the end of this initial period of incubation to fresh medium containing unlabeled palmitate and incubation was continued for an additional 45 min, the proportion of label recovered in lower glyceride was halved and that found in triglyceride commensurately increased. It is likely that this change was due to completion of diglyceride esterification during the second period of incubation. Since it was considered desirable to have as much radioactivity as possible in the triglyceride fraction before determining triglyceride turnover, this sequential incubation system was used in most of the experiments to be described.

Distribution of Triglyceride Radioactivity between Saturated and Unsaturated Triglyceride

After paired pieces of adipose tissue had been incubated in the system described, the glyceride extracts were separated into saturated and unsaturated triglyceride fractions and the proportions of radioactive and total palmitic acid in each fraction were determined (Table 3).

Approximately 50% of the radioactivity was recovered in the saturated triglyceride fraction, which in these experiments contained about 15% of the total palmitic acid. Thus, the specific activity of palmitic acid in this fraction was five times that in the predominantly unsaturated triglycerides. During incubation the specific activity of fatty acid in the medium fell, and this dilution by predominantly unsaturated acids from the tissue probably accounted, at least in part, for the recovery of only 50% of the label in the saturated fraction. This suggestion is supported by the data shown in Table 4; addition of increasing amounts of oleic acid to the incubation medium progressively reduced the proportion of label found in the saturated fraction.

It is unlikely that rapid desaturation of palmitic acid contributed to the recovery of only half the radioactivity in predominantly saturated triglycerides. Previous studies using in vivo (1) and in vitro (17) incubation techniques have failed to demonstrate significant conversion of palmitic acid during intervals similar to those used in the present study.

Prolonged Incubation Studies

A number of studies were performed in order to determine the time required for palmitate-C^{14} in the two tri-

TABLE 4 DISTRIBUTION OF TRIGLYCERIDE RADIOACTIVITY FOLLOWING INCUBATION WITH MIXTURES OF PALMITIC AND OLEIC ACIDS

Experiment	Medium Composition % 16:0	Medium Composition % 18:1	% of Triglyceride Radioactivity Saturated TG	% of Triglyceride Radioactivity Unsaturated TG
A, *B*, *C*, Control	100	0	43 (39–46)	57
A	86	14	28	72
B	54	46	21	79
C	35	65	6	94

In *A* and *C*, one pad was incubated in palmitate-1-C^{14}, the other in palmitate-1-C^{14} and unlabeled oleate for 1 hr. No correction was made for lower glyceride radioactivity in the unsaturated fraction. In *B*, one pad was incubated for sequential 45 min periods in labeled, then unlabeled palmitate; and the other initially with palmitate-1-C^{14} and unlabeled oleate, and then with an identical mixture of nonradioactive palmitic and oleic acids. The unsaturated fraction was corrected for lower glyceride activity. In all experiments the total concentration of fatty acids in the medium varied from 0.9 to 1.3 meq/liter.

TABLE 5 DISTRIBUTION OF TRIGLYCERIDE RADIOACTIVITY FOLLOWING PROLONGED INCUBATION IN GLUCOSE–INSULIN

Experiment	Pad	Incubation Procedure: 1st Incubation	Incubation Procedure: 2nd Incubation	% of Total TG Radioactivity in Saturated TG
A. Fed (3)*	1	45 min palmitate-1-C^{14}; 45 min unlabeled palmitate	0	43 (40–46)
	2	Same	180–240 min glucose–insulin	40 (32–45)
B. Refed (1)	1	Same	0	43
	2	Same	210 min glucose–insulin	42
C. Fed (1)	1	20 min palmitate-1-C^{14}; 20 min unlabeled palmitate	0	43
	2	Same	180 min glucose–insulin	46

Both pads were incubated sequentially in labeled and unlabeled palmitate; one was then extracted and the second transferred to fresh buffer containing glucose and insulin (200 mg/100 ml, 10 mU/ml). The refed animal was fasted for 3 days and then fed for 2 days.

* Number of experiments.

