LIPOGENESIS IN HUMAN AND RAT ADIPOSE TISSUE IN VITRO

by

Alfred Fessler, M.D.

A thesis submitted to the Faculty of Graduate Studies and Research, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Department of Biochemistry,
McGill University,
Montreal.

April, 1966.

Acknowledgements

The project of this thesis was directed by Dr. D. Rubinstein, Associate Professor of Biochemistry, McGill University, and Dr. J. C. Beck, Director of the McGill University Clinic and Physician-in-Chief of the Royal Victoria Hospital. I am deeply indebted to both of them for their advice and encouragement.

I am also most grateful to Dr. Beck for providing me with financial assistance and space in his laboratories.

Dr. Rubinstein has been both a mentor and a friend and I greatly appreciate all his efforts on my behalf.

I wish to thank Dr. J. Dupre for carrying out the experiments with isolated adipocytes and proof-reading a part of this thesis. Drs. P. Weldon and T. Anastassiades kindly contributed to the proof-reading.

My thanks also go to Miss E. Mader who did the typing.

My wife, Agnes, contributed more than a fair share to the completion of this thesis and I dedicate it to her with apologies.

I would also like to acknowledge the support of the Medical Research Council of Canada for twice awarding me with a Fellowship.

Table of Contents

Abbreviations

AMP, ADP, ATP	adenosine mono-, di-, triphosphate
CoA	coenzyme A
DNA	deoxyribonucleic acid
DPN(H)	diphosphopyridine nucleotide (reduced)
FFA	free fatty acids
FMN(H)	flavin mononucleotide (reduced)
g	gravity
α-GP	α-glycerol phosphate
GSH	glutathione (reduced)
3-MG	3-0-methyl glucose
m	milli
M	molar
N	normal
N.L.	neutral lipid
N.S.	not significant at the 0.05 level
Pi	inorganic phosphate
POPOP	1,4-bis-2(5-phenyloxazolyl)-benzene
PP	pyrophosphate
PPO	2,5-diphenyloxazole
TPN(H)	triphosphopyridine nucleotide (reduced)

I. PREFACE

The work described in this thesis is a part of a larger project on the metabolism of mammalian tissues *in vitro* and the effect of hormones thereon, which is under progress at the McGill University Clinic, Royal Victoria Hospital, Montreal.

The original intention had been to first establish parameters for the utilization of glucose for lipid synthesis by human adipose tissue, and then to extend the investigations to tissue from obese and diabetic patients. A technique, developed by Dr. J. Hirsch of the Rockefeller Institute, by which metabolically active fragments of human subcutaneous adipose tissue could be obtained by needle biopsy held great promise for the project (1,2). However, the data obtained with this technique, and kindly supplied by Dr. Hirsch prior to publication, showed that human adipose tissue was in most cases unresponsive to the biosynthetic effects of insulin. This seemed unusual, as the stimulatory effect of insulin on the utilization of carbohydrate by rat adipose tissue has led to views that adipose tissue is a major site for this action of this hormone (3,4). In man, excessive blood insulin concentrations are frequently associated with obesity (5-8). Furthermore, human adipose tissue (1,2,9-11) resembles rat adipose tissue (12,13) in that it carries out lipogenesis from glucose and acetate and the esterification of fatty acids. It has been shown that insulin stimulates the uptake of glucose by human adipose tissue *in vivo* (14), as well as *in vitro* (15,16).

In view of these considerations, the effect of insulin on glucose metabolism by human adipose tissue from surgical patients, and some of the factors which influence it, was investigated. The results obtained in turn led to studies on lipogenesis by rat adipose tissue in vitro.

A part of the results has been presented at scientific meetings:

a) Montreal Physiological Society, December 1964;

b) Canadian Society for Clinical Investigation, January 1965;

c) American Federation for Clinical Research, May 1965; abstract in Clinical Research: 13, 322, 1965 (17).

A short communication entitled "The Effect of Insulin on the Metabolism of Human Adipose Tissue in vitro" has appeared in the Biochim. Biophys. Acta: 106, 199, 1965 (18).

II. INTRODUCTION

Until the appearance of an article by Wertheimer and Shapiro in 1948 (19), adipose tissue evoked little interest as a physiological and biochemical entity. Developments in this field became very rapid after Hausberger et al. (20), Feller (21) and Favarger and Gerlach (22,23) demonstrated lipogenesis in adipose tissue. The vast literature on adipose tissue, accumulated up to 1964, culminated in the appearance of Section 5 of the Handbook of Physiology (24), a volume which lists well over 4000 references. Taken together with the reports which have been published in the last two years, it is obvious that a comprehensive review of adipose tissue metabolism would be more than a monumental task for any one reviewer. The short review presented here is therefore limited to the subject of lipogenesis, i.e. fatty acid synthesis.

A. Mechanisms of Lipogenesis.

Research in lipogenesis up to 1953 established that certain features in this process are common to animal tissues and micro-organisms. The most important of these is undoubtedly the utilization of a two-carbon unit - the acetyl group - as the building stone for fatty acids. Thus, it was shown that acetate or metabolites which form acetate can give rise to fatty acids (25-27), that all the carbons of straight chain, even numbered fatty acids originate from acetate (28-30), and that the two carbons of acetate are incorporated intact (28,31). Fatty acids are produced by the head-to-tail linkage of acetyl groups (30,31). This process was initially regarded as a reversal of the steps in oxidation of fatty acids, a mitochondrial process (32). Subsequent studies with purified enzymes and cell-free preparations indicated that the subcellular localization of certain lipogenic systems and their cofactor requirements are not in agreement with this mechanism of synthesis (33,34). In the last few years, a number of lipogenetic systems, differing from each other in subcellular origin, mechanism of action, cofactor requirements and products have been described (35). These systems are of two types. The first synthesizes fatty acids, especially palmitate, de novo from acetyl-CoA. The second elongates fatty acids of medium or long chain length, and is also associated with the desaturation of fatty acids.

De novo synthesis of fatty acids.

The enzymes which carry out de novo synthesis from acetyl-CoA are chiefly located in the high speed supernatant fraction of homogenates of a variety of cells and tissues such as liver (36,37), brain (38), adipose tissue (33,39), yeast (40) and bacteria (41,42). The discovery of malonyl-CoA as the critical intermediate in de novo synthesis (43) indicated that two constituent reactions are involved in lipogenesis. In the first reaction, the enzyme acetyl-CoA carboxylase catalyzes the fixation of CO_2 to acetyl-CoA to form malonyl-CoA:

$$ATP + CO_2 + \text{Biotin-Enzyme} \xrightarrow{Mn^{++}} CO_2\text{-Biotin-Enzyme} + ADP + Pi \quad 1a.$$

$$CO_2\text{-Biotin-Enzyme} + CH_3CO\text{-SCoA} \xrightarrow{Mn^{++}} \text{Biotin-Enzyme} + COOHCH_2CO\text{-SCoA} \quad 1b.$$

$$ATP + CO_2 + CH_3CO\text{-SCoA} \xrightarrow{Mn^{++}} COOHCH_2CO\text{-SCoA} + ADP + Pi \quad 1.$$

Acetyl-CoA carboxylase, a biotin enzyme, is inhibited by avidin (44,45). Reaction 1 is stimulated by intermediates of the Krebs cycle (44,46). Of these, citrate is by far the most stimulatory on partially purified preparations from rat adipose tissue (44). It has been demonstrated that this stimulation is not due to the formation of metabolites or derivatives of citrate, or to its chelating properties. It is uncertain whether citrate stimulates only the half-reaction 1b (47), or both 1a and 1b (48). In the presence of citrate, acetyl-CoA carboxylase undergoes conformational changes, as indicated by an increase in the sedimentation rate in sucrose gradient centrifugation (49).

The second enzyme system, fatty acid synthetase, catalyzes the condensation of acetyl-CoA with malonyl-CoA to form fatty acids (39). The stoichiometry for the synthesis of palmitate is shown in reaction 2:

$$CH_3COS\text{-}CoA + 7\ COOHCH_2CO\text{-}SCoA + 14\ TPNH \longrightarrow CH_3(CH_2)_{14}COOH + 7\,CO_2 + 8\,CoASH + 14\,TPN + 6\,H_2O \quad 2.$$

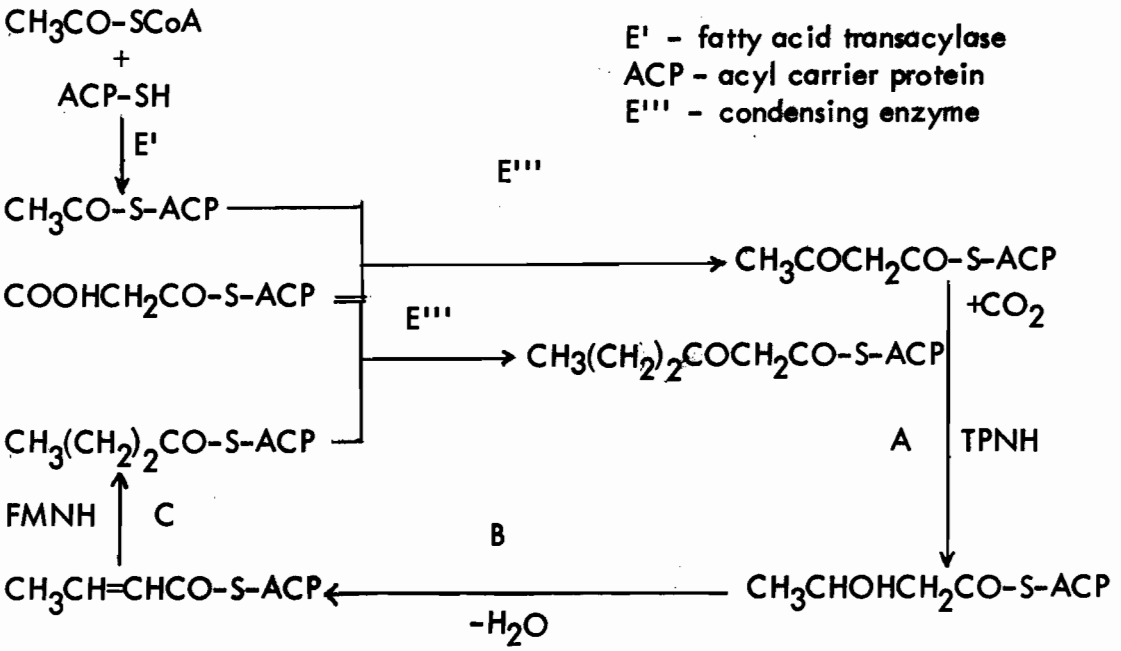
As condensation takes place CO_2 is lost from the free carboxyl of malonyl CoA (38,50). In highly purified preparations of fatty acid synthetase only acetyl-CoA or propionyl-CoA react with malonyl-CoA (40,51). The acetyl-CoA forms the methyl end of the initial condensation product, and fatty acids are formed by stepwise decarboxylation-condensation with malonyl-CoA and reduction of the product (39,52). There are two reductive steps in the conversion of the condensation product, a ß-keto acyl derivative, to the acyl derivative (34). TPNH is an absolute requirement for these reductions by purified fatty acid synthetase from rat adipose tissue and DPNH cannot substitute for TPNH (39). Intermediates in lipogenesis appear to be bound to protein, and CoA derivatives of intermediate products have not been detected (33,40). Unlike the synthetase from bacteria, the enzyme from yeast and animal sources behaves as a complex and attempts to fractionate it have failed (33,35). Figure 1 shows the sequence of reactions in lipogenesis with bacterial (17) and yeast enzyme (53) preparations.

The synthetase complex from E. coli has been fractionated into acyl carrier protein (ACP), acyl-CoA-ACP transacylases, condensing

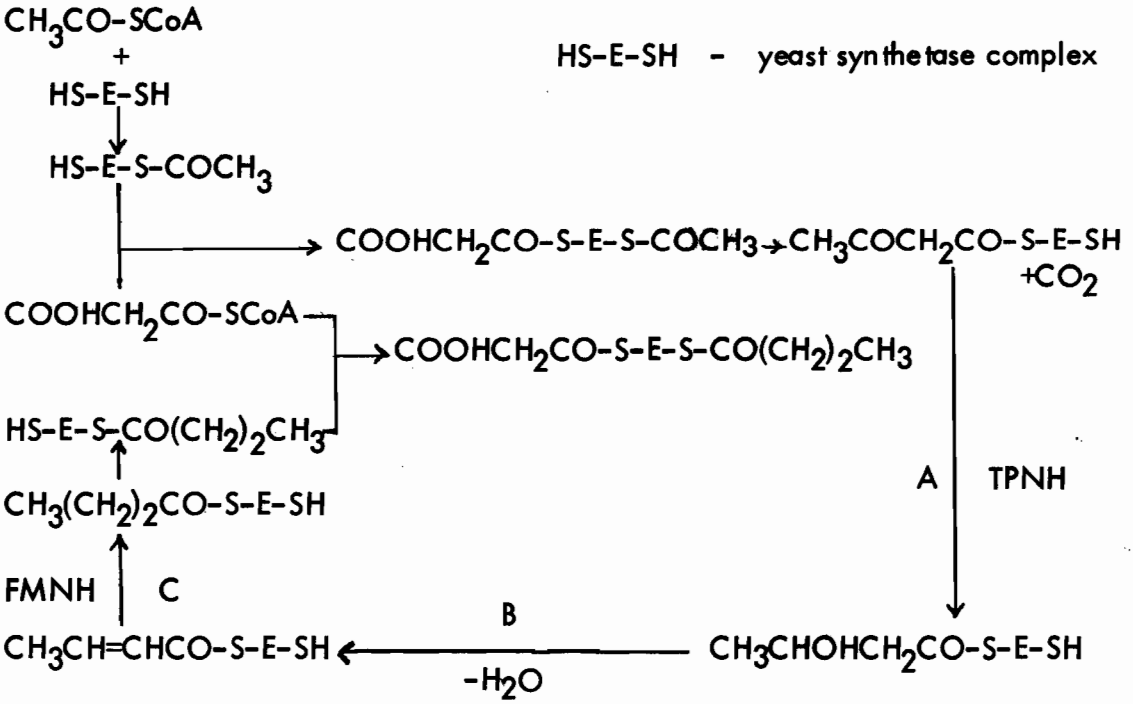
Figure 1

Mechanisms of Action of Fatty Acid Synthetase (33,35).

A. From E. coli



B. From Yeast



A - ß-ketoreductase
B - ß-hydroxylacyl dehydrase
C - enoyl reductase

enzyme, ß-keto-acyl-ACP reductase and enoyl-ACP (de)hydrase; the enoyl-ACP reductase reaction has been demonstrated but the enzyme has not been purified (54). The transacylases convert both acetyl-CoA and malonyl-CoA to the ACP derivatives (55,56). Condensation-decarboxylation of acetyl- and malonyl-ACP is carried out by the condensing enzyme (57). During subsequent steps in lipogenesis, the intermediates remain bound to ACP by thioester linkage (56,58,59). CoA derivatives of intermediates are not utilized for lipogenesis (54). Majerus et al. (59) and Pugh and Wakil (60) have worked out the structure of ACP. As in the case of acetyl-CoA, 4'-phosphopantetheine of ACP provides the sulfhydryl group to which linkage occurs (61).

Lynen (53) has isolated the synthetase complex from yeast as a homogeneous protein. Disruption of the complex leads to loss of activity. According to the scheme proposed by Lynen, there are two types of sulfhydryl groups in the complex, as indicated by HS-E-SH in Figure 1b. A central SH group (HS-E-) reacts with malonyl-CoA and the condensation decarbozylation and reductive steps occur here. The product of these reactions is transferred to a peripheral SH group (-E-SH) before another molecule of malonyl-CoA reacts with the central SH group. The cycle is then repeated until palmitate is formed.

Although attempts to fractionate it have so far been unsuccessful (33), mammalian synthetase has been shown to carry out some of the reactions already described. In contrast to the synthetase from

liver (36,62) and yeast (40), partially purified preparations from rat brain (38,63) and adipose tissue (64) can incorporate acetoacetyl-CoA, ß-hydroxybutyryl-CoA and crotonyl-CoA into long chain fatty acids. Acetoacetyl-CoA is, like acetyl-CoA, incorporated into the methyl-end of fatty acids. If TPNH is omitted from the reaction mixture, elongation does not occur, and condensation-decarboxylation must therefore precede reduction. In animal tissues both reductive steps require TPNH (33). The second reductive step with yeast (40) and bacterial enzyme (41) utilizes reduced FMN generated from TPNH or DPNH.

E. coli ACP derivatives of the four-carbon intermediates can readily substitute for the CoA derivatives in mammalian enzyme systems (56). A protein similar in function to the bacterial ACP has been implicated in lipogenetic systems from animal (65) and plant tissue (66), but mammalian ACP has not yet been identified. It is possible that the ACP derivatives react with mammalian enzymes simply because of the end-group similarities between ACP and CoA. One major difference between bacterial, and the mammalian and yeast synthetase is that the first is soluble, whereas the other two can be sedimented by centrifugation at 140,000 x g (33). On the other hand, the sedimentable enzyme systems also differ from each other. Thus the reductase component co-purifies with the yeast synthetase (53) but not with adipose tissue synthetase (33). Furthermore, the inability of rat liver synthetase to utilize acetoacetyl-CoA for lipogenesis (36), while the rat adipose tissue enzyme readily incorporates this substrate (64), indicates the presence of different synthetases

in the same animal.

The enzymes associated with de novo synthesis of fatty acids, which have been discussed up to this point, are not believed to be of microsomal of mitochondrial origin.

The elongation and desaturation of fatty acids.

Palmitate is the major fatty acid synthesized by purified fatty acid synthetase from supernatant preparations of tissue homogenates (39). However, in intact tissue (69-72) and crude homogenates (11,73) a greater variety of fatty acids is produced although palmitate predominates. Dauben et al. (30) observed that palmitate isolated from tissues of animals injected with acetate-1-^{14}C was uniformly labelled in alternate carbon atoms, while stearate and oleate were more highly labelled in the carboxyl carbon. This suggested the presence of pathways by which fatty acids longer than palmitate could be formed. Harlan and Wakil (67,68) studied lipogenesis with liver microsomes and mitochondria, and found that acetyl-CoA was incorporated into a variety of long chain fatty acids, especially unsaturated acids having a chain length of C18 and C20. The fatty acid produced were of a type and pattern similar to those found in phospholipids (74). As the carboxyl end of these fatty acids was disproportionately heavily labelled, they concluded that elongation of shorter chain fatty acids had taken place. In distinction to the mechanism of de novo synthesis, lipogenesis by cell particles did

not require CO_2 or Mn^{++}, both DPNH and TPNH were suitable as reducing cofactors, and the system was not inhibited by avidin. Some of these observations indicated that acetyl-CoA carboxylase is not involved in the elongation mechanism, and indeed malonyl-CoA was not required. When malonyl-CoA was added to the particulate system or O_2 was eliminated from the gas phase, only saturated fatty acids were formed. In the presence of oleyl-CoA, the incorporation of acetyl-CoA into fatty acids by liver mitochondria is stimulated. The condensation of acyl-CoA with acetyl-CoA was neither ATP-dependent nor sensitive to avidin. A similar system has been described in particles from avocado mesocarp.(75).

Gibson (34) and Wakil (51) have pointed out that the equilibrium constant for the thiolase reaction in the condensation of two molecules of acetyl-CoA is of the order of 10^{-4}, which would limit the reaction in the direction of acetoacetyl-CoA synthesis. The enzymatic mechanisms in the elongation of fatty acids are therefore problematical and their elucidation awaits the purification and characterization of the enzymes involved.

