HANDBOOK OF PHYSIOLOGY

SECTION 5:

Adipose Tissue

ALBERT E. RENOLD

GEORGE F. CAHILL

HANDBOOK OF PHYSIOLOGY

A critical, comprehensive presentation of physiological knowledge and concepts

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SECTION 5: **Adipose Tissue**

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Preface

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ADIPOSE TISSUE, by definition, is composed of aggregates of cells located throughout the body at specific sites predetermined by differentiation, the primary role of these cells being the storage of triglyceride as a potential source of energy. Similar aggregates of triglyceride-containing cells may occur in invertebrates, but adipose tissue does not achieve its fullest development until emergence of homoiothermy in birds and mammals. Since mobility in the animal kingdom is often paramount to survival, transport of energy stores is most efficiently achieved when storage is in the form of lipid. In addition to its greater caloric density, triglyceride storage does not necessitate accumulation of water and ions as does storage of carbohydrate in the form of glycogen. One can calculate, for example, that if an average 70-kg man were to transport as glycogen-containing tissue a caloric reserve equal to that of his adipose tissue, his weight would be doubled. The weight economy of adipose tissue as a site of energy storage is even more striking in migratory birds and insects, and these instructive extremes of physiologic regulation of energy storage are discussed in separate chapters in this volume.

Adipose tissue may also serve other functions, such as insulation against cold, or as a mechanical buffer or lubricating system, as in joints or in the socket of the eye. Its cosmetic role is evident and, in an ecological sense, it may be of importance to the survival of certain species. It may also be a site of heat production during exposure to cold and especially during awakening from hibernation. These functions, however, although important in special circumstances, are nevertheless secondary to the primary role of this tissue in energy storage. To be of use, an energy store must be easily accessible for withdrawals or deposits of energy, and much of this volume will be devoted to the detailed analysis of our present knowledge of the mechanisms of depositing in and withdrawing from the adipose tissue energy bank.

Since earliest times, man has directed much attention to fat, its advantages and its disadvantages. In populations with generally marginal caloric intake, adiposity has been associated with success and wealth, also with good health and a jovial disposition. There is no doubt that the primitive hunter who deposited 100,000 cal as triglyceride before the lean winter was given a far better chance for survival. In the more affluent modern societies, however, where calories are easily obtained, such an energy store is no longer necessary; in fact, statistics now underscore the liabilities of adiposity and its frequent association with degenerative and metabolic diseases.

The purpose of this volume is to assemble current knowledge relating to adipose tissue. A very large body of information has accumulated over the past 40 years, and to be truly complete the volume would have to be several times its present size. The outline was planned with the aim of including most aspects of adipose tissue physiology, supported by morphological, biochemical and certain clinical sections where these appeared relevant to the understanding of the physiology of the tissue. The authors of the various chapters have been selected on the basis of their previous work in each specific area to be covered as well as on the basis of differences in their point of view. With a few exceptions, several rather short chapters covering a particular topic have been preferred to fewer, longer and more inclusive ones. Thus the volume contains chapters which appear to duplicate others, and occasionally opposite opinions are pre-

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sented. This does not surprise us in a field which has grown so rapidly from its birth just a few decades ago, and the many differences of opinion and interpretation are best presented by their different proponents. Even so, the great interest and activity directed toward adipose tissue within the past ten years has attracted many talented investigators whose contributions are significant and of importance. Because of the limits necessarily set to the extent of the volume, a number of omissions had to be made and will be noted.

As placed into its proper perspective by Dr. Wertheimer's introduction, the relative youth of adipose tissue physiology and its rapid growth are perhaps among the most interesting aspects of this field of research. We feel particularly privileged in having been able to include contributions from several original investigators whose curiosity and insight detonated this explosive area of physiology. Two of these are singled out particularly and to them the volume is dedicated.