TABLE 6 DISTRIBUTION OF TRIGLYCERIDE RADIOACTIVITY FOLLOWING SEQUENTIAL INCUBATION WITH PALMITIC AND OLEIC ACIDS

Pad	Incubation Procedure: 1st Incubation	Incubation Procedure: 2nd Incubation	% of Total TG Radioactivity in Saturated TG
1	45 min palmitate-1-C^{14}; 45 min unlabeled palmitate	0	48 (46–59)
2	Same	180 min, unlabeled oleic acid	50 (48–51)

Paired pads from 2 animals were incubated sequentially in radioactive and nonradioactive palmitate; one pad was then extracted and the other transferred to fresh buffer containing unlabeled oleic acid (1.1 mM), glucose, and insulin.

glyceride fractions to approach a distribution equivalent to that of unlabeled palmitic acid. Provided that the newly formed radioactive triglyceride was available for mixing with the existing tissue lipid, transfer of palmitic acid between the saturated and unsaturated fractions by any of the mechanisms previously outlined would be associated with a reduction in the proportion of label present in the saturated fraction and a fall towards unity in the ratio of the specific activities of palmitic acid in the two triglyceride fractions. Alternatively, if intermolecular rearrangement were not to occur, these parameters would remain unaltered.

In the first series of experiments paired pads were incubated in the system previously outlined. One pad was then extracted; the second was transferred to fresh buffer containing glucose and insulin only, and incubation was continued for a further 3–4 hr. The results of these studies, shown in Table 5, Experiment *A*, indicate that during the second incubation the proportion of label in the two fractions remained unaltered; the specific activity of palmitic acid in the saturated fraction thus remained nearly five times that of the unsaturated fraction. Similar results were obtained when tissues from refed animals were employed (Experiment *B*) or when a shorter initial incubation period was used (Experiment *C*). Hence under these experimental conditions no evidence of extensive transfer of radioactive palmitate between the two triglyceride fractions was noted.

Previous studies have shown that in similar incubation systems, equilibration of label between the glyceride esters of the particulate and bulk fat fractions of the adipose cell is complete 4 hr after beginning the incubation (12, 20). Because of the prolonged incubation times used it was thought unlikely that absence of turnover in the present study was due to complete anatomical segregation of the newly synthesized radioactive triglycerides from the bulk lipid. Nevertheless it was considered of interest to determine if mixing could be demonstrated between two varieties of triglyceride that would undoubtedly be located in the same cellular compartment.

Experiments were therefore performed in which, after the initial incubation interval, one pad was extracted and the second transferred to fresh medium containing unlabeled oleic acid, glucose, and insulin; incubation was continued for a further 3 hr. A pool of predominantly unsaturated triglycerides was thus formed which was presumably located in the same area of the cell as the radioactive triglycerides synthesized during the first incubation period. The results of these studies, shown in Table 6, indicate that the proportion of radioactivity in the saturated and unsaturated triglyceride fractions remained constant during the second incubation. Thus transfer of radioactive palmitate between newly synthesized saturated and unsaturated triglycerides had not occurred. The absence of an effect of oleic acid on distribution of triglyceride radioactivity noted in these experiments is in contrast to the marked effect on this distribution found when oleic and palmitic acids were presented to the tissue simultaneously (Table 4). The data presented in Tables 5 and 6 suggest that the establishment of equilibrium between the specific activities of the glyceride esters contained in the particulate

and bulk fat fractions may be achieved without significant intermixing of triglyceride fatty acids.

In Vivo Experiments

It is obviously difficult to extrapolate these in vitro studies to the intact animal. In order to approach the problem under in vivo conditions, to extend the time available for fatty acid transfer, and to eliminate uncertainties due to compartmentalization of newly synthesized triglyceride, a series of experiments was performed using the in vivo incubation technique described by Stein and Stein (12). Although by this procedure fatty acids are introduced into adipose tissue by nonvascular channels, these acids are, after 4 hr, handled in a fashion indistinguishable from the bulk lipid and are located almost exclusively in the bulk fat fraction.

In the first group of experiments both pads were incubated sequentially in labeled and in nonradioactive palmitate, after which either both pads were removed (Table 7, Experiment *A*) or else one was excised and the other replaced in the animal for 24 hr (Experiment *B*). Despite the double incubation procedure, 20% of the incorporated radioactivity was recovered in lower glyceride immediately after incubation; 24 hr later this fraction contained only a small proportion of the label. No redistribution of label between the two triglyceride fractions was evident over the 24 hr interval.

TABLE 7 Distribution of Radioactivity after In Vivo Incubation with Palmitate-1-C^{14}

Experiment	Pad	Time of Excision*	% of Tissue Radioactivity		% of Total TG Radioactivity in Saturated TG	% of Tissue 16:0 in Saturated TG
			LG	TG		
A (2)†	1	0	21	77	36	16
	2	0	18	80	34	15
B (2)	1	0	18	80	43	11
	2	1	7	92	45	13
C (2)	1	1	2	98	41	15
	2	1	3	97	41	16
D (4)	1	1	3	96	44 ± 2	15
	2	3	2	97	40 ± 2	15 ± 1
E (4)	1	1	4	95	43 ± 3	14 ± 2
	2	13	2	98	34 ± 3	14 ± 1

In experiments *A* and *B* both pads were incubated sequentially with labeled and nonradioactive palmitate for 30 min periods. In experiments *C*, *D*, and *E*, the pads were incubated with palmitate-1-C^{14} for 30 min. Mean values are shown together with standard errors where indicated.