Nugteren (76) has recently reported the presence of an elongation system in liver microsomes which is malonyl-CoA and TPNH dependent, and therefore different from that of Harlan and Wakil (67,68). This system elongated medium and long chain saturated acids, as well as long chain polyunsaturated acyl CoA derivatives to higher fatty acids. In contrast to the findings with systems which synthesize fatty acids

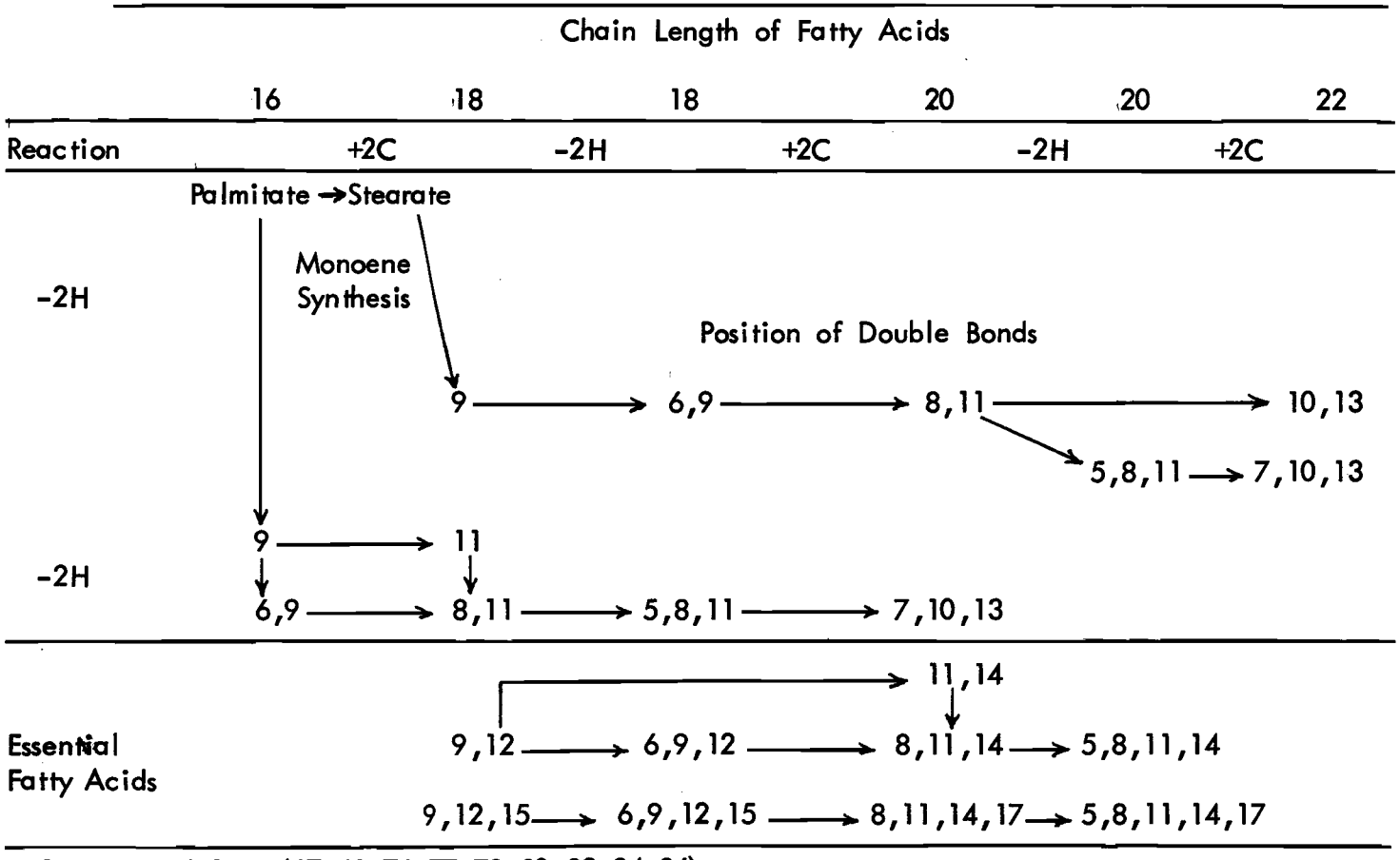
de novo, Nugteren was able to detect CoA esters of the intermediates formed during the chain elongation of myristyl CoA.

On the basis of experiments with intact rats, Mead (77) proposed that elongation is an integral part of the pathway leading to the formation of unsaturated acids. Using liver mitochondria, Harlan and Wakil (67,68) provided evidence that elongation of fatty acids with acetyl-CoA alternates in a stepwise fashion with desaturation as homologous higher fatty acids are formed. The desaturation of saturated long chain fatty acids has been demonstrated in yeast (78) and liver (76,79,80), plant tissue (81) and adipose tissue (69,80). Elovson (80) found that desaturation and elongation are less prominent in extra-hepatic tissues, including adipose tissue, than in liver. Molecular oxygen and TPNH or DPNH are required for the desaturation of saturated fatty acids to monoenes in tissues (69) and cell-free preparations (82, 83). Similar requirements hold for the desaturation of mono-, di- and triunsaturated fatty acids (84-87).

The pathways for the elongation and desaturation of long chain saturated and unsaturated fatty acids in the rat, summarized from the work of several investigators (67,68,76,77,79,80,82-84,86) are given in Figure 2. The scheme is essentially based on the inability of rat tissue to introduce double bonds into the carbon chain between the first double bond and the methyl end of the chain. Thus, oleic acid ($C18:1^{\Delta 9}$) cannot be converted to linoleic acid ($C18:2^{\Delta 9,12}$), an essential fatty

Figure 2

Elongation-Desaturation of Long Chain Fatty Acids in the Rat[a]



a-Summarized from (67,68,76,77,79,80,82-84,86).

acid. Desaturation occurs only towards the carboxyl end, of the fatty acids, γ to previous double bonds.

Although the pathways of lipogenesis have been delineated to some extent on a qualitative basis, little information is as yet available on the interaction between the de novo and the elongation-desaturation systems in animal tissues. However, the predominance of palmitate synthesis from glucose and acetate in animal tissues (50-53), strongly suggest that lipogenesis from these substrates occurs mainly by de novo synthesis. Hollenberg (89) recently reported that 80% of the glucose label incorporated into fatty acids by rat adipose tissue is stored in wholly saturated triglycerides, and that no change occurred in the distribution of fatty acids over a period of weeks. The conversion of saturated to unsaturated fatty acids must therefore be limited, and the principal function of the elongation-desaturation pathway(s) in adipose tissue may be to process short or medium chain fatty acids obtained from dietary sources.

B. The Source of Substrate for Lipogenesis.

Fatty acids are synthesized from acetyl-CoA units, and therefore the source of substrate for lipogenesis is essentially the same as the source of acetyl-CoA. As it stands at the crossroads of cell metabolism, acetyl-CoA can be formed from carbohydrates, amino acids, fatty acids and ketone bodies.

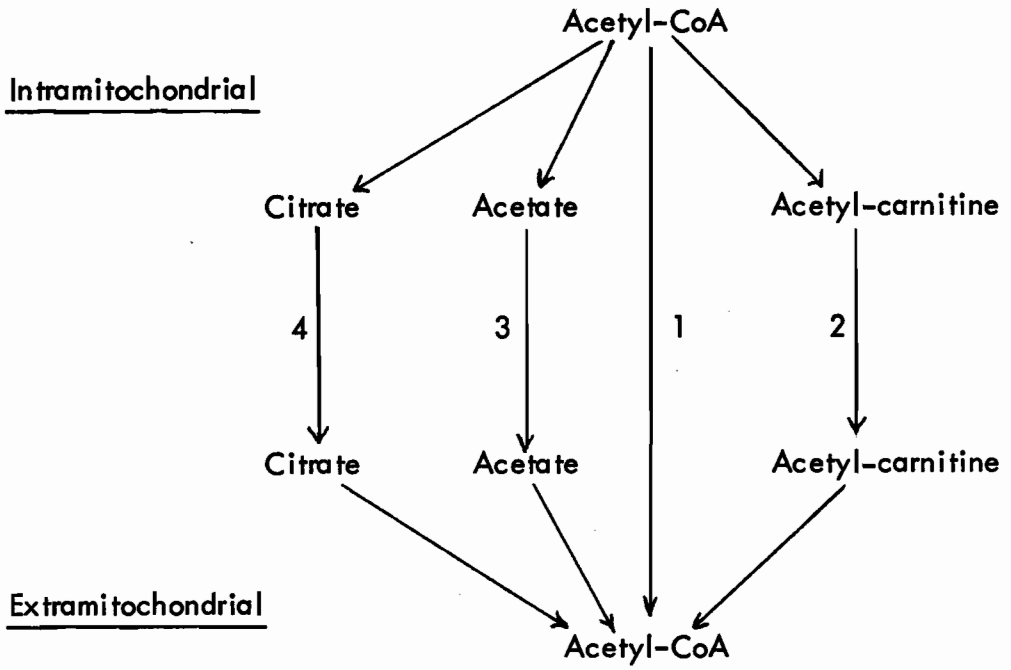
The production of acetyl-CoA from pyruvate derived from glucose is a mitochondrial process (90,91) and acetyl-CoA must be transferred to the extramitochondrial site of lipogenesis (92-94). Figure 3 (modified from Lowenstein) shows the pathways by which the translocation of acetyl-CoA may be accomplished(94).

The first pathway, direct diffusion of acetyl-CoA across the mitochondrial membrane, is too slow to account for the observed rates of lipogenesis. Thus, labelled acetyl-CoA incubated with mitochondria is oxidized much more slowly than acetate (95), and loss of endogenous CoA or acetyl-CoA from isolated intact mitochondria does not occur until they begin to swell after prolonged standing (94). TPN, a coenzyme similar in size and charge to acetyl-CoA, moves only slowly across the mitochondrial membrane (96).

Acetyl carnitine, a high-energy ester of acetate, has been proposed as the product transferred (97-99). In this pathway, acetyl-CoA produced in the mitochondria is converted to acetyl carnitine by the enzyme acetyl-CoA-carnitine transferase, the carnitine ester leaves

Figure 3

Possible Pathways for the Transfer of Acetyl Groups Across the Mitochondrial Membrane.



the mitochondria, and acetyl-CoA is regenerated by extramitochondrial acetyl-CoA-carnitine transferase. Bressler and his co-workers (100-102) showed that carnitine stimulates the conversion of pyruvate to fatty acids by whole liver homogenates about twofold. Pretreatment of animals with carnitine led to increased incorporation of intraperitoneally injected pyruvate-2-^{14}C into fatty acids of liver and adipose tissue. Carnitine had no effect on lipogenesis from citrate in vivo or in vitro, probably due to direct cleavage of citrate by the extramitochondrial citrate cleavage enzyme. These findings are in agreement with a transport function of acetyl carnitine in lipogenesis. However, in contrast to the observations of Fritz (95) and of Norum (97), Lowenstein (94) as well as Klingenberg and his co-workers (103,104), have been unable to demonstrate acetyl-CoA-carnitine transferase activity in homogenate supernatants. Lowenstein (94) has also questioned the role of acetyl carnitine in lipogenesis by rat mammary gland supernatants because of the poor utilization of this substrate for lipogenesis in comparison to citrate and acetate. These conflicting findings remain to be reconciled.

The third form in which acetyl groups may move out of mitochondria is as free acetate. This mechanism requires the hydrolysis of mitochondrial acetyl-CoA to acetate (105), and the diffusion of acetate into the extramitochondrial space where it is re-esterified with CoA by acetyl thiokinase:

$$\text{Acetate} + \text{ATP} + \text{CoA} \longrightarrow \text{Acetyl-CoA} + \text{AMP} + \text{PP}$$

Acetyl thiokinase has been found in the intra- and extramitochondrial compartments of the cell (106).

In recent years, the role of citrate cleavage enzyme, citrate and precursors of citrate in lipogenesis has drawn widespread attention. Srere (107) suggested in 1959 that citrate may be a source of acetyl-CoA for biosynthetic reactions. The cleavage of citrate proceeds by the reaction:

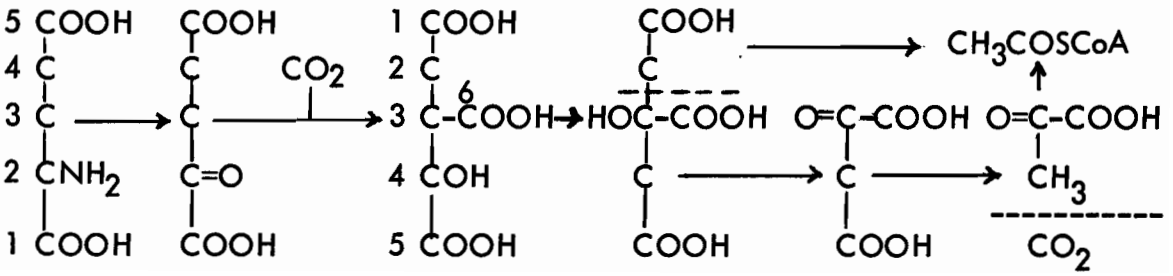
$$\text{Citrate} + \text{ATP} + \text{CoA} \longrightarrow \text{Acetyl-CoA} + \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$

The loss of high energy phosphate per mole of acetyl-CoA generated is half that required for the activation of acetate by thiokinase; the pathway proposed via acetyl carnitine does not require ATP. The incorporation of citrate carbon into fatty acids was subsequently demonstrated in liver (108,110), mammary gland (94, 111) and adipose tissue (112). Bhaduri and Srere (109) and Spencer and Lowenstein (92) have proposed that citrate generated in the mitochondria diffuses into the extramitochondrial space where it is cleaved. The diffusion of citrate across the mitochondrial membrane is not believed to be rate-limiting for lipogenesis (94).

Lowenstein and his co-workers have studied the activity of acetyl thiokinase and citrate cleavage enzyme in livers from normal and diabetic rats under a variety of conditions (93,94). Both enzymes are equally active in tissue from normal animals (94). Starvation and induction of alloxan-diabetes depressed the activities of these enzymes

to the same extent. However, under conditions which promote lipogenesis, such as feeding a diet high in carbohydrate after a 48-hour fast, the increase in the activity of citrate cleavage enzyme was much more marked than that of acetyl thiokinase, and the values exceeded the control levels. Insulin treatment of diabetic animals resulted in similar changes. It is of interest that the activity of both enzymes increased in untreated diabetic animals fed diets high in fructose content. On the basis of these results, Lowenstein has attributed great importance to the generation of acetyl-CoA from citrate for lipogenesis.

Lardy et al. (113) have suggested that glutamate rather than citrate diffuses out of the mitochondria, and is then converted to acetyl-CoA via α-ketoglutarate, oxalosuccinate and citrate, the so-called 'backward reactions' of the Krebs cycle of Madsen et al. (114). This pathway has been demonstrated in slices of mammary gland (114, 115), adipose tissue (112,116) and in perfused liver (117) using specifically labelled glutamate. When glucose was omitted from the incubation medium, little of the glutamate carbon appeared in fatty acids and glutamate was preferentially oxidized to CO_2 (114,116), according to the usual direction of the Krebs cycle. The distribution of glutamate carbon in the intermediates of the 'backward reactions' is given by the following reaction sequence:



Madsen et al. (116) have calculated that in adipose tissue incubated with glucose, 17% of the glutamate metabolized by the Krebs cycle proceeded by the 'backward reaction', and this increased to 34% when insulin was present. Carbon-1 of glutamate was not incorporated into fatty acids. Both carbon-5 and carbon-2 of glutamate appeared in fatty acids in a ratio of 3 to 1, indicating that oxaloacetate derived from glutamate via citrate is also utilized in lipogenesis. Leveille (112) and Hanson have confirmed these findings and have also demonstrated lipogenesis from aspartate-3-^{14}C and glutamate-3,4-^{14}C. In fasted, carbohydrate-refed and in mealfed rats lipogenesis from these substrates was considerably increased.

Hanson and Ziporin (118), using mouse adipose tissue, have studied the conversion of ketone bodies to fatty acids. Factors which stimulate lipogenesis from precursors of acetyl-CoA also stimulate lipogenesis from ketone bodies. It is not known whether ketone bodies are first converted to acetyl-CoA or are directly incorporated into fatty acids.

Feller (119) has reviewed the subject of utilization of amino acids for lipogenesis in adipose tissue. The ability of amino acid carbon to enter lipogenetic pathways appears to depend on the type of products formed from amino acids during their degradation. Of the amino acids tested, leucine, which is metabolized to acetyl-CoA and acetoacetate, was the most actively incorporated. The amino acids which yield acetate

are more actively converted to lipid than those which yield propionate. It is not known to what extent amino acid degradation and other sources of acetyl-CoA contribute to lipogenesis. However, Flatt and Ball (120) have determined that less than 3% of the acetyl-CoA in adipose tissue was of endogenous origin when incubated with glucose and insulin in vitro. In the absence of insulin over 40% of the acetyl-CoA was of endogenous origin.

C. Controlling Factors in Lipogenesis.

Masoro (121) has reviewed the mechanisms by which the homeostatic regulation of lipogenesis in animals is achieved. Lipogenesis is particularly susceptible to alterations in the nutritional and endocrine status of animals. It is suppressed by fasting or feeding of diets high in fat content, in diabetes and in tissues of animals treated with hormones such as epinephrine, thyrotropic hormone and glucagon. Conversely, lipogenesis is stimulated by diets high in carbohydrate content especially when given after a period of fasting, and by feeding the total daily caloric requirements in one meal. The role of insulin in stimulating lipogenesis in animals has been extensively investigated (cf. reviews by Winegrad (122,123)). In this chapter, lipogenesis at the cellular level will be described with reference to the extracellular influences to which this process is subject.

Enzyme activity.

A number or workers have attempted to correlate the lipogenetic activity of rat liver and that of the enzymes associated with it. In 1960, Gibson and Hubbard (124) reported that the activity of fatty acid synthetase decreases with fasting. Subsequently, diminished activity of acetyl-CoA carboxylase was demonstrated in fasted (125) and alloxan-diabetic (126) rats. Korchak and Masoro (127) confirmed these findings but noted that the fatty acid synthetase is about twenty times more active than acetyl-CoA carboxylase in normal as well as fasted rats.

Moreover, 24 hours of fasting led to a 50% fall in the activity of acetyl-CoA carboxylase, but only a 22% drop in fatty acid synthetase. At the same time, lipogenesis from acetate in liver slices fell by 99% (128). It therefore appears unlikely that decreased lipogenesis in fasting is related solely to the loss of acetyl-CoA carboxylase activity (48, 129). Hicks et al. (130) have recently reported that the activities of acetyl-CoA carboxylase and fatty acid synthetase in supernatants of liver rise to supranormal levels on refeeding fasted animals, but that actinomycin D and puromycin inhibited this response. Both enzymes thus appear to undergo adaptive changes.

Cofactor requirements.

1. TPNH

When the requirement for TPNH in lipogenesis was first established, it was proposed that the availability of TPNH may be rate-limiting (131-133). There are several pathways by which extramitochondrial TPNH can be generated:

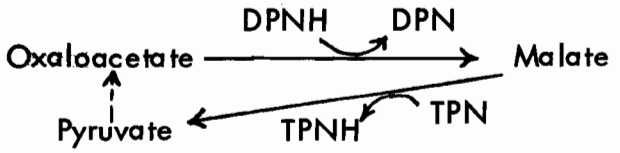
a) substrate-linked reduction of TPN by the pentose cycle dehydrogenases (132), isocitric dehydrogenase (134) and malic enzyme (135);

b) transhydrogenation of DPNH to TPNH, either directly by DPNH transhydrogenase (136) or indirectly in a substrate-linked system (135,137).