Dr. H. E. Wertheimer was born in Germany, in Bühl, in 1893. He received his M.D. degree from Heidelberg in 1920 and the following years was Senior Assistant at the Halle Institute of Physiology under Professor Abderhalden. Between 1926 and 1928 he published several papers on the regulation of the mobilization of fat and on the glucose content of adipose tissue. In 1927 he was appointed Associate Professor at Halle but was dismissed by the government in power in 1934 and emigrated to Jerusalem where he was appointed Professor of Pathological Physiology and Head of the Chemical Laboratory of the Hadassah Hospital, then Professor of Biochemistry and Head of the Department, the Hebrew University-Hadassah Medical School. Dr. Wertheimer published a series of papers in the 1940's on glycogen metabolism in adipose tissue and, in 1948, published, together with Dr. Shapiro, the review on the physiology of adipose tissue which was to usher in the modern and explosive period of research in this field. Since then his department has been persistently productive and Dr. Wertheimer and Dr. Shapiro have been joined by a number of outstanding associates whose interest was directed into this area by the original foresight, interest and enthusiasm of Professor Wertheimer.

Dr. Franz X. Hausberger was born in Mühldorf, Bavaria, in 1908, graduated from the Gymnasium in Munich in 1928 and from the University of Munich

Medical School in 1934. In 1935 he completed his thesis on the innervation of adipose tissue and received his M.D. degree (summa cum laude), a noble and honorable academic start for adipose tissue. During these studies, Dr. Hausberger noted changes in glycogen, fat and water content in the tissue, and these observations led him to consider that adipose tissue was likely to have a discrete and discretely controlled metabolism of its own. In 1935, he was Assistant in Anatomy at the University of Munich Medical School where Dr. Wassermann, also a contributor to this volume, was Professor. Dr. Hausberger finished his clinical training at the Gertrauden Krankenhaus in Berlin. From 1934 to 1946 he was Head of Internal Medicine at Salem Hospital, Koeslin, Germany, then went to the University Hospital, Erlangen, in charge of the Division of Endocrinology before moving to the United States in 1949. Since 1950, Dr. Hausberger has been associated with the Department of Anatomy, Jefferson Medical College of Philadelphia, Pennsylvania, where he is currently Professor of Anatomy.

In addition to these two founding fathers of adipose tissue physiology, contributors to this volume include Dr. F. Wassermann who pioneered the anatomical and embryological development of adipose tissue, and Dr. C. Tedeschi who has performed the most extensive studies into the various aspects of its pathology.

The volume is divided into five sections. The first section presents some general concepts of the physiology of fat synthesis and storage as related to adipose tissue, in addition to some comparative studies with special emphasis on the role of fatty tissues in birds, fish and insects. The second section is devoted to the microscopic and molecular anatomy of the tissue, thereby encompassing studies with the light and the electron microscope, embryogenesis and chemical composition. The third section attempts to describe the various enzymatic pathways known to exist in adipose tissue and their relative activities. The fourth section discusses the mechanisms (hormonal, nutritional and neural) which control the dynamic processes described in the third section, while the fifth and final section relates these mechanisms, often first defined in isolated tissue preparations, to the integrated physiology of the living organism.

The bibliography has become extensive. Instead of single bibliographies at the end of each chapter, which would have resulted in much duplication, all have been combined and cross-indexed into a single bibliography arranged according to senior authors and year of publication. The editors fully realize that any one author may have more than one reference to his name in any one year. However, the titles of all papers have been included and should facilitate the selection of the article referred to in any one instance. The cross-indexing of authors and subjects, although necessarily limited, should also expedite any bibliographical search. The final reason for combining the bibliographies into a single one is the inclusion of many articles not referred to in any of the chapters. The editors make no claims of completeness for this bibliography on the subject of adipose tissue, but they would not be too surprised if it were not too far from being complete, approximately up to the end of the year 1963. With all its shortcomings, this bibliography is offered to workers in the field as the result of much effort by the editors and their collaborators, greatly aided by many contributors of individual chapters. The editors are especially indebted to Dr. B. Mosinger of Prague, Czechoslovakia, for helping in the compilation of the contributions from Eastern Europe, these being less accessible and less familiar to us.