* Days after incubation.

† Number of animals.

In the second series of experiments both pads were incubated in labeled palmitate and then replaced in the animal; 24 hr later both pads were either removed (Table 7, Experiment *C*) or one was excised and the other taken out 3 or 13 days after incubation (Experiments *D* and *E*). This approach allowed control observations to be made at a time when almost all the radioactivity was present in the triglyceride fraction. A small fall in the proportion of radioactivity recovered in the saturated triglyceride fraction was noted 72 hr after incubation (Experiment *D*); although this fall was more marked 13 days after incubation (Experiment *E*), the specific activity of palmitic acid in the saturated fraction at this time was still more than three times that in the predominantly unsaturated triglyceride pool. Recirculation of label during the 13 day period was negligible.

Although transfer of palmitic acid between the two triglyceride fractions was considered to be the most likely explanation of the change noted after 13 days, it was possible that differences between the two species in fractional turnover rates or conversion of palmitic acid to unsaturated acids could have contributed to the results obtained. To explore these possibilities, a series of experiments was performed in which the tissues were incubated in vivo in a mixture of palmitate-1-C^{14} and nonradioactive oleate; the proportions of each acid in the mixture were so chosen that the specific activities of palmitic acid in the saturated and unsaturated triglyceride fractions were equal.

Under these experimental conditions a relatively greater fall in the specific activity of palmitic acid in the saturated fraction could be due only to a more rapid turnover of this fraction or to extensive conversion of palmitic acid to unsaturated acids. The results of these studies, shown in Table 8, indicate that the ratio of the specific activities of palmitic acid in the two fractions remained unaltered 12 or 13 days after incubation. Although these data do not exclude the possibility that differences in turnover rates exist or that conversion of palmitic acid occurs, they suggest that these factors did not influence the redistribution of radioactivity noted over the 13 day interval. It is of interest in this regard that previous in vivo studies have shown that no desaturation of palmitic acid occurs up to 90 days after incorporation of the acid into adipose tissue (1).

DISCUSSION

The present study offers only an initial approach to the problem of intermolecular rearrangement of fatty acids in adipose tissue triglyceride. The techniques used did not permit observations of fatty acid turnover within the saturated and unsaturated triglyceride fractions, and the disposition of fatty acids synthesized within the tissue has not been determined. Although the scope of the study is

thus limited, the present data have, nonetheless, relevance to several aspects of adipose tissue metabolism.

Little is known about the relative importance of the various processes which can regulate the distribution of fatty acids between the many triglyceride species present in fat tissue. As previously mentioned, it has been proposed that this distribution is determined by random inter- and intramolecular exchange of fatty acids and that this random distribution is restricted only by the solubility of the saturated triglycerides (10). It is probable that an equilibrium reaction of this type involving most of the triglyceride molecules in the droplet would have resulted in fairly rapid equilibration of the specific activity of palmitic acid in the two triglyceride fractions. The results of the present study, while not eliminating the possibility that slow, limited exchange of acids occurs in adipose tissue, strongly suggest that the distribution of fatty acids between various types of triglyceride is not determined by rapid, extensive transesterification. Previous studies involving hydrolysis of mammalian triglyceride by pancreatic lipase have also failed to support a random pattern of esterification of fatty acids, since a nonrandom intramolecular arrangement of fatty acids was consistently found (21).

Recent studies of triglyceride turnover in adipose tissue indicate that approximately 40 days are required for half the triglyceride of this tissue to be completely hydrolyzed and re-formed (2). The prolonged period of time required for intermolecular rearrangement of fatty acids noted in the present study is thus more in keeping with a process involving hydrolysis of triglyceride, mixing of fatty acids, and reesterification of a portion of this pool than with a mechanism involving rapid exchange of acids. It is thus conceivable that the fatty acid composition of a triglyceride molecule in adipose tissue may remain unaltered from the time of synthesis until it is hydrolyzed by tissue lipases or until the acids are converted to other types.