The pentose cycle is particularly active in lipogenetic tissues such as liver and adipose tissue (132), and a direct relationship between pentose cycle activity and lipogenesis has been demonstrated (120,138).

The pentose cycle dehydrogenases, glucose-6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase, are susceptible to nutritional changes, falling in starvation (137,139) and increasing with refeeding (140) or meal-feeding (112,141). Direct evidence of the importance of the pentose cycle in providing TPNH for lipogenesis has been provided by Katz et al. (142), who demonstrated that label from glucose-3-^{3}H is quantitatively utilized for fatty acid synthesis. However, carbon balance studies on adipose tissue incubated with labelled glucose have revealed that the pentose cycle can only supply about 65% of the TPNH requirement under conditions of maximal lipogenesis (120,138). Other sources of TPNH must, therefore, be available.

Pande et al. (137) and Young et al. (135) have drawn attention to the role of the malic enzyme in TPNH formation. They have proposed that the TPN-linked malic enzyme is coupled with the DPN-linked malic dehydrogenase in liver and adipose tissue:



The oxaloacetate needed for this reaction sequence could be regenerated from pyruvate by pyruvate carboxylase (143) or via citrate. Pande et al. argue that the high activity of malic enzyme in adipose tissue, which does not carry out gluconeogenesis can be explained by the function of this enzyme in transhydrogenation of DPNH to TPNH. This is supported

by the observation that malic enzyme, like the pentose cycle dehydrogenases, is particularly sensitive to fasting (137), refeeding diets high in carbohydrate content (135), or meal-feeding (112). In contrast, isocitric dehydrogenase does not undergo adaptive changes (112,137). Jacob (117) was unable to detect DPNH transhydrogenase in homogenates of human and rat adipose tissue.

Currently, TPNH production is not considered to be rate limiting for lipogenesis. Thus, studies of the concentration of TPNH in liver have revealed that it is not diminished by fasting (144,145) and in diabetes (146). Flatt and Ball (120) and Katz et al. (138) have demonstrated that the extramitochondrial production of reduced pyrimidine nucleotides from glucose exceeds the requirements of lipogenesis in adipose tissue from normal and refed animals in the absence or presence of glucose in vitro. Indeed, Flatt and Ball have obtained evidence that a lack of oxidized cofactors may limit the utilization of glucose in insulin-stimulated adipose tissue (147).

2. ATP

As previously discussed, ATP is required for the conversion of acetate and citrate to acetyl-CoA. Masoro, Korchak and Porter (148) showed that the inhibitory effect of liver microsomes obtained from fasted animals on the acetyl-CoA carboxylase catalyzed step in lipogenesis is partly attributable to increased microsomal ATPase activity. Myant and Iliffe (149) observed that liver extracts from thyroxine-treated rats have a reduced capacity to synthesize malonyl-CoA which could be reversed by addition of ATP.

3. Citrate.

Citrate stimulates the activity of acetyl-CoA carboxylase. The possibility has been considered that the extramitochondrial concentration of citrate may control the rate of lipogenesis (33). To the author's knowledge no direct evidence for the control of lipogenesis by citrate has been reported. Although the concentrations of citrate in tissues fall within the range stimulating acetyl-CoA carboxylase (33, 44), a large part of the cellular citrate may be located in the mitochondria (150) and thus be inaccessible to the enzyme. Furthermore, citrate levels are elevated in tissues from fasted rats (151,152) and chicks (153) and in diabetic rats (151). At the present time, the role of citrate as a cellular regulator of lipogenesis remains undetermined.

The inhibition of lipogenesis by fatty acids and their CoA derivatives.

It has been established that lipogenesis from glucose and acetate is depressed in livers from rats fed diets high in fat content (154-157). This effect is very rapid. Hill et al. (158) showed that within one hour after the administration of fat, lipogenesis from acetate is reduced. Bortz et al. (159) observed a loss in lipogenetic activity in liver homogenates when rats were given corn oil two hours before sacrifice, even though carbohydrate metabolism proceeded normally.

Fatty acids inhibit lipogenesis in intact tissue and homo-

genates in vitro. Langdon (160) found that palmitate inhibits lipogenesis in liver homogenates, but as CoA partly reversed this effect he attributed it to competition for CoA by fatty acyl thiokinase. This conclusion was not in agreement with the earlier findings of Porter and Long (161) who demonstrated that palmityl-CoA inhibits lipogenesis by pigeon liver preparations, and palmityl-CoA deacylase partially reverses this effect. Using a purified enzyme system, Bortz and Lynen (162) demonstrated that palmityl-CoA and stearyl-CoA at a concentration of 10^{-6} M inhibit acetyl-CoA carboxylase, while the free fatty acids were ineffective at this concentration. Palmityl-CoA and stearyl-CoA were the most inhibitory in the series of CoA derivatives tested, and, as these are the last small molecules in the synthetic sequence before incorporation into complex lipids, it was suggested that they act as feedback regulators. The level of palmityl-CoA in various tissues is sufficiently high to consider it as a controlling factor in lipogenesis (163,164). Furthermore, changes in diet or hormonal conditions lead to alterations in tissue palmityl-CoA concentrations and rate of lipogenesis consistent with a regulatory function (165). Bortz et al. (159) localized the defect in lipogenesis induced by feeding corn oil at the acetyl-CoA carboxylase step.

Other inhibitory effects of fatty acids or their CoA derivatives on the lipogenic enzymes have been reported, but require considerably higher concentrations (10^{-4} M) of the inhibitory compounds than observed by Bortz and Lynen (162). Korchak and Masoro (166) reported that fatty

acids inhibit lipogenesis in particle-free supernatants which lack fatty acyl thiokinases, suggesting that fatty acids need not be converted to the CoA derivative to be inhibitory. In their system, myristate was the best inhibitor tested and both acetyl-CoA carboxylase and fatty acid synthetase were inhibited. Robinson et al. (167) observed that palmityl-CoA inhibits the condensation of acetyl-CoA and malonyl-CoA. Steiner (168), using mitochondria-free preparations from brown adipose tissue, noted that fatty acids inhibit lipogenesis from acetate but not acetyl-CoA, and suggested that acetyl thiokinase is inhibited by fatty acids.

Wieland and Weiss (169) and Tubbs (170) reported that low concentrations of palmityl-CoA inhibit the citrate cleavage enzyme. They postulated that this inhibition may regulate the generation of citrate and hence lipogenesis. However, Srere (171) provided evidence that the interaction between palmityl-CoA and highly purified citrate cleavage enzyme is not of physiological significance. He observed that the inhibition depends on the molar ratio between palmityl-CoA and the enzyme. 16 moles of palmityl-CoA had to be bound per mole of enzyme to achieve an inhibition of 27%, and the inhibition was irreversible. Glucose-6-phosphate dehydrogenase is also inhibited by long chain acyl CoA derivatives (172,173). Taketa and Pogell (173) have recently shown that many enzymes are inhibited by low concentrations of palmityl-CoA. For instance, glutamic and malic dehydrogenase were

inhibited at a concentration of 10^{-7} M. The significance of these findings remains to be evaluated.

Complex lipid synthesis.

Lorch et al. (174) have suggested that the removal of inhibitory end products, e.g. fatty acyl-CoA derivatives, may control the rate of lipogenesis. The esterification of fatty acyl-CoA with α-glycerolphosphate to form glycerides is a control mechanism of this type. Howard and Lowenstein (175,176) have shown that α-glycerolphosphate stimulates lipogenesis when microsomes, which contain the acyl transferases required, are added to cell-free preparations of liver and mammary gland. This effect was uninfluenced by changes in nutritional state or the induction of alloxan diabetes. At optimal concentrations of microsomal protein, one half maximal stimulation of lipogenesis was obtained with 0.37 mM α-glycerolphosphate, a value comparable with those found in vivo. However, the degree of stimulation exceeded the values expected on the basis of the amount of fatty acyl-CoA removed from the system (176). To circumvent this discrepancy, Howard and Lowenstein proposed that some fatty acyl-CoA is stored as the ACP derivative, leading to a deficiency of free ACP required for lipogenesis from acetyl-CoA, and that α-glycerolphosphate regenerates free ACP by removal of fatty acyl-CoA. One possible defect in this argument is that it is based on the theoretically calculated content of fatty acyl-CoA in the system employed, and does not take into account the fatty acyl-CoA produced from endogenous free fatty acids.

D. Lipogenesis and Adipose Tissue Metabolism.

The preeminence of adipose tissue in lipogenesis has been established both *in vitro* and *in vivo* (177). Stetten and Boxer (178) calculated that rats maintained on a diet high in carbohydrate content but low in total calories convert 30% of the carbohydrate into fatty acids. Katz *et al.* (138), studying the distribution of glucose carbon in products formed by rat epididymal fat pads under a variety of dietary and hormonal conditions (Table I) found that 21% of the glucose carbon taken up is incorporated into fatty acids by tissue from rats fed ad libitum. When adipose tissue was stimulated by insulin *in vitro* 36% of the glucose carbon appeared in fatty acids and the percentage of glucose carbon utilized for lipogenesis was over 40% of the uptake when tissue from fasted-refed rats was employed.

The pathways of glucose metabolism in adipose tissue appear to be geared for lipogenesis when the animal is in positive energy balance, i.e. when the caloric intake exceeds the energy requirements. Thus, in adipose tissue glycogen synthesis is relatively unimportant (179), glycolysis is an essentially unidirectional process (12, 180), and gluconeogenesis does not occur (180, 181). The absence of glucose-6-phosphatase (182,183), fructose-1,6-diphosphatase (183) and phosphoenolpyruvate carboxykinase (135) from adipose tissue undoubtedly contributes in delineating this pattern of glucose metabolism in adipose

TABLE I (138)

Effect of diet and hormones on distribution of glucose carbon in products formed by rat epididymal fat tissue

Diet and hormone	Relative glucose utilized	Glucose carbon converted to						
		Carbon dioxide				Fatty acids	Glycerol	Lactate
		Pentose cycle	Pyruvate decarboxylation	Krebs cycle	Total			
		%	%	%	%	%	%	%
Fasted-refed, insulin	770	12	24	2	38	45	5	12
Fasted-refed, no hormone	460	13	22	3	38	42	7	13
Fed *ad libitum*, insulin	240	11	20	3	34	36	11	19
Fed *ad libitum*, no hormone	100	8	13	8	29	21	20	30
Fasted-refed, growth hormone	330	6	14	18	38	19	20	23
Fed *ad libitum*, epinephrine	250	3	10	17	30	7	40	23

tissue. The presence of an active pentose shunt pathway for the generation of TPNH is characteristic for tissues which carry out lipogenesis (132). Katz et al. (138) showed that of the glucose utilized by adipose tissue 11% is metabolized in the pentose cycle in the absence of insulin, and 20% in the presence of insulin in vitro, and that there is a marked correlation between pentose shunt activity and lipogenesis. The adaptability of enzymes associated with lipogenesis to alterations in the dietary or endocrine state of the animal provides further evidence for the important role of lipogenesis in adipose tissue.

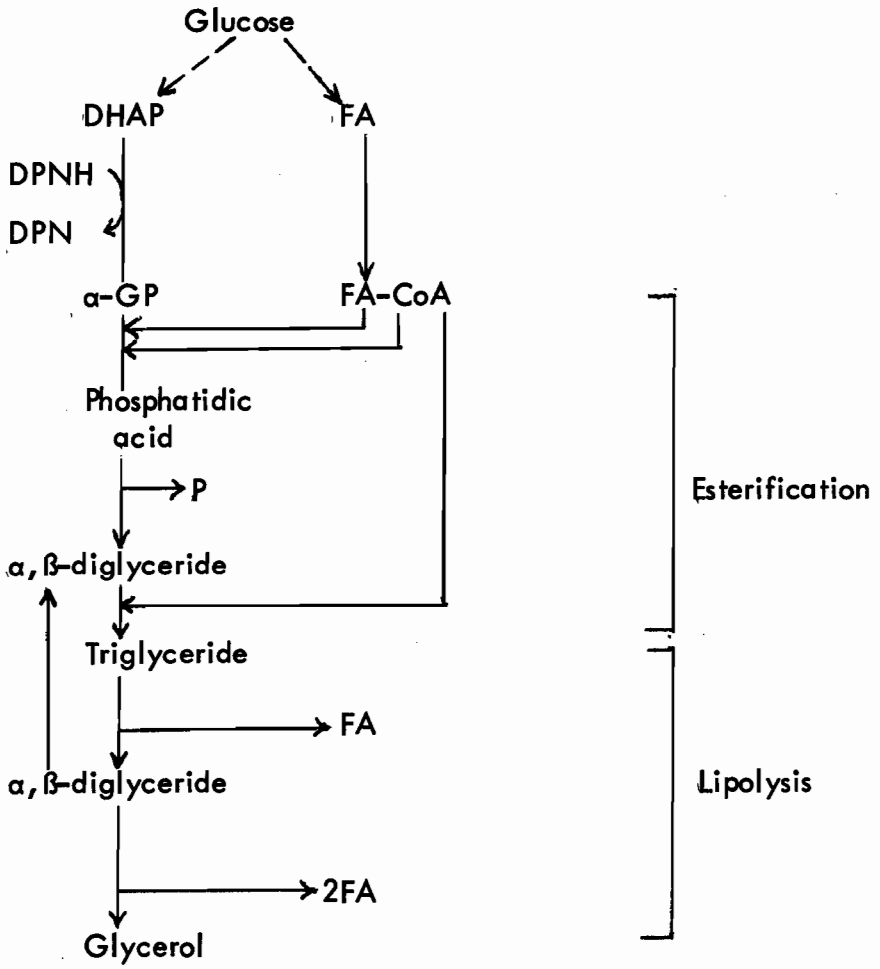
Patkin and Masoro (184) and De Freitas and Depocas (185) have provided evidence that lipogenesis from glucose in adipose tissue does not represent an important pathway for glucose metabolism in rats. Animals infused with 300 mg glucose per hour converted less than 5% of the administered glucose to fatty acids (185). However, it is important to note that the rats used in these studies were old animals, as Benjamin et al. (186) noted a marked decline in lipogenesis with aging; lipogenesis is ten times greater in adipose tissue from young rats (105-124 g, 44 days old) than old rats (280-285 g, 81 days old). Thus, it is possible that De Freitas and Depocas underestimated lipogenesis from glucose due to the age of their rats.

The processes by which fatty acids are stored in or released from adipose tissue are in dynamic equilibrium (12). During times of positive energy balance the deposition of fatty acids of exogenous or

endogenous origin as triglyceride predominates, whereas the breakdown of lipid stores is stimulated when the energy demands of the organism exceed the supply of substrate or lipolytic agents act on adipose tissue (12). The details of these processes have been reviewed elsewhere (187, 188). The pathways of triglyceride synthesis and lipolysis are summarized in Figure 4.

Figure 4.

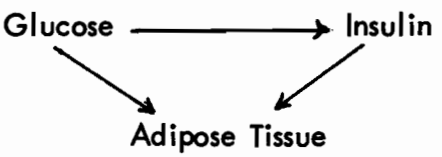
Triglyceride Synthesis and Lipolysis in Adipose Tissue.



In contrast to liver (189) and brown adipose tissue (190), glycerol phosphokinase activity in white adipose tissue appears to be insignificant (191,192). As glycerol cannot be reutilized, the α-glycerolphosphate required for esterification of fatty acids is formed from glucose. In the presence of an adequate supply of glucose, stimulation of lipolysis, e.g. by epinephrine (193,194) or growth hormone (195), leads to increased glyceride glycerol synthesis. Cahill et al. (194) suggested that this increase is secondary to the accumulation of FFA in the tissue. It has been demonstrated that glyceride glycerol synthesis and esterification are enhanced when tissue from normal rats is incubated with fatty acids (194). However, in adipose tissue from fasted rats there is a net release of FFA even though glyceride glycerol synthesis from glucose is relatively unaltered (188). At the same time, the uptake of glucose and the oxidation of carbon 1 of glucose and lipogenesis are markedly diminished (196). Thus, the production of α-glycerolphosphate is maintained in the face of large changes in glucose utilization and takes precedence over lipogenesis from glucose. As glucose is available to adipose tissue in vivo even in the fasted state, other factors must be involved in the regulation of adipose tissue metabolism. The evidence at hand indicates that both lipid deposition and breakdown are under control of a complex interaction of nutritional, hormonal and neurohumoral influences (cf. 24 for reviews).

Adipose tissue is one of the main target organs for insulin

(3,4), the release of which is dependent on the blood glucose concentration (197). The interaction between blood glucose, insulin and adipose tissue is such as to favor energy storage as lipid when food is present in excess (123).



The availability of glucose to cellular processes requires the transfer of glucose across the cell membrane, considered to be the rate limiting step in glucose utilization by adipose tissue (198-201). Crofford and Renold (198,199) have shown that the major action of insulin on adipose tissue is to facilitate the transport of glucose by a mobile, stereospecific carrier. Thus, insulin controls the rate of glucose utilization prior to the initial steps of its metabolism. In turn, many of the effects of insulin are dependent on the presence of glucose. For instance, the utilization of acetate and pyruvate for lipogenesis is not stimulated by insulin unless glucose is available (202).

The changes in the pattern of dissimilation of glucose carbon in the presence of insulin, as shown in Table I are not necessarily a consequence of increased glucose entry into the cell, as proposed by Renold and his co-workers (195,203). Leonards and Landau (204) found that insulin stimulates the oxidation of C-1 of glucose and lipogenesis,

whereas high concentrations of medium glucose favor the oxidation of C-6 and glyceride glycerol synthesis. In addition, some actions of insulin are unrelated to its transport function. Jungas and Ball (205) observed that insulin inhibits the lipolytic breakdown of triglycerides in adipose tissue treated with adrenaline and incubated in the absence of glucose. Others have confirmed this observation (206,207). Zierler and Rabinowitz (208), using an in vivo perfusion technique, found that very small amounts of insulin can lead to diminished release of FFA from the superficial compartment of the human forearm without stimulating glucose uptake, suggesting that the effect of insulin on lipolysis may precede its action on glucose utilization.

While insulin is considered to be the sole hormonal agent responsible for lipid deposition, a large number of hormones stimulate lipolysis. These include epinephrine (209,210), norepinephrine (210), adrenocorticotropic hormone (211,212), glucagon (213,214), thyroid stimulating hormone (215), melanocyte stimulating hormone (216) vasopressin (216), pituitary 'Fraction H' (216) and peptides I and II (217), and growth hormone (218). The first five hormones in this list have been shown to stimulate adipose tissue lipase (188). Adrenal glucocorticoids are weakly lipolytic (219,220), but also have the property of inhibiting glucose uptake (221-223). Fat-mobilizing substances have been extracted from the urine of fasted rats and man (224). It has also been found that an intact sympathetic nervous system is required to

maintain blood FFA levels (225).

Adipose tissue metabolism is thus the resultant of nutritional neural and endocrine influences. The balance between lipolysis and neutral lipid synthesis is part and parcel of the function of adipose tissue in regulating the storage and release of substrate. On the other hand, "the major homeostatic function of lipogenesis is to store as fat the chemical energy of carbohydrate ingested in excess of the immediate energy requirements of the organism" (121). In view of the diurnal variations in food intake, it is not surprising that these processes are carefully controlled.

III. EXPERIMENTAL PROCEDURE

Source and Preparation of Adipose Tissue.