One final comment must be made. The volume consists of 69 chapters from as many laboratories in several countries. Several chapters have been translated. In requesting these chapters the authors were asked for speedy delivery since it was and is the editors' strong belief that in this moving area of investigation the value of the volume will be greatly enhanced by keeping the interval between writing and publication as brief as possible. This has resulted in considerable unevenness in the quality of the presentations, particularly of their literary aspects. This unevenness will be apparent to all. To remedy it, beyond the rather modest extent which has been nonetheless achieved, would have meant a very considerable delay in publication. It was the editors' deliberate decision to speed the volume on its way rather than to aim for greater perfection or at least greater unity of expression. For this decision we stand responsible, without apology. As pointed out by Dr. Wertheimer, this is the first handbook treatment given to this area of physiology and we believe that its value will reside in helping in the orientation of new workers in this growing field, rather than in the timeless balance of its comprehensive analyses and syntheses.

The editors wish to express their appreciation first to the Handbook Editorial Committee of the American Physiological Society, Dr. Maurice B. Visscher, Dr. A. Baird Hastings, Dr. John Pappenheimer and Dr. Hermann Rahn for encouraging them to proceed with this volume. Second, the editors would like to thank several of the authors and certain others who were of great assistance in the original organization of the outline. These include Dr. H. E. Wertheimer, Dr. Franz X. Hausberger, Dr. Eric G. Ball, Dr. Pierre Favarger, Dr. Don Fawcett, Dr. Eugene Kennedy, Dr. Daniel Steinberg, Dr. Lars Carlson and the late Dr. Frank Engel. Special recognition should go to Miss Patricia Conley who superbly handled the major portion of secretarial duties and to Dr. Bernard Jeanrenaud who indexed the bibliography. Lastly, thanks should be expressed to Miss Virginia Safford, Mrs. Louise Loughman and Mademoiselle Claire-Lise Moriaud, secretaries to the editors in Boston and Geneva, and to Miss Josette Leuba and Mrs. Harriet Giovagnoli for their assistance in the preparation of the volume.

CHAPTER 2

Introduction—a perspective

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THIS SECTION on the physiology of adipose tissue presents a special case in this series. In all other sections, the authors can refer to previous handbooks or textbooks as a basis for presenting their own work and as a guide for evaluating new knowledge and concepts. But the subject of adipose tissue physiology has never before been treated as a special section in any handbook. In fact, it is not even mentioned in most of them. Today, adipose tissue is a metabolic organ of primary importance. This means the actual detection of a new highly active organ with an evaluation of its metabolic activities for the first time. Such a development is unusual in physiology. For this reason the editors of the *Handbook of Physiology* resolved that such a subject should be presented in a special form, namely, in a series of short chapters written by the leading workers in a broad and comprehensive manner and to present indeed the whole existing up-to-date knowledge. This entry into a rather new field with the exploration of new pathways is in itself a challenge.

The introduction to such a section cannot be the usual bridge between previously published handbooks on the one hand, and a presentation of important current developments on the other hand. It must first of all justify the inclusion of this very new subject in a section of some 800 pages, documented by some 4000 references in the present *Handbook*. For this reason several questions must be asked:

1) What was the general opinion about adipose tissue some 30 years ago when the last series of handbooks appeared, and what is it today in 1964? *2*) What experimental findings led to the appearance of the first review on the physiology of adipose tissue in 1948? *3*) What caused the explosive development to which we are witness since 1948? *4*) Can we say today that a sound basis for this new field has really been established? This question is important since the Editorial Committee has especially stressed the popularity of the subject. One may wonder whether this popularity is only of a transitory nature or whether it truly reflects the feeling that here before us is a new stimulating subject of great importance which will continue to expand at the same pace in the future. It is well known that the development of a new field is usually accompanied by a great deal of enthusiasm which is succeeded by some disappointments and ultimately leads to a stabilization. The same type of pattern has been seen in many other fields and this raises the question as to whether we have indeed reached this period of more or less final stabilization. The last point to be considered at this stage then is *5*) What may be the direction of further developments in the future?