If transesterification does not occur to a significant extent in adipose tissue, the concentrations of the various triglyceride species in this tissue must be determined by other factors, such as the composition of the fatty acids presented to the esterifying system, the interconversion of fatty acids, and possibly differences in fractional turnover rates between triglyceride types. The importance of free fatty acid composition is obvious and is well exemplified by the data presented in Table 4; this composition can be influenced by the pattern of acids synthesized and that produced by hydrolysis of adipose tissue and plasma triglyceride. The type of triglyceride formed will also be affected by differences between free acids in rates of release from and esterification in adipose tissue (19).

The results of the present study are in contrast to those noted when the turnover of exogenous lipoprotein triglyceride within adipose tissue was examined (17). In these experiments the fatty acids of predominantly saturated lipoprotein triglyceride were incorporated within hours into other species of triglyceride as a result of hydrolysis of the lipoprotein triglyceride by lipoprotein lipase and reesterification of the fatty acid products. Hence the fractional turnover rate of lipoprotein triglyceride taken up by adipose tissue is probably more rapid than that of stored triglyceride. This provides another piece of evidence that discrete pathways regulate the hydrolysis of triglyceride from these two sources.

TABLE 8 DISTRIBUTION OF RADIOACTIVITY AFTER IN VIVO INCUBATION WITH PALMITATE-1-C^{14} AND NONRADIOACTIVE OLEATE

Pad	Time of Excision	% of Total Radioactivity in Saturated TG	% of Tissue 16:0 in Saturated TG	S.A. Palmitic Acid Saturated TG / Unsaturated TG
1	1	12	13	0.9
2	12 or 13	11	13	0.8

Pads from 4 animals were incubated for 30 min in the usual medium containing 1.0 mmole/liter of fatty acids of which 30% was palmitate-1-C^{14} and 70% unlabeled oleate. Mean values are shown.

The author is grateful to Maria Faluhelyi and Ruth Osiek for their assistance. Dr. A. Angel and Dr. D. Roncari rendered valuable aid and criticism at various stages of the study.

This work was supported by Public Health Service Grant AM HE 07212-04 and Grant MT-972 from the Medical Research Council of Canada.

Manuscript received June 29, 1964; *accepted September* 2, 1964.

REFERENCES

1. Stein, Y., and O. Stein. *Biochim. Biophys. Acta* **60:** 58, 1962.
2. Gorin, E., and E. Shafrir. *Biochim. Biophys. Acta* **70:** 109, 1963.
3. Gordon, R. S., Jr., and A. Cherkes. *Proc. Soc. Exptl. Biol. Med.* **97:** 150, 1958.
4. White, J. E., and F. L. Engel. *J. Clin. Invest.* **37:** 1556, 1958.
5. Hollenberg, C. H., M. S. Raben, and E. B. Astwood. *Endocrinology* **68:** 589, 1961.
6. Rizack, M. A. *J. Biol. Chem.* **236:** 657, 1961.
7. Raben, M. S., and C. H. Hollenberg. *J. Clin. Invest.* **39:** 435, 1960.
8. Bally, P. R., G. F. Cahill, Jr., B. Leboeuf, and A. E. Renold. *J. Biol. Chem.* **235:** 333, 1960.
9. Steinberg, D., M. Vaughan, and S. Margolis. *J. Biol. Chem.* **236:** 1631, 1961.
10. Vander Wal, R. J. *J. Am. Oil Chemists' Soc.* **40:** 242, 1963.
11. Hollenberg, C. H. *Am. J. Physiol.* **197:** 667, 1959.
12. Stein, Y., and O. Stein. *Biochim. Biophys. Acta* **54:** 555, 1961.
13. Dole, V. P. *J. Clin. Invest.* **35:** 150, 1956.
14. Hirsch, J., and E. H. Ahrens, Jr. *J. Biol. Chem.* **233:** 311, 1958.

15. Borgström, B. *Acta Physiol. Scand.* **25:** 101, 1952.
16. Reiser, R., M. J. Bryson, H. S. Carr, and K. A. Kuiken. *J. Biol. Chem.* **194:** 131, 1952.
17. Rodbell, M. *J. Biol. Chem.* **235:** 1613, 1960.
18. Van Handel, E., and D. B. Zilversmit. *J. Lab. Clin. Med.* **50:** 152, 1957.
19. Hollenberg, C. H., and A. Angel. *Am. J. Physiol.* **205:** 909, 1963.
20. Kerpel, S., E. Shafrir, and B. Shapiro. *Biochim. Biophys. Acta* **46:** 495, 1961.
21. Mattson, F. H., and E. S. Lutton. *J. Biol. Chem.* **233:** 868, 1958.