Human adipose tissue was obtained from non-diabetic patients at laparotomy under general anesthesia. The anesthetics were pentobarbital and fluothane or halothane; ether was never employed. The adipose tissue was excised as soon as possible after the start of the operation, usually within 60 minutes. Subcutaneous fat was taken from the edge of the abdominal incision, and omental adipose tissue from the perimeter of the greater omentum. Immediately after removal the tissue was placed into Krebs-Ringer bicarbonate buffer (226), gassed with 95% O_2:5% CO_2 to pH 7.4 and kept at room temperature. Adjacent strips of tissue up to 2 mm in thickness and weighing 150 to 250 mg were cut from the operative specimens with fine scissors. The time interval between removal of the tissue and the start of the incubation was usually 15 to 30 minutes.

Epididymal fat pads were taken from male hooded rats, Royal Victoria Hospital strain, maintained on a chow diet and weighing 140-160 g. The animals were anesthetized with 15 mg pentobarbital. The fat pads were rinsed with Krebs-Ringer bicarbonate buffer, spread out on moistened filter paper and the thick proximal portions of the pads were cut off and discarded. Pads were halved by a cut in their longitudinal axis. Before being weighed or incubated the pads were gently blotted

with filter paper.

Isolated cells were prepared as described by Rodbell (227) by incubating pieces of rat adipose tissue in a medium of 3 μMoles of glucose and 10 mg collagenase per ml of albumin-bicarbonate buffer for 60 minutes.

Incubation of Intact Tissue and Isolated Adipocytes.

Tissue was incubated in Krebs-Ringer bicarbonate medium gassed with 95% O_2 : 5% CO_2 to pH 7.4 in 25 ml Erlenmeyer flasks fitted with center wells. Incubations were carried out at 37°C in a waterbath shaker. The substrate used was either glucose alone or glucose and acetate. Labelled substrate had a final specific activity of approximately 0.02 μc/μMole for ^{14}C and 0.2 μc/μMole for ^{3}H. Gelatin, 1 g/100 ml medium, was added in prolonged incubations of intact rat adipose tissue. When isolated cells were incubated, the medium contained 4 g albumin/100 ml.

At the end of the incubation period, 0.3 ml of hyamine-10X (Packard Instrument Co.) was injected through the rubber cap into the center well of each flask, and 0.5 ml of 6 N HCl into each main compartment. After another 30 minutes, the hyamine carbonate was transferred to counting vials.

Lipid extraction and saponification: the adipose tissue was

removed from the flask, blotted, rinsed twice in 250 ml of 0.9% NaCl and placed into chloroform:methanol (2:1). The tissue was homogenized in chloroform:methanol and the lipid extracted by the method of Folch et al. (228). The lipid extract was divided into two portions. One portion was brought to dryness under a stream of nitrogen in a tared counting vial and the lipid content determined gravimetrically before adding scintillator for counting. The second portion was dried and then saponified with 5 ml of 0.05 N KOH in 85% ethanol for 90 minutes at 75°C. Fatty acids were extracted by the method of Borgstrom (229), using n-heptane as the solvent. A part of the heptane extract was evaporated and counted. Better than 95% of the radioactivity in tripalmitin-^{14}C-palmitate was consistently recovered by this method.

In the case of the isolated cells, the lipids were extracted by the method of Dole (230). Otherwise, the lipid extracts were processed in the same way as those of the intact tissue.

Lipogenesis in Homogenates.

Adipose tissue was homogenized in cold 0.25 M sucrose (1 part tissue by weight to 2 volumes of sucrose) with a glass hand homogenizer for two minutes. In order to obtain preparations free of mitochondria, the homogenates were centrifuged at 12,000 x g at 2°C for 20 minutes. The homogenates separated into three layers: congealed

fat at the top, a rosy, slightly turbid middle layer,and cell and connective tissue debris at the bottom. The middle layer was aspirated into a syringe and immediately dispensed into the reaction tubes.

The reaction mixture used for assaying lipogenesis from acetyl-CoA contained: potassium phosphate 83 mM, pH 6.6; $MgCl_2$ 10 mM; $MnCl_2$ 1 mM; $KHCO_3$ 25 mM; GSH 2 mM; citrate 10 mM; isocitrate 5 mM; TPN 2 mM; ATP 10 mM; DL-α-glycerol phosphate (α-GP) 10 mM; NaF 40 mM; acetyl-CoA 0.2 mM including acetyl-1-^{14}C-CoA 10^5 dpm; homogenate supernatant 0.2 ml; final volume 1.2 ml. The pH was 6.6 both before and after incubation. Incubation was carried out in 20 ml extraction tubes for 30 minutes at 37°C in a waterbath shaker. The gas phase was air. The reaction was stopped with 5 ml isopropanol-n-heptane-9 N H_2SO_4 (40:10:1) and lipids were extracted by the method of Dole (230). The heptane extracts were dried in counting vials with a stream of nitrogen.

The system described was based on that of Martin and Vagelos for partially purified enzymes (44), but several modifications were made. As shown in Table II, lipogenesis was more active with phosphate buffer, and it was substituted for the imidazole buffer used by Martin and Vagelos. TPNH was replaced by TPN and isocitrate without change in lipogenetic activity of the system. α-GP was added to stimulate esterification of endogenous or newly-formed fatty acids (175,176) and NaF was used to inhibit lipolysis (231). In the absence

TABLE II

Lipogenesis from Acetyl-1-^{14}C-CoA in 12,000xg Supernatants of Rat Adipose Tissue Homogenates.

System	% of Control
Complete*	100
-TPN, -isocitrate, +TPNH	100
+insulin 0.1 unit/ml	100
-phosphate, +imidazole buffer	23
-α-GP	66
-citrate	66
-α-GP, -citrate, -NaF	25
-citrate, +CoA 0.025 mM	4
-α-GP, -citrate, -NaF, +CoA 0.025 mM	2

*Complete system contained: potassium phosphate 83 mM, pH 6.6; $MgCl_2$ 10 mM; $MnCl_2$ 1 mM; $KHCO_3$ 25 mM; GSH 2 mM; citrate 10 mM; isocitrate 5 mM; TPN 2 mM; ATP 10 mM; DL-α-GP 10 mM; NaF 40 mM; acetyl-CoA 0.2 mM including acetyl-1-^{14}C-CoA 10^5 dpm; homogenate supernatant 0.2 ml. Final volume 1.2 ml. Time of incubation - 30 minutes.

of α-GP or citrate lipogenesis was reduced by 34%; when both were omitted lipogenesis fell to 25% of the values with the complete system. Low concentrations of CoA (0.025 mM) markedly inhibited lipogenesis both in the absence and presence of α-GP. Insulin had no effect on lipogenesis from acetyl-CoA, as noted by Martin and Vagelos (33).

As shown in Figure 5, the system was optimal with respect to GSH, ATP and pH. The reaction was linear over a 30 minute period of incubation, with acetyl-CoA concentrations from 0.1 to 0.8 mM, and with homogenate concentrations equivalent to from 25 to 100 mg of tissue/tube.

The mean and standard error (S.E.) for seven determinations each with homogenates from tissues pooled from two rats was 32 ± 4.3 mμMoles of acetyl-CoA incorporated into lipids/100 mg tissue in 30 minutes.

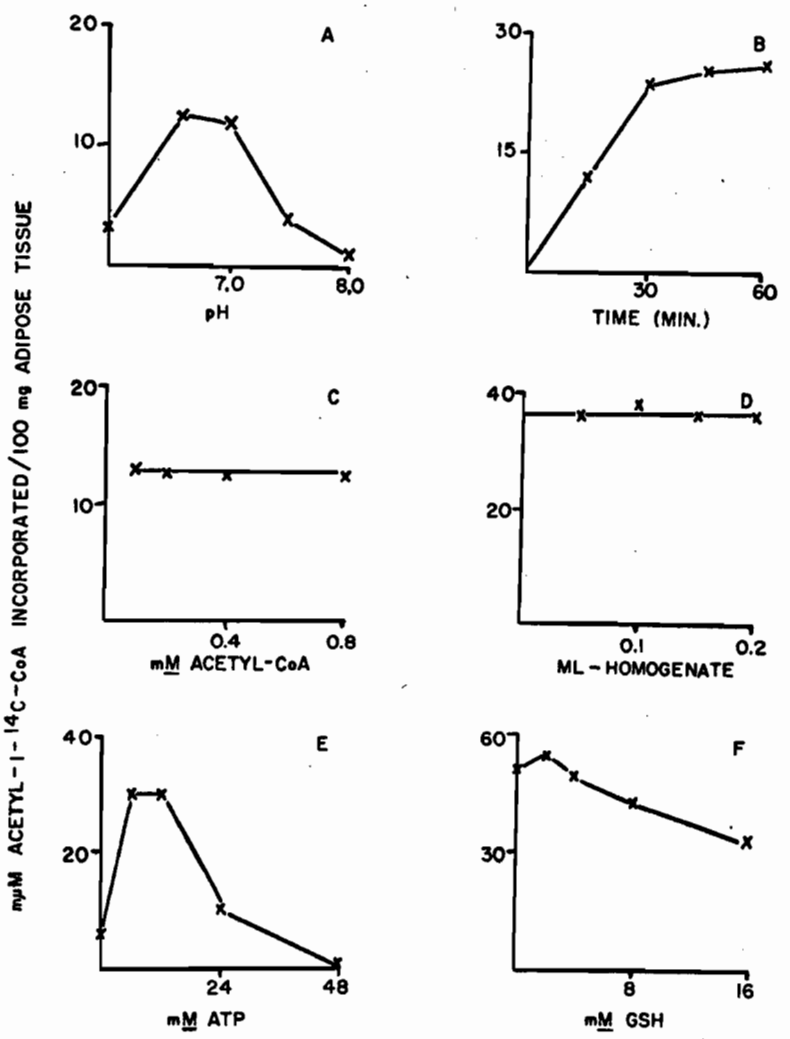
Analytical Procedures.

1. DNA content of adipose tissue.

The fluorometric ultramicromethod of Kissane and Robins (232) was adapted for the determinations of DNA in the 100 μg range. The defatted tissue residue was washed twice with absolute ethanol and then left to dry under reduced pressure in a dessicator. 0.2 ml of a

Figure 5

Incorporation of Acetyl-1-^{14}C-CoA into Lipids by the 12,000 x g Supernatant of Rat Adipose Tissue Homogenates.



cf. Table II and Text

freshly prepared solution of 3,5-diaminobenzoic acid dihydrochloride (300 mg of DABA 2 HCl/ml water) was added to the tissue residue and heated for 60 minutes at 60°C in a water bath. The tubes were allowed to cool, 5 ml of 0.6 N perchloric acid was added, the tubes were shaken with a Vortex mixer, and 0.3 ml was transferred to Coleman tubes. 4 ml of 0.6 N perchloric acid was added to the Coleman tubes and they were read against the blank in a Turner Fluorimeter with a #47B primary filter and a #2A12 secondary filter. The standard curves were linear up to 160 µg and reproducible during the time the determinations were carried out. The recovery of added DNA was 96 ± 2% in six determinations. The variation between quadruplicate replications was usually less than 5% from the mean.

Table III shows the values obtained for human omental and subcutaneous adipose tissue.

TABLE III

DNA Content of Human Adipose Tissue.

Tissue	Number of determinations	µg DNA/g lipid mean ± S.E.	p
Omental	27	508 ± 39	< 0.001
Subcutaneous	13	211 ± 14	

Each determination was the mean of triplicates or quadruplicates.

Omental adipose tissue contained significantly more DNA than subcutaneous adipose tissue. The DNA content of subcutaneous adipose tissue shown here is in close agreement with the values obtained by Hirsch and Goldrick (1) who used a diphenylamine method.

2. Free fatty acid content of tissues.

Adipose tissue was homogenized in the extraction mixture described by Dole (230) and centrifuged to remove debris. The fatty acids were extracted and titrated by the method of Dole and Meinertz (233).

The FFA content of the left and right fat pads from the same animal was found to be identical. In 6 fed rats the mean tissue FFA concentrations were 2.52 and 2.47 μEq/g tissue in the left and right pads respectively. In adipose tissue from rats fasted for 48 hours the corresponding values were 5.53 and 5.36 μEq/g tissue.

Uptake of Non-Metabolizable Sugars.

Pieces of adipose tissue were incubated in a medium consisting of 5 mM sorbitol, 5 mM 3-0-methyl glucose, 20 μc sorbitol-1-^{3}H, 1 μc 3-0-methyl-^{14}C-glucose, 80 mg albumin in 2 ml Krebs-Ringer bicarbonate buffer, pH 7.4. The incubations were carried out at 37°C in counting vials. The sugar spaces were determined as described

by Crofford and Renold (198).

Radioactive Counting Procedures.

All counting was done by scintillation spectrometry. Initially a Packard Tri-Carb #3314 and later a #4000 was employed. Aqueous solutions, such as incubation media, were counted in dioxane phosphor (234). Lipids and hyamine carbonate were counted in toluene phosphor (0.3% PPO and 0.01% POPOP in toluene). Corrections were applied to account for quenching and differences in efficiency related to the use of different phosphors for counting the medium and samples. At optimal machine settings the efficiency of the dioxane phosphor was 65%, that of the toluene phosphor 85%.

The machine settings for the simultaneous counting of ^{3}H and ^{14}C in dioxane phosphor were adjusted so that spillage of ^{3}H into the ^{14}C channel was less than 0.04%. This was accomplished at efficiencies of 41% for ^{14}C and 19% for ^{3}H, with a ^{14}C spillage of 28%. When calculating the actual counts for each isotope it was usually not necessary to take the ^{3}H spillage into account, even when the ratio of ^{3}H : ^{14}C was 20 : 1, and the need for simultaneous equations was obviated.

Materials

All materials were obtained from commercial sources: ATP, CoA, TPN, TPNH, GSH - Sigma Chemical Company; acetyl-CoA (lithium salt), 3-0-methyl glucose - Calbiochem; 3,5-diamino-benzoic acid dihydrochloride - Eastman Organic Chemicals; collagenase - Worthington Biochemical Corporation; glucose-1-^{14}C, -6-^{14}C, -U-^{14}C and acetate-1-^{14}C - Merck, Sharpe & Dohme; acetyl-1-^{14}C-CoA, 3-0-methyl-^{14}C-glucose, sorbitol-1-^{3}H, acetate-^{3}H - New England Nuclear Corporation.

Glucagon-free insulin was kindly donated by E. Lilly & Co.

Albumin (Armour Pharmaceutical Company) was dissolved in five volumes of distilled water, dialized thrice for 12 hours and passed through a Seitz filter before use.

IV. RESULTS

A. Human Adipose Tissue.

1. The effect of insulin on the metabolism of human adipose tissue in vitro.

The influence of medium glucose concentration on the incorporation of label from glucose-1-^{14}C into neutral lipid was studied in the absence and presence of insulin. Glucose concentration of 2.7 and 10.7 mM were chosen to represent the physiological extremes. The results in Table IV show that incorporation into neutral lipid was greater at 10.7 than at 2.7 mM glucose, but only in the absence of insulin.

TABLE IV

Effect of Insulin on Neutral Lipid Synthesis from Glucose-1-^{14}C by Human Omentum.

Glucose mMolar	Insulin 0.1 unit/ml	Neutral Lipid mμMoles/g N.L.	p
2.7	-	579 ± 60[a]	
			< 0.02
10.7	-	899 ± 59	
2.7	+	1107 ± 99	
			N.S.
10.7	+	1220 ± 56	

Incubation time - 2 hours.
a-Mean of three determinations ± S.E.

However, insulin stimulated lipid synthesis more markedly at the lower glucose concentration. Subsequent studies with insulin were therefore carried out with 2.7 mM glucose.

The effect of insulin on the metabolism of glucose-1-^{14}C by omental and subcutaneous adipose tissue is shown in Table V. In both types of adipose tissue, the addition of 0.1 unit insulin/ml of medium significantly increased the incorporation of label into CO_2, neutral lipid and fatty acids.

When insulin was present the incorporation of label into CO_2 by omentum increased from a mean of 565 to 992, and by subcutaneous adipose tissue from 456 to 1086 mμMoles glucose/mg DNA. Neutral lipid synthesis increased from 759 to 1371 and from 753 to 1693 mμMoles/mg DNA in omental and subcutaneous tissue respectively.

There was no significant difference between the two tissues in terms of CO_2 production or neutral lipid synthesis from glucose, in the absence or presence of insulin. However, in the absence of insulin, the ratio of carbon 1 to carbon 6 of glucose oxidized to CO_2 - a rough measure of pentose cycle activity - was lower in subcutaneous than in omental adipose tissue. Thus, in nine experiments with omentum the mean value for this ratio ± the standard error (S.E.) was 4.1 ± 0.4 and in five experiments with subcutaneous adipose tissue the ratio was 2.7 ± 0.4, a difference which is significant at the 0.005 level.

TABLE V

Effect of Insulin on the Utilization of Glucose-1-^{14}C by Human Adipose Tissue in vitro.

	Control	Insulin 0.1 unit/ml	Increase with insulin	p
	mμMoles/mg DNA			
Omentum				
CO_2 (11)[b]	565	992	427 ± 83[a]	<0.001
Neutral Lipids (11)	759	1371	612 ± 65	<0.001
Fatty Acids (14),	69	162	93 ± 33	<0.02
Subcutaneous (7)				
CO_2	456	1086	630 ± 123	<0.005
Neutral Lipids	753	1693	940 ± 97	<0.001
Fatty Acids	12	44	32 ± 13	<0.05

Medium glucose - 2.7 mM. Incubation time - 2 hours.
a-Mean ± S.E.
b-Number of duplicate determinations.

Fatty acid synthesis from glucose-1-^{14}C increased from 69 to 162 in omental and from 12 to 44 mμMoles/mg DNA in subcutaneous adipose tissue when insulin was present. Although not indicated in Table V, lipogenesis was significantly more active in omental than in subcutaneous adipose tissue, both in the absence and presence of insulin (p 0.05).

As insulin stimulates lipogenesis from acetate in the presence of glucose in rat adipose tissue (235), it was of interest to determine whether this can also be demonstrated with human adipose tissue. Lipogenesis from acetate-1-^{14}C and glucose-1-^{14}C was studied in omental adipose tissue from six subjects (Table VI). Insulin stimulated lipogenesis from both substrates in five out of the six experiments; lipogenesis could not be demonstrated in tissue from D.F. The data presented here also show the variability in lipogenesis encountered with human adipose tissue. Thus, lipogenesis from glucose-1-^{14}C ranged from 0 to 382 and from 0 to 634 mμMoles/mg DNA in the absence and presence of insulin respectively. With acetate-1-^{14}C as substrate the range was even wider. The absence of demonstrable lipogenesis was also noted in other experiments carried out under the same conditions. Out of a total of 24 experiments with omental and subcutaneous adipose tissue, lipogenesis could not be demonstrated in seven when insulin was absent. Six of these tissues were also studied in the presence of insulin, and lipogenesis could then be demonstrated in three samples.

TABLE VI

Fatty Acid Synthesis by Human Omental Adipose Tissue

Patient	Glucose-1-^{14}C		Acetate-1-^{14}C	
	Control	+Insulin[a]	Control	+Insulin
	mμMoles/mg DNA			
B.M.	382	634	816	1426
D.F.	0	0	0	0
J.W.	230	338	2297	4369
D.P.	0	46	177	653
S.G.	17	38	40	356
M.M.	0	415	89	445

Glucose alone or glucose + acetate - 2.7 mM.
Incubation time - 2 hours.
a-0.1 unit/ml

2. Effect of glucose infusion in vivo on lipid synthesis and response to insulin of adipose tissue in vitro.

As the patients from whom adipose tissue was obtained in the previous studies had been fasted for 10 to 14 hours preoperatively, the anabolic activity of the tissues may have been impaired. The possibility that the metabolism of adipose tissue in vitro could be influenced by the administration of glucose was therefore investigated.