I. THE OPINION ON ADIPOSE TISSUE 30 YEARS AGO AND TODAY

The general picture of adipose tissue physiology as it existed some 30 years ago can only be reconstructed from the meager literature available at that time. Fleming's opinions were widely accepted. Adipose tissue was considered to be a connective tissue filled, by chance, with droplets of fat. The cells were not believed to participate actively either in the accumulation of fat or in its remobilization during periods of starvation. Only a small number of capillaries could be observed in the usual histological sections of adipose tissue and it was almost universally accepted that it was devoid of nerves. It presumably belonged to a class of tissues whose metabolism could not be measured. The fat stores were believed to be purely passive in nature and not in any way involved in the general energy metabolism of the body. Its main task was believed to be that of insulating the body against heat loss and of providing mechanical support for certain tissues.

The underestimation of the importance of adipose tissue at that time is readily understood. Histologically, 95% of the adipose tissue appeared to be a foam-like aggregation of fat droplets and the remaining 5% to consist of connective tissue and blood vessels. The metabolically active part of the tissue which constitutes about 2% of the wet weight, is almost invisible. It hardly seemed possible that such a tiny quantity of living matter could have such a widespread influence on the metabolism of the whole body.

Recent electron microscope photographs reveal an entirely different picture. It is possible to observe large numbers of mitochondria associated with the fatty droplets which obviously suggests the presence of a very active living structure consuming energy at a rapid rate. When these fat cells are observed during periods of refeeding, after starvation, the rims of living cytoplasm show a dense accumulation of glycogen-containing granules. This clearly demonstrates that during the accumulation of new fat, an active process is occurring within the fat cells themselves. It may also be accepted, on the basis of recent metabolic evidence, that the fat cell consists of two compartments. A large one serving as a relatively inert storage site in which the exchange or turnover is very slow, and a second, smaller compartment, consisting of lipids in a very rapid state of turnover and closely related metabolically to the serum and liver lipids. If one further assumes that every such cell is in direct contact with a capillary network whose dimensions are of the same order of magnitude as those found in muscle and further, that each cell is innervated by the sympathetic nervous system, then a completely different impression of adipose tissue is obtained. Thus today the fat cell is not considered merely as a connective tissue cell filled at random with fat droplets, but as a cell belonging to a specific organ differentiated even in the embryological state and representing an active center of energy metabolism. By precise sympathic-adrenal regulation, free fatty acids are continually leaving this organ as energy-rich material to be oxidized, especially by muscle, heart, etc. A certain normal sympathetic tonus is believed to be the steady-state stimulus causing the normal release of these free fatty acids. Increased stimulation can result from hunger, muscular activity, cold or acclimation to cold, even a change of position as from a lying to a standing one and, finally, from physical or mental strain. Pituitary peptides may be another controlling mechanism in the mobilization of free fatty acids, though the extent to which they may participate is not yet known. It is however quite clear that considerable differences exist between species with respect to the balance between various regulatory mechanisms.

The deposition of fat by adipose tissue is considered today to be one of the most important aspects of energy metabolism. This process is regulated by and localized within the adipose tissue itself and not by the liver as was previously believed. This lipogenesis from glucose is regulated by the state of nutrition; it is decreased to a minimum in carbohydrate deficiency and accelerated considerably during carbohydrate availability. It is further regulated by insulin or, more precisely, by the ratio of insulin to sympathetic-adrenal tonus. In diabetes, lipogenesis decreases according to the severity of the disease. Not only does adipose tissue re-accumulate fat after periods of starvation but, even more important, it is capable of accumulating fat in the steady state after normal feeding. Since most animals are intermittent eaters, consuming mainly carbohydrates, they are confronted with the problem of storing temporarily much of the chemical energy ingested as food. In the steady state, approximately 30% of the carbohydrate ingested is temporarily converted to fat which not only occupies less volume but also weighs considerably less, per calorie of stored chemical energy, than either carbohydrates or proteins. The following example may give an idea of the metabolic potential of adipose tissue. A 70-kg man has about 10% of his total body weight as fat, i.e., about 7 kg, and from the above proportions, the active compartment of the adipose tissue would amount to about 150 g. Assuming that this man consumes 450 g of carbohydrate per day, then one-third of this, or 150 g, may be transformed daily into fat. This shows quite strikingly that adipose tissue protoplasm each day may transform its own weight of carbohydrate into fat.