Six subjects received intravenous infusions of 80 g of glucose over a two-hour period preoperatively. Lipid synthesis from glucose-1-^{14}C was then studied in vitro. The incorporation of label into neutral lipids and the response to insulin in tissues from infused and noninfused patients are compared in Table VII. Tissues from both groups of patients appeared to give quantitatively similar results for neutral lipid synthesis, as seen from the widely overlapping range of values. However, the response to insulin was significantly reduced in both subcutaneous and omental tissue from infused patients. In the presence of insulin, the average increase in neutral lipid synthesis above control levels fell from 612 to 371 and from 925 to 453 mμMoles glucose incorporated/mg DNA in omental and subcutaneous tissue respectively. These results suggest that the adipose tissue from patients who had received glucose may have been partially stimulated by endogenous insulin.

On the other hand, fatty acid synthesis appeared to be

TABLE VII

Effect of Glucose Infusion on the Incorporation of Glucose-1-^{14}C into Neutral Lipid in vitro.

	Neutral Lipid			
	Control	Insulin 0.1 unit/ml	Increase with insulin	p
	mμMoles/mg DNA			
Omentum				
No infusion (11)[c]	759[a] (430-1157)	1371 (773-1852)	612 ± 65[b]	
Infusion (6)	1089 (577-2074)	1461 (814-2493)	371 ± 76	<0.05
Subcutaneous				
No infusion (7)	753 (431-1277)	1693 (1133-2353)	940 ± 97	
Infusion (6)	1336 (538-2044)	1897 (978-2299)	453 ± 115	<0.02

Medium glucose - 2.7 mM. Incubation time - 2 hours.
a-Mean and range
b-Mean ± S.E.
c-Number of duplicate determinations.

uninfluenced by glucose infusion (Table VIII). A statistically significant difference in lipogenesis between tissues from the two groups could not be demonstrated although the mean values were considerably higher in tissue from infused than from noninfused subjects in the presence as well as in the absence of insulin in vitro. For example, with omentum from the noninfused group 69 mμMoles of glucose/mg DNA were incorporated into fatty acids, whereas omentum from infused subjects incorporated 403 mμMoles/mg DNA. This apparent difference was not statistically significant. In addition, there was no difference between tissues from the two groups in the lipogenetic response to insulin. A large part of the difficulty in analyzing these results is attributable to the wide range of the values encountered.

However, further evidence that glucose infusion leads to an alteration of glucose metabolism in adipose tissue was obtained from studies of the oxidation of carbon-1 and carbon-6 of glucose to CO_2. The values for the ratio of carbon-1 to carbon-6 obtained with omental adipose tissue from noninfused subjects in the absence and presence of insulin, and from infused subjects in the absence of insulin are given in Table IX. It is well known that the carbon-1 to carbon-6 ratio of glucose incorporated into CO_2 is increased by insulin in rat adipose tissue (235). A similar response is demonstrated here with human omentum. The ratio increased from 4.1 to 9.4 in omentum from noninfused subjects when insulin was added to the medium. With omentum from infused

TABLE VIII

Effect of Glucose Infusion on the Incorporation of Glucose-1-^{14}C into Fatty Acids *in vitro*.

	Fatty Acids			
	Control	Insulin 0.1 unit/ml	Increase with insulin	p
	mμMoles/mg DNA			
Omentum				
No infusion (14)[c]	69[a] (0-382)	162 (0-634)	93 ± 33[b]	N.S.
Infusion (6)	403 (154-1244)	622 (306-1794)	219 ± 76	
Subcutaneous				
No infusion (7)	12 (0-32)	44 (0-131)	32 ± 13	N.S.
Infusion (6)	184 (4-914)	284 (20-1143)	100 ± 56	

Medium glucose - 2.7 mM. Incubation time - 2 hours.
a-Mean and range.
b-Mean ± S.E.
c-Number of duplicate determinations.

TABLE IX

Ratio of C-1 to C-6 of Glucose Converted to CO_2 by Human Omentum.

	No. of Expts.	C_1/C_6	p	
Control	9	4.1±0.4[a]	<0.001	<0.05
+Insulin in vitro[b]	4	9.4±1.0	N.S.	
Glucose infusion	4	8.2±1.4		

a-Mean ± S.E.
b-0.1 unit/ml

patients in the absence of insulin in vitro the ratio was 8.2 which is significantly higher than the value for omentum from noninfused subjects in the absence of insulin. On the other hand, there was no significant difference between the value for tissue from noninfused subjects stimulated by insulin in vitro and omentum from infused patients. These observations suggest that the metabolism of glucose by adipose tissue was stimulated by infusing patients with glucose prior to tissue sampling.

3. Factors influencing lipogenesis in vitro.

As the metabolism of glucose in vitro could be stimulated by the infusion of glucose in vivo, an attempt was made to see if a similar phenomenon can be reproduced in vitro.

In three initial experiments (Table X), omentum was preincubated for one hour with unlabelled glucose (10.7 mM) before being transferred to medium containing glucose-U-^{14}C for another hour. Control tissues were not preincubated. It was observed that lipogenesis was greater in the preincubated tissues than in the controls.

TABLE X

The Effect of Preincubation on Lipogenesis from Glucose-U-^{14}C.

Experiment	Control	Preincubation
	mμMoles/g N.L.[a]	
1	5	20
2	17	30
3	2	8

Medium glucose - 10.7 mM. Incubation and preincubation - 1 hour.
a-Mean of duplicate determinations.

These results suggested that lipogenesis from glucose may not vary directly with the length of incubation. The incorporation of glucose label into lipids was therefore studied as a function of time. Strips of omentum were incubated in medium containing glucose-U-^{14}C for three hours. Samples were taken for analysis at the end of each hour. The rates of lipid synthesis in the first and third hour of incubation are summarized in Table XI (see Appendix, Figure 8 for complete data). Because of the spread of the values obtained in this series of experiments, the tissues were divided into three groups, depending on the rate of neutral lipid synthesis:

A. less than 300 mμMoles glucose incorporated/g neutral lipid per hour - four samples;

B. between 300 and 2000 mμMoles/g neutral lipid per hour - four samples;

TABLE XI

Effect of Length of Incubation on Lipid Synthesis from Glucose-U-^{14}C by Human Omentum.

Patients[a]	Neutral Lipid			Fatty Acids			Glyceride Glycerol		
	Initial[b]	Final[b]	p	Initial	Final	p	Initial	Final	p
	mμMoles/g N.L.			mμMoles/g N.L.			mμMoles/g N.L.		
A	238± 20[c]	217± 26	N.S.	6±2.1	44±9.4	<0.01	232± 19	173±29	N.S.
B	573±138	1005±248	N.S.	85±33	732±200	<0.02	478±115	283±98	N.S.
C	2940	2637		2412	2318		303	319	

Medium glucose - 10.7 mM.
a-Group A - 4 patients: T.S., R.P., and C.M.
B - 4 patients: L.F., C.L., and T.J.
C - 1 patient: R.L.
b-Initial - first hour of incubation; Final - third hour of incubation.
c-Mean ± S.E.; all determinations were done in duplicate or triplicate.

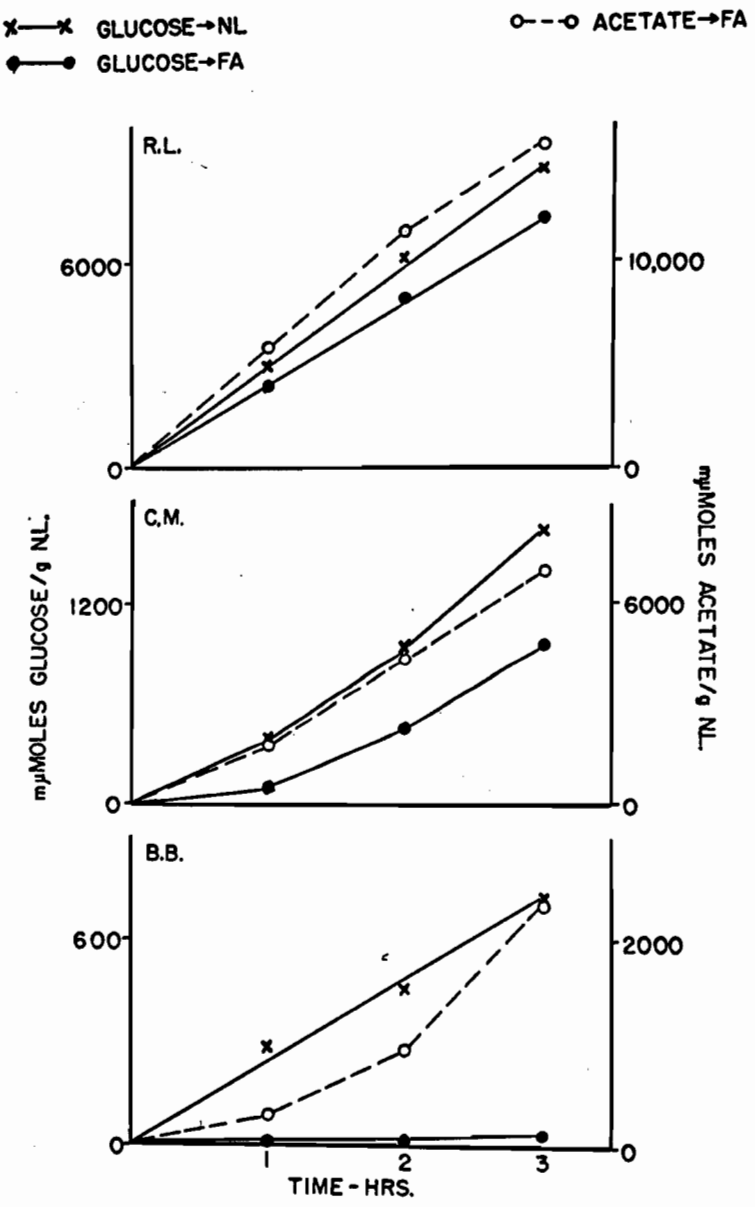
C. more than 2000 mμMoles/g neutral lipid per hour - one sample.

Except for the very active sample of omentum in group C, the rate of lipogenesis increased over the three hours of incubation. In tissues with low activity (group A), incorporation of glucose into fatty acids increased from a mean of 6 to 44 mμMoles/g neutral lipid per hour. The more active tissues in group B gave values of 85 and 732 mμMoles/g neutral lipid in the first and third hours of incubation respectively. At the same time, there was no significant change in glyceride glycerol synthesis, although it showed a tendency to fall as incubation progressed. In six of the nine tissues studied the incorporation of glucose into neutral lipid was approximately linear with time; the other three, which were in group B, showed an increasing rate of neutral lipid synthesis. These observations indicate that the pattern of glucose utilization for lipid synthesis changes with the length of incubation, although this may not be obvious from the incorporation of glucose into neutral lipid.

The effect of the length of incubation on lipogenesis from acetate-^{3}H was studied with omentum from three of the nine patients in this series. The incorporation of glucose and acetate into neutral lipid and fatty acids are plotted against time in Figure 6. In tissue from C.M. (group B) and R.L. (group C) lipogenesis from acetate was approximately linear with time, whereas lipogenesis from glucose in-

Figure 6

Effect of Time on Lipid Synthesis by Human Omentum.



creased with time in tissue from C.M. only. With tissue from B.B. (group A) where neutral lipid synthesis was 'low' but linear, lipogenesis from both glucose and acetate continued to rise from the first to the third hour of incubation. Thus, there seem to be several patterns of glucose and acetate metabolism in human omentum in vitro.

As the slow initial rates of lipogenesis could represent inhibition by high levels of endogenous fatty acids (FFA), tissue FFA concentrations were determined in two experiments and found to remain constant throughout the three-hour period of incubation (Table XII). These results suggest that the increasing rates of lipogenesis are not related to endogenous FFA concentrations. However, both of the samples used in these experiments were from patients arbitrarily classified as falling into 'group B'; it is therefore uncertain whether the fatty acid levels in omentum from other patients do not change with time.

In order to examine the effects of the length of incubation on adipose tissue metabolism further, and to circumvent the difficulties inherent in studies of human adipose tissue, subsequent experiments were carried out using the epididymal fat pad of the rat.

TABLE XII

FFA Concentrations in Human Omentum.

Time of Incubation (min.)	L.S.	R.P.
	μEq/g N.L.	
0	2.4	2.9
30	2.1	2.0
60	1.9	2.3
120	2.2	2.5
180	2.1	2.7

Medium glucose - 10.7 mM; no insulin.

B. Rat Adipose Tissue.

Herrara et al. (236,237) reported that the utilization of glucose by the epididymal fat pad of the rat increases on prolonged incubation. This finding was similar to the observations with human adipose tissue described in the previous chapter and prompted an investigation of the effect of preincubation in rat adipose tissue. The results of these studies are presented here.

1. The effect of preincubation on lipid synthesis in rat epididymal fat pads.

The effect of preincubation on lipogenesis and glyceride glycerol synthesis from glucose was studied in half pads. The half pad which served as the control was incubated without label for 30 minutes; the other half of the ipsilateral pad was preincubated for 360 minutes. The pads were then transferred to medium containing glucose-U-^{14}C and incubated for two hours.

Table XIII shows the results obtained with adipose tissue from fed rats and rats fasted for 48 hours. In tissue from fed rats lipogenesis increased from a mean of 55 to 107 mμMoles/100 mg neutral lipid following preincubation when incubation was carried out in 43 mM glucose in the absence of insulin. With relatively physiological concentrations of medium glucose (10.7 mM) and insulin (0.05 munits/ml)

TABLE XIII

Effect of Preincubation on Fatty Acid and Glyceride Glycerol Synthesis from Glucose-U-^{14}C by Adipose Tissue from Fed and 48 Hour-Fasted Rats.

	Fed			Fasted	
Glucose mM	10.7		42.7	10.7	
Insulin munits/ml	0.05(8)[a]	80(6)	0.05(6)	0.05(4)	80(4)
	mμMoles/100 mg N.L.			mμMoles/100 mg N.L.	
Fatty Acids					
Control	88	652	55	17	80
Preincubated	186	614	107	52	87
Difference ±S.E.	98±25	-38±100	52±12	35±4.5	7±4.8
p	<0.01[b]	N.S.	<0.01	<0.005	N.S.
Glyceride glycerol					
Control	89	348	52	136	210
Preincubated	138	390	83	204	174
Difference ±S.E.	49±7	42±42	31±5	68±14	-36±11
p	<0.001	N.S.	<0.005	<0.02	<0.05

Flask contents: 5 ml. Preincubation medium: glucose 10.7 mM, gelatin 1% and insulin as indicated. Incubation medium also contained glucose-U-^{14}C 0.02 μc/μMole. Half fat pads preincubated for 30 minutes are referred to as the 'Controls'; others, preincubated for 360 minutes, are labelled 'Preincubated'. The time for final incubations was 2 hours.

a-Number of duplicate determinations.

b-Mean ± S.E.

preincubation led to an increase in lipogenesis from 88 to 186 and from 17 to 52 mμMoles/100 mg neutral lipid in tissue from fed and fasted rats, respectively. However, at high medium insulin concentrations (80 munits/ml) preincubation had no effect on lipogenesis, suggesting that preincubation had an insulin-like effect on glucose utilization by adipose tissue. Preincubation had the same effect on glyceride glycerol synthesis as on lipogenesis, with the exception that glyceride glycerol synthesis decreased in tissue from fasted rats incubated with 80 munits of insulin/ml.

As transport into the adipocyte is rate-limiting for the utilization of glucose but not for acetate, and in view of the previous findings with human adipose tissue (cf. Figure 6), it was of interest to study the effect of preincubation on lipogenesis from acetate in rat adipose tissue. The results in Table XIV demonstrate that preincubation did not stimulate lipogenesis from acetate-1-^{14}C in tissue from fed rats at either of the two insulin concentrations used. However, tissue from fasted rats incubated with 0.05 munits insulin/ml showed increased lipogenesis following preincubation; this effect did not occur when the medium insulin concentration was raised to 80 munits/ml.

The necessity for including insulin or glucose in the media used for the preincubation was next investigated. In these studies the media contained 10.7 mM glucose but insulin was omitted. The results in Table XV indicate that insulin is not required to demonstrate the

TABLE XIV

Effect of Preincubation on Lipogenesis from Acetate-1-^{14}C.

			Fatty Acids			
Rats	n[a]	Insulin	Control	Preinc.	Change with preinc.	p
		munits/ml	mμMoles/100 mg N.L.			
Fed	6	0.05	188	186	-2 ± 13[b]	N.S.
	6	80	605	606	1 ± 13	N.S.
Fasted	11	0.05	27	57	30 ± 5	<0.001
	11	80	83	98	15 ± 10	N.S.

Conditions and preincubation medium as in Table XIII. Incubation time - 2 hours. Incubation medium: glucose 10.7 mM, acetate 10.7 mM and acetate-1-^{14}C 0.02 μc/μMole.
a-Number of determinations
b-Mean ± S.E.

TABLE XV

Effect of Preincubation without Insulin on the Utilization of Glucose-U-^{14}C by Adipose Tissue from Fed Rats.

	Control	Preinc.	Change with preinc.	p
	mμMoles/100 mg N.L.			
CO_2	187	307	120 ± 39[a]	<0.025
Glyceride glycerol	112	172	60 ± 7	<0.001
Fatty acids	152	256	104 ± 35	<0.025

Conditions and preincubation medium as in Table XIII but without insulin in incubation and preincubation medium. Incubation time - 2 hours.
a-Mean ± S.E. in seven determinations.

stimulation of CO_2 production, fatty acid synthesis and glyceride-glycerol synthesis from glucose-U-^{14}C by preincubation. Furthermore, the effect of preincubation on lipogenesis could be demonstrated at low glucose concentrations (1.33 mM) and even without glucose in the preincubation medium (Table XVI).

The effect of preincubation on isolated fat cells was studied in two experiments (Table XVII). In the first, the substrate was glucose-1-^{14}C and the preincubation lasted 4.5 hours. Incorporation of label into CO_2, total lipids and fatty acids increased 159%, 57% and 241% above controls respectively. However, glyceride glycerol synthesis decreased by 49%. The second experiment was carried out with glucose-6-^{14}C and after six hours of preincubation lipogenesis was 84% higher than in non-preincubated cells. Insulin was not present in these experiments.

2. The effect of preincubation on tissue FFA concentration.

The possibility that the effect of preincubation on lipogenesis is a result of elevated concentrations of FFA in the control tissues was investigated.

The FFA concentrations in whole fat pads, either unincubated or incubated for 30 and 360 minutes with 10.7 mM glucose and 0.05 munits insulin/ml were compared. The conditions employed in these ex-

TABLE XVI

Effect of Preincubation without Insulin of Glucose or at Low Glucose Concentration on Lipogenesis from Glucose-U-^{14}C in Adipose Tissue from Fed Rats.

Preinc. Medium Glucose mM	Fatty Acids		
	Control	Preinc.	Increase
	mμMoles/100 mg N.L.		
1.33	58[a]	213	155
	215	228	13
	29	89	60
0[b]	40	58	18
	63	292	229
	28	69	41

Each value is the mean of duplicate determinations. Conditions as in Table XIII but without insulin in preincubation or incubation medium. Glucose concentrations in preincubation medium as shown.
a-Mean of duplicate determinations.
b-Controls preincubated in 2.7 mM glucose for 30 minutes; the experimental samples were preincubated for 330 minutes without glucose then transferred to medium containing 2.7 mM glucose for 30 minutes before incubation with glucose-U-^{14}C.

TABLE XVII

Effect of Preincubation on Glucose Metabolism of Isolated Adipocytes from Fed Rats.

	Preinc.	Control	Preinc.	Change
	hr.	mμMoles/100 mg N. L.[a]		%
Glucose-1-^{14}C	4.5			
CO_2		231	597	159
Neutral lipid		430	675	57
Fatty acids		156	534	241
Glyceride glycerol		274	141	-49
Glucose-6-^{14}C	6			
Fatty acids		106	195	89

The medium contained 3.5% albumin and 8 mM glucose.
Incubation time - 2 hours.
a-Mean of duplicates.

periments were the same as those used to study the effect of preincubation on lipogenesis (cf. Table XVIII).

The results in Table XVIII indicate that the FFA concentration in adipose tissue from fasted rats continued to fall after 30 minutes of incubation. In contrast, tissue from fed rats achieved stable concentrations of FFA by this time. The stimulation of lipogenesis by preincubation in tissue from fasted, but not fed rats may therefore partly result from a fall in tissue FFA levels.

3. The effect of preincubation on lipogenesis from acetyl-CoA in homogenates of adipose tissue.

As preincubation may lead to an increase in lipogenesis due to an increase in the activity of the lipogenic enzymes or the appearance of stimulatory substances, it was of interest to see if this could be demonstrated with homogenates of adipose tissue.

Half of the fat pads from controlateral sides were combined, two halves serving as controls and the other two as experimental samples. The controls and experimentals were preincubated for 30 and 360 minutes respectively, with 10.7 mM glucose and 0.05 munits insulin/ml as previously described. At the end of the preincubation periods the 12,000 xg supernatants were prepared and lipogenesis from acetyl-1-^{14}C was determined (cf. Experimental Procedure). The values obtained are shown in Table XIX.

TABLE XVIII

Effect of Length of Preincubation on FFA Concentration in Adipose Tissue from Fed and 48 Hour-Fasted Rats.

	Period of Incubation		FFA			
	A	B	A	B	Difference (B-A)	p
	minutes		μEq/g tissue			
Fed (6)[a]	0	30	3.41	2.80	0.62 ± 0.19[b]	<0.025
	30	360	2.86	2.73	0.13 ± 0.13	N.S.
Fasted (6)	0	30	5.06	4.10	0.96 ± 0.30	<0.025
	30	360	4.09	2.87	1.22 ± 0.39	<0.05

Medium: glucose 10.7 mM; gelatin 1%; insulin 50 μunits/ml.
a-Number of determinations.
b-Mean ± S.E.

TABLE XIX

Lipogenesis from Acetyl-1-^{14}C-CoA in the 12,000xg Supernatants of Rat Adipose Tissue Homogenates.

Expt.	Control	Preincubated
	mμMoles/100 mg tissue[a]	
1	36	31
2	50	45
3	25	27
4	25	25
5	65	50
6	12	10
Mean	35	31

See Experimental Procedure for details.
a-Means of duplicates.

It can be seen that preincubation had no effect on lipogenesis from acetyl-CoA. Indeed, in four out of six experiments prolonged preincubation resulted in a slight decrease in lipogenesis and the average rate of incorporation fell from 35 to 31 mμMoles/100 mg tissue.

4. The relation between preincubation and glucose transport.

It was shown previously that the oxidation of glucose to CO_2 and its utilization for glyceride glycerol and fatty acid synthesis all increase on prolonged incubation. The possibility was therefore considered that the permeability of the adipocyte changes during the time of preincubation, resulting in a secondary rise in glucose utilization. If this is the case, two things should happen: first, it should be possible to prevent the rise in glucose utilization by partially blocking the glucose transport system; second, the transport of a non-metabolizable sugar which competes with glucose for transport into the adipocyte should increase.

The first possibility was investigated by using phloretin to block the transport of glucose. Frerichs and Ball (238) have provided evidence that phloretin at a concentration of 0.1 mM does not interfere with the metabolism of endogenous glycogen in adipose tissue from fasted-refed rats while glucose transport is curtailed. More recently,

Crofford and Renold (199) reported that phloretin selectively inhibits glucose transport in adipose tissue.

The preincubation and incubation of adipose tissue from fed rats was carried out as previously described with medium glucose at 10.7 mM but without insulin. In addition, the medium used for the incubations contained 0.1 or 1.0 mM phloretin. 0.1 mM phloretin inhibited neutral lipid synthesis by 60%. The data presented in Table XX show that phloretin prevented the rise in lipogenesis and glyceride glycerol synthesis usually seen following preincubation. This was observed at both of the concentrations of phloretin employed. These results suggested that the effect of preincubation depends on the integrity of the glucose transport system.

The second possibility, that the activity of the glucose transport system increased on preincubation, was studied by measuring the rate of accumulation of a non-metabolizable sugar, 3-0-methyl-^{14}C-glucose (3-MG), in the intracellular compartment of adipose tissue from fed rats. Half pads were preincubated in medium containing 10.7 mM glucose and 4 g albumin/100 ml; insulin was not present. Following the usual periods of preincubation, the tissues were rinsed for 45 seconds in albumin-Krebs-Ringer bicarbonate medium, blotted lightly and transferred to glass counting vials containing the radioactive markers but neither glucose nor insulin (cf. Experimental Procedure). The incubations lasted for 6, 10, 20 and 120 minutes. The results are shown in Figure 7

TABLE XX

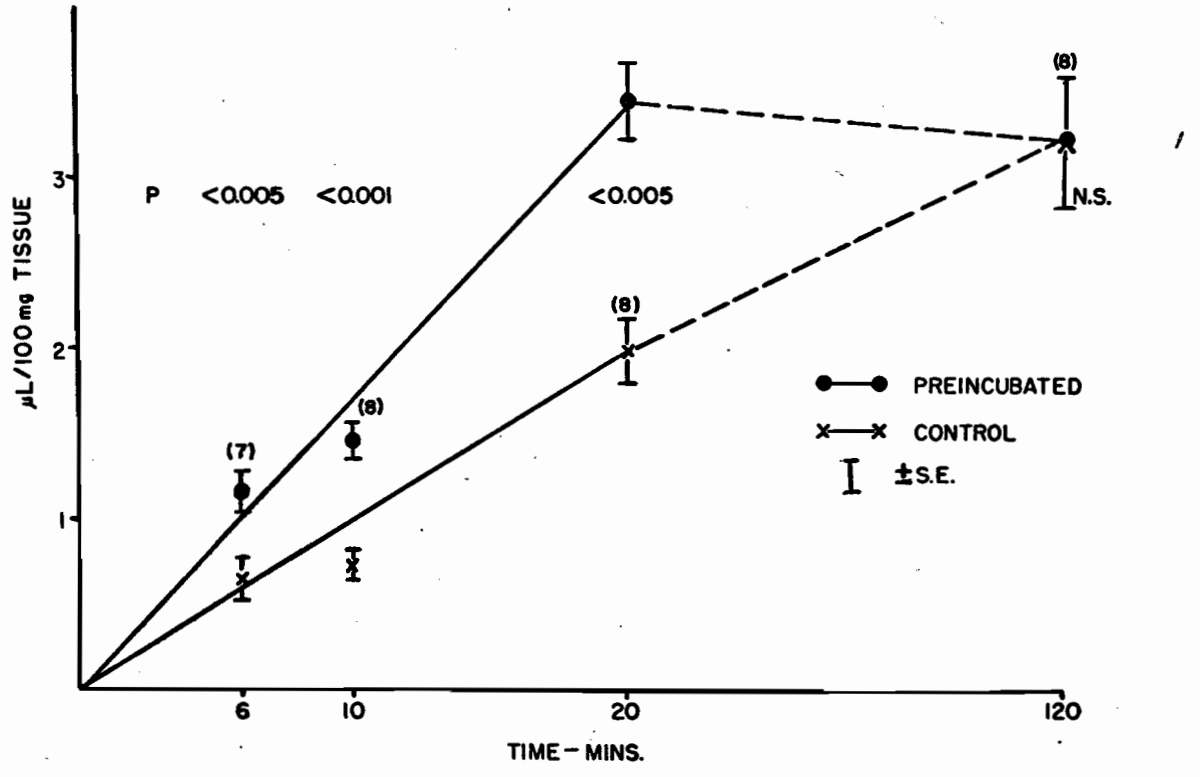
Effect of Phloretin and Preincubation on Lipid Synthesis from Glucose-U-^{14}C in Adipose Tissue from Fed Rats.

Phloretin	n[a]	Product	Control	Preinc.	Change	p
mM			mμMoles/100 mg tissue			
0.1	3	FA	17.0	19.6	2.6±1.0[b]	N.S.
		G-G[c]	26.6	40.6	14.0±4.0	N.S.
1.0	9	FA	3.7	2.0	-1.7±1.0	N.S.
		G-G	12.5	10.1	-2.4±1.9	N.S.

Conditions as in Table XIII. Medium glucose - 10.7 mM, no insulin. Phloretin was dissolved in absolute ethanol. All flasks contained 0.5% ethanol. Incubation time - 2 hours.
a-Number of duplicate determinations
b-Mean ± S.E.
c-Glyceride glycerol

Figure 7

Accumulation of 3-0-Methyl Glucose in the Intracellular Space of Rat Adipose Tissue.



See Text and 'Experimental Procedure'.

(cf. Appendix, Table XXII). It was found that preincubation significantly increased the rate of accumulation of 3-MG in the intracellular space after 6, 10 and 20 minutes of incubation, indicating that preincubation had stimulated the transport system. This effect disappeared in tissue incubated for 120 minutes presumably because equilibration between the intracellular and extracellular compartments had taken place by this time. As 3-MG occupies the entire intracellular water space if enough time is allowed for equilibration (198), these results also indicate that there is no change in the volume of the intracellular space with prolonged incubation.

V. DISCUSSION

Much of the work on the physiology and biochemistry of adipose tissue has been carried out using the epididymal fat pad of the rat (24). Prompted largely by the possibility of species difference, a few studies on the metabolism of human adipose tissue have appeared in recent years (1,2,9-11,15,16). These demonstrated that human adipose tissue in carries out the esterification and release of fatty acids and the formation of fatty acids from glucose and acetate.

In the present work a comparison was made between human omental and subcutaneous adipose tissue. When based on the DNA content, it was found that rates of neutral lipid synthesis and oxidation of glucose to CO_2 were similar in both tissues. On the other hand, Hamosh et al. (10), expressing neutral lipid synthesis and the rate of esterification of FFA on the basis of tissue wet weight, found that subcutaneous adipose tissue was more active than omental adipose tissue. If one calculates their results on the basis of the DNA content shown in Table III, it is found that both tissues carry out neutral lipid synthesis and esterification at the same rate.

However, in the view of the lower ratio of carbon 1 to carbon 6 of glucose oxidized to CO_2, the pentose cycle appears to be less active in subcutaneous adipose tissue. This correlates with our

findings as well as with those of Hamosh et al. (10) and Gellhorn and Benjamin (70) that average values of lipogenesis are lower for subcutaneous than omental adipose tissue.

The evidence that human adipose tissue responds to insulin by a stimulation of glucose utilization has, in the past, been equivocal (1,2), although a number of workers have noted that insulin stimulates the uptake of glucose by human adipose tissue in vitro (15, 16) and in vivo (14). The data presented here (Table V) clearly show that insulin stimulates the utilization of glucose by both omental and subcutaneous adipose tissue. More specifically, insulin stimulated CO_2 production, neutral lipid synthesis and lipogenesis from glucose and lipogenesis from acetate in vitro. It has also been demonstrated that the effect of insulin on neutral lipid synthesis is partially decreased at high medium glucose concentrations (Table IV). This finding is consistent with the hypothesis of Levine and Goldstein (239) that the main action of insulin is in the translocation of glucose into the cell interior.

The effect of insulin on human adipose tissue in vivo could also be demonstrated indirectly by infusion of glucose prior to removal of the adipose tissue. Thus, a decrease in insulin-stimulated neutral lipid synthesis was demonstrated in subcutaneous and omental adipose tissue from infused patients (Table VII). The infusion also led to an increase in the ratio of carbon 1 to carbon 6 of glucose oxidized

to CO_2 (Table IX). This increase was quantitatively similar to that encountered when tissue from non-infused patients was stimulated by insulin in vitro, suggesting that the infusion of glucose had brought about the release of insulin which then stimulated the omental tissue. That this could be demonstrated in vitro is probably due to the tight binding of insulin to adipose tissue, as observed by Ball and Jungas (240). The nutritional state of the tissue donor is therefore an important factor in determining the responsiveness of adipose tissue to the effects of insulin in vitro. The inability of Hirsch and Goldrick (1,2) to obtain a consistent increase in glucose utilization in the presence of insulin may partially be explained on this basis, as many of their tissue donors had received a test meal two hours before sampling. The difference between the complete absence of an insulin effect in their hands and the diminished but still demonstrable response after glucose infusion in our experiments may be related to the route of glucose administration. Thus, McIntyre et al. (241) and Dupre (242) showed that oral glucose was more effective in increasing the blood insulin level and the rate of glucose disappearance than glucose given intravenously.

Several other factors also influence the responsiveness of adipose tissue to insulin in vitro. The integrity of the adipocyte membrane appears to be a prerequisite for the action of insulin. Hirsch and Goldrick (1) provided evidence that minced or needled rat adipose

tissue is less responsive to insulin than intact tissue. It is therefore conceivable that shreds of human adipose tissue sampled by needle biopsy possess damaged cell membranes on which insulin cannot act. Another factor in determining the responsiveness to insulin is the type of albumin used for incubations. Recant and Alp (243) have shown that certain preparations of albumin have insulin-like activity, and Kahlenberg and Kalant (15) ran into problems of this type in their studies on the effect of insulin on human adipose tissue.

One of the major problems in studying human adipose tissue was the great variability in the lipogenetic activity of this tissue. Similar difficulties were also encountered by other workers in this field (1,9). Using human subcutaneous adipose tissue, Gellhorn and Marks (9) obtained values ranging between 0 and 68 mμMoles of acetate incorporated into lipid per gram wet weight per hour. Hirsch and Goldrick (1) reported that the coefficient of variation (i.e. the standard deviation/mean x 100) for lipogenesis from acetate in samples from nine subjects was 88.5%; in other words, the standard deviation was almost as large as the mean itself. However, the day to day fluctuations in samples from the same patient gave a coefficient of variation of 66% and they attributed much of the variability in lipogenesis to changes in the daily rate. It is clear from their date that lipogenesis must have been very low or even absent in some of their samples. Similar observations were also made in the present study, as

can be seen from Tables V and VII. In spite of differences in experimental conditions the values obtained here were of the same order as those observed by other workers. These are summarized in Table XXI.

Hirsch and Goldrick (17) calculated that an average of 0.7 g of fatty acid is synthesized from acetate per day, but concede that this is a minimal value because of dilution of substrate by endogenous pools. As the actual rate of lipogenesis cannot be calculated accurately from available information, it may be more profitable to determine the lipogenetic capacity of adipose tissue, i.e. the maximal rate of synthesis. Using the rate of lipogenesis in the most active sample of tissue studied (Table XI, Fig. 6, subject R.L.), and assuming uniform activity of the entire adipose tissue depot, and 6.5 kg lipid in a 10 kg depot, it can be calculated that more than 60 g glucose could be converted to fatty acids per day:

Lipogenesis: rate <u>in vitro</u>	2.4 µMoles glucose converted to fatty acids/g lipid/hour
: in 24 hours	57.6 µMoles/g lipid
: in 6.5 kg lipid	374 mMoles
	= 67.5 g glucose

As discussed previously, lipogenesis is less active in subcutaneous tissue than in omentum. However, these comparisons were based on average

TABLE XXI

Lipid Synthesis from Glucose and Acetate

Author	Medium Glucose	Glucose Incorporation		Acetate Incorporation
	mg %	N.L.	F.A.	N.L.
		mμMoles/g N.L./2 hours		
Omentum				
Gellhorn and Benjamin (70)	360	-	-	200
Hamosh et al.(10)[a]	200	-	-	3510
	200	3550	-	-
Fessler	50[b]	380	35	-
	50[b,c]	545	201	-
	50	-	-	386
	200	1310	685	-
Subcutaneous				
Gellhorn and Benjamin (70)	360	-	-	3
Hirsch and Goldrick (1)	90	-	-	183
	90	186	30	-
Hamosh et al. (10)[a]	200	-	-	150
	200	1870	-	-
Fessler	50[b]	150	4	-
	50[b,c]	267	40	-
	50	-	-	12

a-Time of incubation not given but 2 hours assumed.
b-Glucose-1-^{14}C; all others glucose-U-^{14}C where applicable.
c-Glucose infusion before sampling (cf. Tables V and VI).

values for lipogenesis. As shown in Table VIII the extreme value for lipogenesis in subcutaneous adipose tissue from a patient who had received intravenous glucose was 1143 mμMoles of glucose converted to fatty acids/mg DNA, whereas the extreme value for omentum was 1794 mμMoles/mg DNA. Thus, the rate of lipogenesis in subcutaneous tissue can, under certain conditions, approach the rates encountered with omental adipose tissue.

The actual rate of lipogenesis may be even greater than indicated above. When lipogenesis from glucose by omentum from this patient (R.L.) was measured in the presence of acetate, the rate of lipogenesis increased from 2.4 to 4.0 mμMoles/g neutral lipid per hour. This increase is similar to the observations of Flatt and Ball who noted that acetate stimulated lipogenesis from glucose by rat adipose tissue in the presence but not in the absence of insulin (250). If the stimulation by acetate represents a physiologic mechanism, then this patient could theoretically synthesize 112 g fatty acid from glucose per day.

The variability in lipogenesis between the tissues from various patients has led us to classify them tentatively into three groups (Table XI). Group A (four patients) consisted of adipose tissues having very little or no lipogenesis and low neutral lipid synthesis (< 300 mμMoles/g neutral lipid/hour) from glucose. Upon preincubation for two hours a moderate increase in the amount of lipogenesis was noted. These effects resemble those seen with adipose tissue from fasted rats. Group B (four patients) consisted of tissues showing a rate of lipogenesis from

glucose ten times and a rate of neutral lipid synthesis twice that of group A. Lipogenesis in this group resembled the type of activity seen in tissues from fed rats. The actual per cent increase in lipogenesis following incubation was similar for the two groups. The third group C consists thus far of the patient R.L., whose tissue showed an extremely rapid rate of lipogenesis and neutral lipid synthesis from glucose which did not change after two hours of incubation. Thus this tissue is analogous to the adipose tissue from fed rats maximally stimulated with insulin (Table XIII). In order to gain some insight into the mechanism responsible for the increase in lipogenesis following incubation of human adipose tissue, the phenomenon was reproduced with rat adipose tissue, using epididymal fat pads from fed and fasted animals. The use of rat tissue had the advantages of being more readily available, having less biological variation between tissue donors and being amenable to controlled conditions of diet, weight, etc.

In the case of the fasted rats a possible explanation of the preincubation phenomenon may lie in the observation that the FFA content of rat adipose tissue is elevated in starvation (Table XVIII), and that FFA or their CoA derivatives may inhibit lipogenesis under these conditions (cf. p. 26). It was found that with tissues from fasted animals the 30 minute preincubation period routinely used in all studies was not sufficient to stabilize the tissue FFA level. However, in tissue from fed rats the FFA content did not change with incubation. Never-

theless, it is clear that prolonged incubation stimulated lipogenesis from glucose as readily in tissues from fed as from fasted animals. As shown in Table XIII, a 48 hour fast led to an 80% fall in lipogenesis in control tissues. However, after preincubating tissues for 6 hours, lipogenesis was still 72% lower in 'fasted' than in 'fed' adipose tissue. Consequently, it is unlikely that the FFA concentration in tissue from fasted animals is an important cause of the preincubation effect. A parallel situation may exist in the human adipose tissue. In two of the experiments with human tissue in which the FFA level was measured it was found to be stable throughout the three-hour incubation. However, both tissues fell into group B, resembling tissues from fed animals. Therefore, the possibility that the FFA level contributed to the lower activity in the tissues of group A cannot be ruled out.

The question now arises as to what the effect of preincubation on adipose tissue metabolism actually represents. The adaptive hyperlipogenesis observed on refeeding fasted animals is associated with an increase in the activity of enzymes related to lipogenesis (244). No such changes seem to occur during preincubation in vitro. It was shown in Table XIX that lipogenesis from acetyl-CoA in the presence of malonyl-CoA, which includes the rate limiting acetyl-CoA carboxylase step, is unaltered by preincubation. In addition, Herrera et al. (236)

who studied the effect of preincubation on hexokinase, pentose cycle dehydrogenases, glycerol phosphate dehydrogenase, and fatty acid synthetase, were unable to demonstrate an increase in the activities of any of these enzymes after preincubating adipose tissue from fasted rats for 24 hours. They also observed that the preincubation effect was not diminished when tissues were incubated in the presence of puromycin to block enzyme synthesis, and proposed that preincubation leads to the accumulation of glycolytic intermediates which stimulate lipogenesis.

A more likely explanation for the effect of preincubation on lipogenesis, and for which evidence is provided here, is that prolonged incubation leads to a breakdown of tissue barriers to glucose transport. Thus, we have shown that preincubation leads to an increase in the rate of transport of the non-metabolizable sugar 3-0-methyl glucose (Figure 7), and that the preincubation effect is abolished when glucose transport is partially blocked by 0.1 mM phloretin (Table XX). In addition, the preincubation effect could be demonstrated in the absence of insulin or glucose during the period of preincubation, indicating that stimulatory glycolytic intermediates play no role in producing the effect. Another point favoring the change in membrane permeability is that the effect of preincubation on tissues both from fed and fasted animals was overcome by high concentrations of insulin in the preincubation

medium (Table XIII). While this section of the thesis was being written, there appeared a report by Galton and Fain (245) which supports the views proposed here, and provides data showing that the sensitivity of isolated adipocytes to insulin diminishes after four hours of incubation, although the lipogenetic activity of the adipocytes remained unaffected. Our observation that lipogenesis from acetate by tissues from fed rats is not stimulated by preincubation (Table XIV) indicates that the access of some substrates to the lipogenetic system of the cell, rather than the activity of the lipogenetic system per se, is rate-limiting in fatty acid synthesis.

One can only speculate on the nature of the membrane changes induced by insulin or preincubation. Some of the evidence at hand indicates that membrane structure is altered. Rieser and Rieser noted that insulin has proteolytic properties (246) and that trypsin and chymotrypsin can, like insulin, promote glycogen synthesis and the accumulation of 3-0-methyl glucose by diaphragm in vitro (247). Barnett and Whitney (248) observed that trypsin in low concentrations stimulates glucose uptake by rat fat pads in vitro. Recently, Rodbell (249) showed that phospholipase C stimulates glucose uptake and oxidation as well as the incorporation of amino acid into proteins by isolated adipocytes. These actions were a function of the phospholipase C concentration but disappeared in the presence of insulin. Rodbell proposed that insulin

and phospholipase C produce their effects by altering the configuration of cell membrane lipoproteins from a lamellar to a micellar form.

It can be concluded therefore that a number of factors which alter the cell membrane of the adipocyte all lead to increased glucose transport and dissimilation. A susceptibility to changes of this type may be characteristic of the human adipocyte, and may account in part for the variability in lipogenesis and the difficulties in demonstrating the metabolic effects of insulin encountered with human adipose tissue.

VI. SUMMARY

1. Human omental and subcutaneous adipose tissue responds to the presence of insulin in vitro by an increase in the oxidation of glucose to CO_2 and the incorporation of glucose carbon into neutral lipids and fatty acids.

2. The insulin-stimulated neutral lipid synthesis of both types of human adipose tissue was diminished by the intravenous infusion of glucose into tissue donors prior to the sampling of adipose tissue. It was also demonstrated that the ratio of carbon 1 to carbon 6 of glucose oxidized to CO_2 by omental tissue is increased by glucose infusion or by the presence of insulin in vitro.

3. Lipogenesis from glucose by human omentum was found to be exceedingly variable and in most instances did not increase linearly with the length of incubation. The rate of neutral lipid synthesis increased or remained constant while the rate of lipogenesis increased during a three-hour period of incubation.

4. Epididymal fat pads from fed rats showed increased neutral lipid and fatty acid synthesis from glucose but not from acetate after six hours of preincubation. The level of FFA was found to be the same in the unpreincubated and in the preincubated tissues. The stimulatory effect of prolonged incubation on glucose utilization from these tissues occurred independently of the presence of glucose or insulin in the

preincubation medium. This effect was also demonstrated with isolated adipocytes.

5. Epididymal fat pads from rats fasted for 48 hours responded to preincubation by an increase in lipogenesis both from glucose and acetate and a decrease in FFA content.

6. Preincubation had no effect on lipogenesis in adipose tissue from fed or fasted rats when incubated with high concentration of insulin.

7. It was shown that the inhibition of lipogenesis induced by fasting is the same before and after prolonged incubation, and evidence that prolonged incubation does not correct the defect in lipogenesis by fasting was presented.

8. Preincubation had no effect on lipogenesis from acetyl-CoA in the 12,000 x g supernatant of adipose tissue homogenates.

9. It was shown that the activity of the glucose transport system is stimulated by preincubation and can account for all the effects of preincubation observed, provided that lipogenesis is not inhibited by endogenous fatty acids. This was discussed in relation to membrane changes induced by lipolytic and proteolytic enzymes.

VII. CLAIMS TO ORIGINALITY

The author considers that all points given in the summary are original contributions to scientific knowledge.

VIII. BIBLIOGRAPHY

1. Hirsch, J., and Goldrick, R.B., J. Clin. Invest., 43, 1776, 1964.

2. Goldrick, R.B., and Hirsch, J., J. Clin. Invest., 43, 1793, 1964.

3. Cahill, G.F., Jeanrenaud, B., Leboeuf, B., and Renold, A.E., Ann. N.Y. Acad. Sci., 82, 403, 1959.

4. Renold, A.E., Winegrad, A.I., Jeanrenaud, B., and Martin, D.B., The Mechanism of Action of Insulin, Blackwell Scientific Publications, Oxford, 1960, p. 153.

5. Kekwick, A., Pawan, G.L.S., and Chalmers, T.M., Lancet, 2, 1157, 1959.

6. Rabinowitz, D., and Zierler, K.L., J. Clin. Invest., 41, 2173, 1962.

7. Wolff, O.H., Quart. J. Med., 24, 109, 1955.

8. Karam, J.H., Grodsky, G.M., and Forsham, P.H., Diabetes, 12, 197, 1963.

9. Gellhorn, A., and Marks, P.A., J. Clin. Invest., 40, 925, 1961.

10. Hamosh, M., Hamosh, P., Bar-Maor, J.A., and Cohen, H., J. Clin. Invest., 42, 1648, 1963.

11. Jacob, M.I., Biochim. Biophys. Acta, 70, 231, 1963.

12. Vaughan, M., J. Lipid Res., 2, 293, 1961.

13. Jeanrenaud, B., Metabolism, 10, 535, 1961.

14. Rabinowitz, D., and Zierler, K.L., J. Clin. Invest., 41, 2191, 1962.

15. Kahlenberg, A., and Kalant, N., Can. J. Biochem., 42, 1623, 1964.

16. Pozza, G., Ghidoni, A., and Basilico, C., Lancet, 1, 836, 1963.

17. Fessler, A., Rubinstein, D., and Beck, J.C., Clin. Res., 13, 322, 1965, (Abstract).

18. Fessler, A., Rubinstein, D., and Beck, J.C., Biochim. Biophys. Acta, 106, 199, 1965.

19. Wertheimer, E., and Shapiro, B., Physiol. Review, 28, 451, 1948.

20. Hausberger, F.X., Milstein, S.W., and Rutman, R.J., J. Biol. Chem., 208, 431, 1954.

21. Feller, D.D., J. Biol. Chem., 206, 171, 1954.

22. Favarger, P., and Gerlach, J., Helv. Physiol. Pharmacol. Acta, 12, 15, 1954.

23. Favarger, P., and Gerlach, J., Helv. Physiol. Pharmacol. Acta, 12, 70, 1954.

24. Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965.

25. Magnus-Levy, A., Beitr. Chem. Physiol. Path., 2, 261, 1902.

26. Smedley Maclean, I., and Hoffert, D., Biochem. J., 20, 343, 1926.

27. French, T.H., and Popjack, G., Biochem. J., 49, 3, 1951.

28. Rittenberg, D., and Bloch, K., J. Biol. Chem., 160, 417, 1945.

29. Zabin, I., J. Biol. Chem., 189, 355, 1951.

30. Dauben, W.G., Hoerger, E., and Peterson, J.W., J. Am. Chem. Soc., 75, 2347, 1953.

31. Popjak, G., French, T.H., Hunter, G.D., and Martin, A.J.P., Biochem. J., 48, 612, 1951.

32. Lynen, F., Fed. Proc., 12, 683, 1953.

33. Martin, D.B., and Vagelos, P.R., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 211.

34. Gibson, D.M., Progress in the Chemistry of Fats and other Lipids, Ed. Holman, R.T., Lundberg, W.O., and Malkin, T., MacMillan, New York, 1963, Vol. 6, p. 117.

35. Vagelos, P.R., Ann. Rev. Bioch., 33, 139, 1964.

36. Brady, R.O., Bradley, R.M., and Trams, E.G., J. Biol. Chem., 235, 3093, 1960.

37. Wakil, S.J., Porter, J.W., and Gibson, D.M., Biochim. Biophys. Acta, 24, 453, 1957.

38. Brady, R.O., J. Biol. Chem., 235, 3099, 1960.

39. Martin, D.B., Horning, M.G., and Vagelos, P.R., J. Biol. Chem., 236, 663, 1961.

40. Lynen, F., Fed. Proc., 20, 941, 1961.

41. Goldman, P., Alberts, A.W., and Vagelos, P.R., J. Biol. Chem., 238, 1255, 1963.

42. Lennarz, W.J., Light, R.J., and Bloch, K., Proc. Nat. Aca. Sci. U.S., 48, 840, 1962.

43. Wakil, S.J., J. Am. Chem. Soc., 80, 6465, 1958.

44. Martin, D.B., and Vagelos, P.R., J. Biol. Chem., 237, 1787, 1962.

45. Wakil, S.J., and Gibson, D.M., Biophys. Acta, 41, 122, 1960.

46. Waite, M., and Wakil, S.J., J. Biol. Chem., 237, 2750, 1962.

47. Waite, M., and Wakil, S.J., J. Biol. Chem., 238, 77, 1963.

48. Lynen, F., Matsuhashi, M., Numa, S., and Schweizer, E., The Control of Lipid Metabolism, Ed. Grant, J.K., Biochem. Soc. Symp. No. 24, Oxford, 1963, Academic Press, London, 1963, p. 43.

49. Vagelos, P.R., Alberts, A.W., and Martin, D.B., J. Biol. Chem., 238, 533, 1963.

50. Vagelos, P.R., and Alberts, A.W., J. Biol. Chem., 235, 2786, 1960.

51. Wakil, S.J., J. Lipid Res., 2, 1, 1961.

52. Horning, M.G., Martin, D.B., Karmen, A., and Vagelos, P.R., J. Biol. Chem., 236, 669, 1961.

53. Lynen, F., Redoxfunktionen Cytoplasmischer Strukturen, Symp. Leitung Th. Bucher, Marburg/Lahn, Deut. Ges. Physiol. Chem., Österr. Biochem. Ges., Wien, 1962, quoted in (35).

54. Vagelos, P.R., Alberts, A.W., and Majerus, P.W., Ann. N.Y. Acad. Sci., 131(1), 177, 1965.

55. Majerus, P.W., Alberts, A.W., and Vagelos, P.R., Proc. Nat. Acad. Sci. U.S., 51, 1231, 1964.

56. Alberts, A.W., Majerus, P.W., Talamo, B., and Vagelos, P.R., Biochemistry 3, 1563, 1964.

57. Alberts, A.W., Fed. Proc., 24, 290, 1965.

58. Majerus, P.W., Alberts, A.W., and Vagelos, P.R., J. Biol. Chem., 240, 618, 1965.

59. Majerus, P.W., Alberts, A.W., and Vagelos, P.R., J. Biol. Chem., 240, 4723, 1965.

60. Pugh, E.L., and Wakil, S.J., J. Biol. Chem., 240, 4727, 1965.

61. Majerus, P.W., Alberts, A.W., and Vagelos, P.R., Proc. Nat. Acad. Sci. U.S., 53, 410, 1965.

62. Gibson, D.M., Titchener, E.B., and Wakil, S.J., Biochim. Biophys. Acta, 30, 376, 1958.

63. Robinson, J.D., Bradley, R.M., and Brady, R.O., J. Biol. Chem., 238, 528, 1963.

64. Robinson, J.D., Bradley, R.M., and Brady, R.O., Biochemistry, 2, 191, 1963.

65. Larrabee, A.R., McDaniel, E.G., Bakerman, H.A., and Vagelos, P.R., Proc. Nat. Acad. Sci. U.S., 54, 267, 1965.

66. Overath, P., and Stumpf, P.K., J. Biol. Chem., 239, 4103, 1964.

67. Harlan, W.R., and Wakil. S.J., J. Biol. Chem., 238, 3216, 1963.

68. Harlan, W.R., and Wakil, S.J., Biochem. Biophys. Res. Comm., 8, 131, 1962.

69. Benjamin, W., and Gellhorn, A., J. Biol. Chem., 239, 64, 1964.

70. Gellhorn, A., and Benjamin, W., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 399.

71. Hirsch, J., and Goldrick, R.B., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 455.

72. Evans, J.D., and Norcia, L.N., J. Lipid Res., 5, 395, 1964.

73. Scheig, R., Alexander, N.M., Klatskin, G., J. Lipid Res., 7, 188, 1966.

74. Getz, G.S., Bartley, W., Stripe, F., Notton, B.M., and Renshaw, A., Biochem. J., 80, 176, 1961.

75. Barron, E.J., and Stumpf, P.K., J. Biol. Chem., 237, PC 613, 1962.

76. Nugteren, D.H., Biochim. Biophys. Acta, 106, 280, 1965.

77. Mead, J.F., Fed. Proc. 20, 952, 1961.

78. Bloomfield, D.K., and Bloch, K., Biochim. Biophys. Acta, 30, 220, 1958.

79. Holloway, P.W., and Wakil, S.J., J. Biol. Chem., 239, 2489, 1964.

80. Elovson, J., Biochim. Biophys. Acta, 106, 291, 1965.

81. Hawke, J.C., and Stumpf, P.K., J. Biol. Chem., 240, 4746, 1965.

82. Bloomfield, D.K., and Bloch, K., J. Biol. Chem., 235, 337, 1960.

83. Gellhorn, A., and Benjamin, W., Biochim. Biophys. Acta, 84, 167, 1964.

84. Holloway, P.W., Peluffo, R., and Wakil, S.J., Biochem. Biophys. Res. Comm., 12, 300, 1963.

85. Harris, R.V., and James, A.T., Biochim. Biophys. Acta, 106, 456, 1965.

86. Stoffel, W., Biochem. Biophys. Res. Comm., 6, 270, 1962.

87. Yuan, C., and Bloch, K., J. Biol. Chem., 236, 1277, 1961.

88. Nugteren, D.H., Biochim. Biophys. Acta, 60, 656, 1962.

89. Hollenberg, C.H., J. Clin. Invest., 45, 205, 1966.

90. Green, D.E., and Fleischer, S., Metabolic Pathways, Vol. 1, Ed. Greenberg, D.M., Academic Press, New York, 1960, p. 41.

91. Green, D.E., and Gibson, D.M., Metabolic Pathways, Vol. 1, Ed. Greenberg, D.M., Academic Press, New York, 1960, p. 301.

92. Spencer, A.F., and Lowenstein, J.M., J. Biol. Chem., 237, 3640, 1962.

93. Kornacker, M.S., and Lowenstein, J.M., Biochem. J., 94, 209, 1965.

94. Lowenstein, J.M., Recent Research on Carnitine, Ed. Wolf, G., M.I.T. Press, Cambridge, Mass., 1965, p. 97.

95. Fritz, I.B., Advances in Lipid Research, 1, 285, 1963.

96. Purvis, J.L., and Lowenstein, J.M., J. Biol. Chem., 236, 2794, 1961.

97. Norum, K.R., Acta Chem. Scand., 17, 896, 1963.

98. Fritz, I.B., and Yue, K.T.N., Am. J. Physiol., 206, 531, 1964.

99. Norum, K.R., and Bremer, J., Biochim. Biophys. Acta, 78, 77, 1963.

100. Bressler, R., and Katz, R., J. Biol. Chem., 240, 622, 1965.

101. Bressler, R., and Katz, R., J. Clin. Invest., 44, 840, 1965.

102. Bressler, R., Katz, R., and Wittels, B., Ann. N.Y. Acad. Sci., 131(1), 207, 1965.

103. Klingenberg, M., and Bode, C., Recent Research on Carnitine, Ed. Wolf, G., M.I.T. Press, Cambridge, Mass., 1965, p. 87.

104. Beenakkers, A.M., and Klingenberg, M., Biochim. Biophys. Acta, 84, 205, 1964.

105. Gergely, J., Methods in Enzymology, Vol. 1, Ed. Colowick, S.P., and Kaplan, N.O., Academic Press, New York, 1965, p. 602.

106. Hele, P., J. Biol. Chem., 206, 671, 1954.

107. Srere, P.A., J. Biol. Chem., 234, 2544, 1959.

108. Srere, P.A., and Bhaduri, A., Biochim. Biophys. Acta, 59, 487, 1962.

109. Bhaduri, A., and Srere, P.A., Biochim. Biophys. Acta, 70, 221, 1963.

110. Horowitz, I., Beck, J.C., and Rubinstein, D., J. Biol. Chem., in press.

111. Kallen, R.G., and Lowenstein, J.M., Fed. Proc., 21, 289, 1962.

112. Leveille, G.A., and Hanson, R.W., J. Lipid Res., 7, 46, 1966.

113. Lardy, H.A., Shrago, E., Young, J.W., and Paetkau, V., Science, 144, 564, 1964.

114. Madsen, J., Abraham, S., and Chaikoff, I.L., J. Biol. Chem., 239, 1305, 1964.

115. Abraham, S., Madsen, J., and Chaikoff, I.L., J. Biol. Chem., 239, 855, 1964.

116. Madsen, J., Abraham, S., and Chaikoff, I.L., J. Lipid Res., 5, 548, 1964.

117. D'Adamo, A.F., and Haft, D.E., J. Biol. Chem., 240, 613, 1965.

118. Hanson, R.W., and Ziporin, Z.Z., J. Lipid Res., 7, 56, 1966.

119. Feller, D.D., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 363.

120. Flatt, J.P., and Ball, E.G., J. Biol. Chem., 239, 675, 1964.

121. Masoro, E.J., J. Lipid Res., 3, 149, 1962.

122. Winegrad, A.I., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 319.

123. Winegrad, A.I., Insulin and Lipid Metabolism, in Actions of Hormones on Molecular Processes, Ed. Litwack, G., and Kritchevsky, D., Wiley & Sons, 1964, p. 382.

124. Gibson, D.M., and Hubbard, D.D., Biochem. Biophys. Res. Comm., 3, 531, 1960.

125. Numa, S., Matsuhashi, M., and Lynen, F., Biochem. Z., 334, 203, 1961.

126. Wieland, O., Neufeldt, I., Numa, S., and Lynen, F., Biochem. Z., 336, 455, 1963.

127. Korchak, H.M., and Masoro, E.J., Biochim. Biophys. Acta, 58, 354, 1962.

128. Masoro, E.J., Cohen, A.I., Panagos, S.S., Am. J. Physiol. 180, 341, 1955.

129. Masoro, E.J., Ann. N.Y. Acad. Sci., 131(1), 199, 1965.

130. Hicks, S.E., Allmann, D.W., and Gibson, D.M., Biochim. Biophys. Acta, 106, 441, 1965.

131. Langdon, R.G., J. Biol. Chem., 226, 615, 1957.

132. Siperstein, M.D., Am. J. Med., 26, 685, 1959.

133. Tepperman, H.M., and Tepperman, J., Diabetes, 7, 478, 1958.

134. Lowenstein, J.M., J. Biol. Chem., 236, 1217, 1961.

135. Young, J.W., Shrago, E., and Lardy, H.A., Biochemistry, 3, 1687, 1964.

136. Lowenstein, J.M., J. Biol. Chem., 236, 1213, 1961.

137. Pande, S.V., Khan, R.P., and Venkitasubramanian, T.A., Biochim. Biophys. Acta, 84, 239, 1964.

138. Katz, J., Landau, B.R., and Bartsch, G.E., J. Biol. Chem., 241, 727, 1966.

139. Hollifield, G., and Parson, W., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 393.

140. Ball, E.G., and Jungas, R.L., Biochemistry, 2, 586, 1963.

141. Hollifield, G., and Parson, W., J. Clin. Invest., 41, 245, 1962.

142. Katz, J., Rognstad, R., and Kemp, R.G., J. Biol. Chem., 240, PC 1484, 1965.

143. Utter, M.F., and Keech, D.B., J. Biol. Chem., 235, PC 17, 1960.

144. Pande, S.V., Parvin, R., and Venkitasubramanian, T.A., Biochim. Biophys. Acta, 100, 344, 1965.

145. Patterson, D.S.P., Biochim. Biophys. Acta, 84, 198, 1964.

146. Dickens, F., Glock, G.E., and McLean, P., Ciba Foundation Symp. on the Regulation of Cell Metabolism, Ed. Wolstenholme, G.E.W., and O'Connor, C.M., Little, Brown and Co., Boston, Mass., 1959, p. 150.

147. Flatt, J.P., and Ball, E.G., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 273.

148. Masoro, E.J., Korchak, H.M., and Porter, E., Biochim. Biophys. Acta, 58, 407, 1962.

149. Myant, N.B., and Iliffe, J., Control of Lipid Metabolism, Ed. Grant, J.K., Biochem. Soc. Symp. No. 24, Academic Press, London, 1963, p. 145.

150. Schneider, W.C., Striebich, M.J., and Hogeboom, G.H., J. Biol. Chem., 222, 969, 1956.

151. Parmeggiani, A., and Bowman, R.H., Biochem. Biophys. Res. Comm., 12, 268, 1963.

152. Garland, P.B., quoted in Tubbs, P.K., and Garland, P.B., Biochem. J., 93, 550, 1964.

153. Hill, C.H., Garren, W.H., and Kelly, J.W., Poultry Sci., 39, 117, 1960.

154. Masoro, E.J., Chaikoff, I.L., Chernick, S.S., and Felts, J.M., J. Biol. Chem., 185, 845, 1950.

155. Hausberger, F.X., and Milstein, S.W., J. Biol. Chem., 214, 483, 1955.

156. Whitney, J.E., and Roberts, S., Am. J. Physiol., 181, 446, 1955.

157. Hill, R., Linazasoro, J.M., Chevallier, F., and Chaikoff, I.L., J. Biol. Chem., 233, 305, 1958.

158. Hill, R., Webster, W., Linazasoro, J.M., and Chaikoff, I.L., J. Lipid Res., 1, 150, 1960.

159. Bortz, W., Abraham, S., and Chaikoff, I.L., J. Biol. Chem., 238, 1266, 1963.

160. Langdon, R.G., In Lipid Metabolism, Ed. Bloch, K.E., Wiley & Sons, New York, 1960, p. 138.

161. Porter, J.W., and Long, R.W., J. Biol. Chem., 233, 20, 1958.

162. Bortz, W.M., and Lynen, F., Biochem. Z., 337, 505, 1963.

163. Tubbs, P.K., and Garland, P.B., Biochem. J., 89, 25P, 1963.

164. Bortz, W.M., and Lynen, F., Biochem. Z., 339, 77, 1963.

165. Tubbs, P.K., and Garland, P.B., Biochem. J., 93, 550, 1964.

166. Korchak, H.M., and Masoro, E.J., Biochim. Biophys. Acta, 84, 750, 1964.

167. Robinson, J.D., Brady, R.O., and Bradley, R.M., J. Lipid Res., 4, 144, 1963.

168. Steiner, G., J. Lipid Res., submitted for publication, 1966.

169. Wieland, O., and Weiss, L., Biochem. Biophys. Res. Comm., 13, 26, 1963.

170. Tubbs, P.K., Biochim. Biophys. Acta, 70, 608, 1963.

171. Srere, P.A., Biochim. Biophys. Acta, 106, 445, 1965.

172. Eger-Neufeldt, I., Teinzer, A., Weiss, L., and Wieland, O., Biochem. Biophys. Res. Comm., 19, 43, 1965.

173. Taketa, K., and Pogell, B.M., J. Biol. Chem., 241, 720, 1966.

174. Lorch, E., Abraham, S., and Chaikoff, I.L., Biochim. Biophys. Acta, 70, 627, 1963.

175. Howard, C.F., and Lowenstein, J.M., Biochim. Biophys. Acta, 84, 226, 1964.

176. Howard, C.F., and Lowenstein, J.M., J. Biol. Chem., 240, 4170, 1965.

177. Favarger, P., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 19.

178. Stetten, D., and Boxer, G.E., J. Biol. Chem., 155, 231, 1944.

179. Shafrir, E., Shapiro, B., and Wertheimer, E., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 313.

180. Landau, B.R., Katz, J., Bartsch, G.E., White, L.W., and Williams, H.R., Ann. N.Y. Acad. Sci., 131(1), 43, 1965.

181. Rose, G., and Shapiro, B., Biochim. Biophys. Acta, 18, 504, 1955.

182. Vaughan, M., J. Biol. Chem., 235, 3049, 1960.

183. Weber, G., Banerjee, G., and Ashmore, J., Biochem. Biophys. Res. Comm., 3, 182, 1960.

184. Patkin, J.K., and Masoro, E.J., Can. J. Physiol. Pharmacol., 42, 101, 1964.

185. De Freitas, A.S.W., and Depocas, F., Can. J. Biochem., 43, 437, 1965.

186. Benjamin, W., Gellhorn, A., Wagner, M., and Kundel, H., Am. J. Physiol., 201, 540, 1961.

187. Vaughan, M., and Steinberg, D., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 239.

188. Steinberg, D., and Vaughan, M., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 335.

189. Bublitz, G., and Kennedy, E.P., J. Biol. Chem., 211, 951, 1954.

190. Treble, D.H., and Ball, E.G., Fed. Proc., 22, 357, 1963.

191. Shapiro, B., Chowers, I., and Rose, G., Biochim. Biophys. Acta, 23, 115, 1957.

192. Wieland, O., and Suyter, M., Biochem. Z., 329, 320, 1957.

193. Lynn, W.S., MacLeod, R.M., and Brown, R.H., J. Biol. Chem., 235, 1904, 1960.

194. Cahill, G.F., Lebeouf, B., and Flinn, R.B., J. Biol. Chem., 235, 1246, 1960.

195. Winegrad, A.I., Shaw, W.N., Lukens, F.D.W., and Stadie, W.C., Am. J. Clin. Nutr., 8, 651, 1960.

196. Jeanrenaud, B., and Renold, A.E., J. Biol. Chem., 234, 3082, 1959.

197. Metz, R., Diabetes, 9, 89, 1960.

198. Crofford, O.B., and Renold, A.E., J. Biol. Chem., 240, 14, 1965.

199. Crofford, O.B., and Renold, A.E., J. Biol. Chem., 240, 3237, 1965.

200. Froesch, E.R., and Ginsberg, J.L., J. Biol. Chem., 237, 3317, 1962.

201. Di Pietro, D.L., Biochim. Biophys. Acta, 67, 305, 1963.

202. Winegrad, A.I., and Renold, A.E., J. Biol. Chem., 233, 267, 1958.

203. Renold, A.E., Crofford, O.B., Stauffacher, W., and Jeanrenaud, B., Diabetologial, 1, 4, 1965.

204. Leonards, J.R., and Landau, B.R., Arch. Biochem. Biophys., 91, 194, 1960.

205. Jungas, R.L., and Ball, E.G., Biochemistry, 2, 383, 1963.

206. Mahler, R., Stafford, W.S., Tarrant, M.E., and Ashmore, J., Diabetes, 13, 297, 1964.

207. Froesch, E.R., et al., quoted in Diabetologial, 1, 4, 1965.

208. Zierler, K.L., and Rabinowitz, D., J. Clin. Invest., 43, 950, 1964.

209. Gordon, R.S., and Cherkes, A., Proc. Soc. Exp. Biol. Med., 97, 150, 1958.

210. White, J.E., and Engel, F.L., Proc. Soc. Exp. Biol. Med., 99, 375, 1958.

211. White, J.E., and Engel, F.L., J. Clin. Invest., 37, 942, 1958.

212. Hollenberg, C.H., Raben, M.S., and Astwood, E.B., Endocrinology, 68, 589, 1961.

213. Steinberg, D., Shafrir, E., and Vaughan, M., Clin. Res., 7, 220, 1959.

214. Hagen, J.H., J. Biol. Chem., 236, 1023, 1961.

215. Freinkel, N., J. Clin. Invest., 40, 476, 1961.

216. Rudman, D., Brown, S.J., and Malkin, M.F., Endocrinology, 72, 527, 1963.

217. Astwood, E.B., Barrett, R.J., and Friesen, H., Proc. Nat. Acad. Sci. U.S., 47, 1525, 1961.

218. Raben, M.S., and Hollenberg, C.H., Ciba Foundation Colloquia on Endoc., 13, 89, 1960.

219. Jeanrenaud, B., and Renold, A.E., J. Biol. Chem., 235, 2217, 1960.

220. Fain, J.N., Scow, R.O., and Chernick, S.S., J. Biol. Chem., 238, 54, 1963.

221. Philipps, G.R., Herrera, M.G., and Renold, A.E., Biochim. Biophys. Acta, 106, 234, 1965.

222. Munck, A., and Koritz, S.B., Biochim. Biophys. Acta, 57, 310, 1962.

223. Munck, A., Biochim. Biophys. Acta, 57, 318, 1962.

224. Stevenson, J.A.F., and Beaton, J.R., Ann. N.Y. Acad. Sci., 131(1), 189, 1965.

225. Brodie, B.B., Maickel, R.P., and Stern, D.N., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 583.

226. Umbreit, W.W., Burris, R.H., and Stauffer, J.F., Manometric Techniques, 3rd Ed., Burgess Publ. Co., Minneapolis, Minn., 1957, p. 145.

227. Rodbell, M., J. Biol. Chem., 239, 375, 1964.

228. Folch, J., Lees, M., and Sloane Stanley, G.H., J. Biol. Chem., 226, 497, 1957.

229. Borgström, B., Acta Physiol. Scand., 25, 111, 1952.

230. Dole, V.P., J. Clin. Invest., 35, 150, 1956.

231. Steinberg, D., Vaughan, M., and Margolis, S., J. Biol. Chem., 236, 1631, 1961.

232. Kissane, J.M., and Robins, E., J. Biol. Chem., 233, 184, 1958.

233. Dole, V.P., and Meinertz, H., J. Biol. Chem., 235, 2595, 1960.

234. Bray, G.A., Anal. Chem., 1, 279, 1960.

235. Winegrad, A.I., and Renold, A.E., J. Biol. Chem., 233, 273, 1958.

236. Herrera, M.G., Philips, G.R., and Renold, A.E., Biochim. Biophys. Acta, 106, 221, 1965.

237. Herrera, M.G., and Renold, A.E., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 375.

238. Frerichs, H., and Ball, E.G., Biochemistry, 3, 981, 1964.

239. Levine, R., and Goldstein, M.S., Rec. Prog. Hormone Res., 11, 343, 1955.

240. Ball, E.G., Jungas, R.L., Rec. Prog. Hormone Res., 20, 183, 1964.

241. McIntyre, N., Holdsworth, C.D., Turner, D.S., Lancet, 2, 20, 1964.

242. Dupre, J., Lancet, 2, 672, 1964.

243. Recant, L., and Alp, H., Ann. N.Y. Acad. Sci., 131(1), 334, 1965.

244. Tepperman, J., and Tepperman, H.M., Ann. N.Y. Acad. Sci., 131(1), 404, 1965.

245. Galton, D.J., and Fain, J.N., Biochem. J., 98, 557, 1966.

246. Rieser, P., and Rieser, C.H., Biochem. Biophys. Res. Comm., 17, 373, 1964.

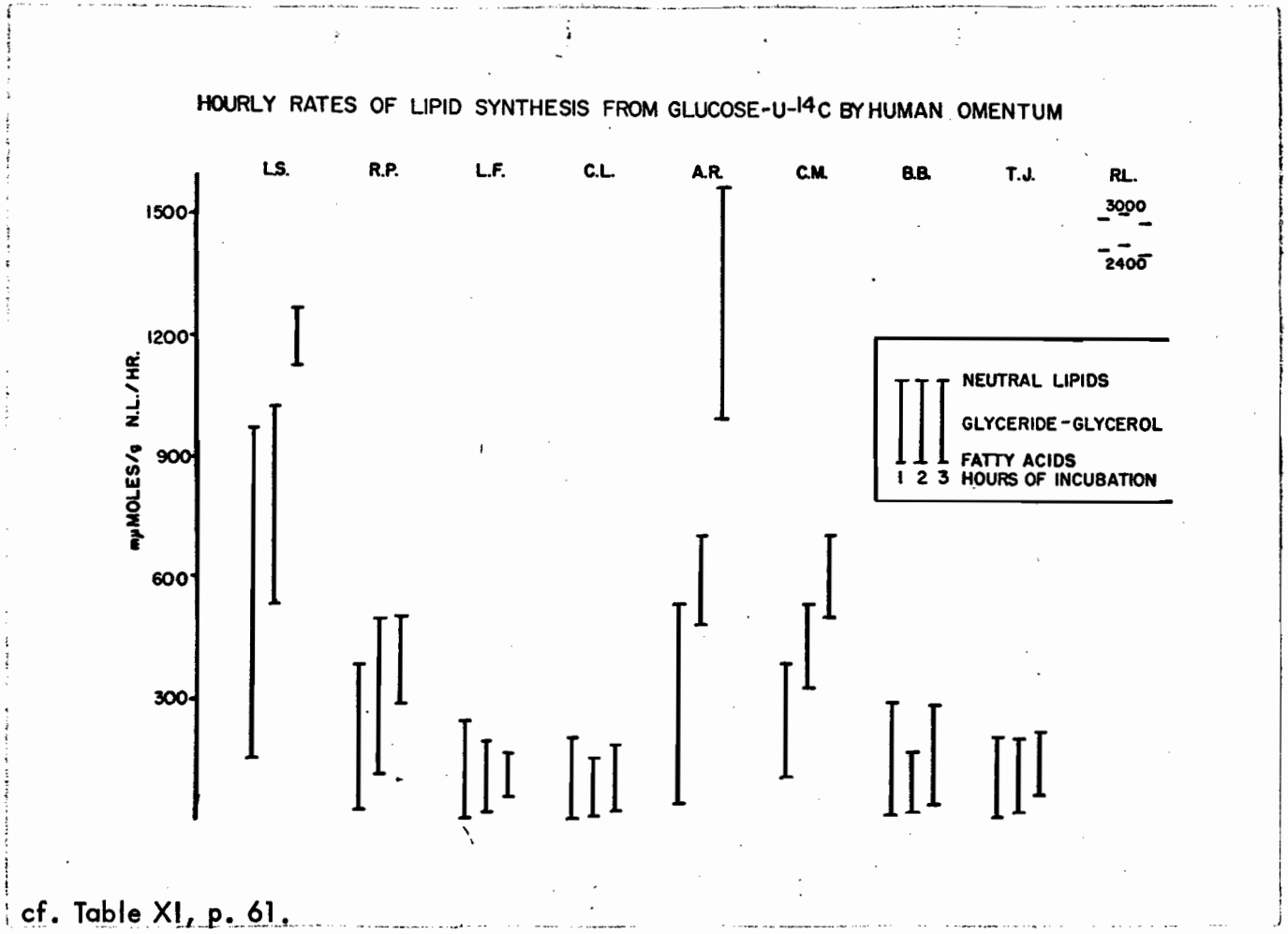
247. Rieser, P., and Rieser, C.H., Proc. Soc. Exptl. Biol. Med., 116, 669, 1964.

248. Barnett, C.A., and Whitney, J.E., Proc. Soc. Exptl. Biol. Med., 119, 866, 1965.

249. Rodbell, M., J. Biol. Chem., 241, 130, 1966.

250. Flatt, J.P., and Ball, E.G., Handbook of Physiology, Section 5: Adipose Tissue, Ed. Renold, A.E., and Cahill, G.F., Jr., American Physiological Society, Washington, 1965, p. 273.

Figure 8



cf. Table XI, p. 61.

TABLE XXII

Accumulation of 3-0-Methyl Glucose in the Intracellular Space of Rat Adipose Tissue.

	µl/100 mg Tissue											
	6 Minutes			10 Minutes			20 Minutes			120 Minutes		
	Control	Preinc.	Δ	Control	Preinc.	Δ	Control	Preinc.	Δ	Control	Preinc.	Δ
1	0.24	1.12	0.88	0.29	1.07	0.78	1.50	3.03	1.53	2.44	2.41	-0.03
2	0.76	1.10	0.34	0.73	1.49	0.76	2.74	3.01	0.27	1.95	2.15	+0.20
3	1.12	1.61	0.49	0.77	1.84	1.07	2.15	2.78	0.63	2.30	2.25	-0.05
4	0.58	1.56	0.98	1.02	1.49	0.47	2.38	2.85	0.47	2.51	2.16	-0.35
5	0.97	1.17	0.20	1.06	1.89	0.83	1.68	3.37	1.69	4.12	3.98	-0.14
6	0.45	0.90	0.45	0.51	1.56	1.05	1.32	4.08	2.66	4.53	4.71	+0.18
7	0.44	0.70	0.26	0.82	1.05	0.23	1.59	4.32	2.73	3.69	4.02	+0.33
8				0.68	1.30	0.62	2.56	4.27	1.71	4.26	4.13	-0.13
Mean	0.65	1.16	0.51	0.73	1.46	0.73	1.99	3.46	1.47	3.22	3.22	0
S.D.	0.31	0.33	0.30	0.25	0.31	0.29	0.54	0.65	0.94	1.00	1.08	-
S.E.	0.12	0.12	0.11	0.09	0.11	0.11	0.19	0.23	0.33	0.36	0.38	-
p			$<.005$			$<.001$			$<.005$			N.S.