The magnitude and reproducibility of the insulin effect on adipose tissue, especially on its lipogenic activity, suggests that adipose tissue is one of the major sites of insulin action (Winegrad & Renold, 1958). So great is its sensitivity to insulin that it is now the tissue most commonly used for the bioassay of minimum amounts of insulinlike activity in serum. Conversely, adipose tissue is very sensitive to insulin deprivation. In this respect it is somewhat more sensitive than liver and exceedingly more sensitive than muscle (Migliorini & Chaikoff, 1963). Twelve times more insulinlike activity can be demonstrated in serum if it is incubated with an extract of rat adipose tissue than with any other tissue. There is considerable evidence today that insulin occurs in the blood both in a bound or complex form and also in a free form (Chapter 68). The relative proportions of these two forms appear to be of considerable physiological and pathological importance. Adipose tissue may actively participate in keeping a balance between the bound form and the free (active) form which could explain the great responsiveness of adipose tissue to insulin (Antoniades & Gundersen, 1961). Adipose tissue metabolism has assumed such great importance today that it has become almost routine, when testing metabolic processes in different tissues, to include not only muscle and liver, but adipose tissue as well.

Concepts on the role of adipose tissue in heat regulation have also changed considerably in the past few years. Adipose tissue, especially subcutaneous fat, was always considered an insulating tissue (as indeed it is). It is probably not fortuitous that subcutaneous adipose tissue appears in evolution simultaneous with the appearance of the homoiothermy. Today there is sufficient evidence to indicate that fat depots may function actively, rather than passively, in heat control (Chapter 9). Free fatty acids released from adipose tissue under neurohormonal control are re-esterified and re-incorporated through a burst of oxygen consumption and ATP utilization suggesting a continuous expenditure of energy with the concomitant generation of heat. The idea that adipose tissue is not merely a simple insulating material but resembles more closely an electric blanket seems very plausible today (Smith & Hock, 1963).

What about the mechanical function of adipose tissue? It is known that adipose tissue occurs in sites where mechanical support is needed (e.g., paw-padding and orbital fat) and the metabolic activity of this adipose tissue is much lower than, for instance, that of mesenteric fat. Subcutaneous fat is an example of an adipose tissue whose metabolic activity falls between these two extremes. On the whole, however, it seems that the major role of most of the adipose tissues is a metabolic one (Aronovsky et al., 1963).

2. WHAT EXPERIMENTAL FINDINGS LED TO THE APPEARANCE OF THE FIRST REVIEW ON THE PHYSIOLOGY OF ADIPOSE TISSUE IN 1948?

At the time when the importance of adipose tissue was generally underestimated not everyone was inclined to agree with the official opinion. Howell, for instance, concluded that "It is to be borne in mind that we know little or nothing concerning the physiology of the tissue most directly concerned in the deposition of fat, namely, the fat cells themselves" (Howell, 1941). At the same time, Wells published a critical review of the histological, embryological and pathological aspects of adipose tissue. The title itself, "Adipose Tissue—The Neglected Subject," describes very clearly what the auther wished to say (Wells, 1940). Another example may be found in the writings of Rosenfeld (1940) whose ideas are based on a simple observation: Overfeeding of geese with carbohydrates causes accumulation of fat in adipose tissue but it is neither accompanied by lipemia nor by accumulation of fat in muscle, heart or kidney. Since there was no evidence for transport of fat from liver to adipose tissue, Rosenfeld (1940) concluded that the site of conversion of carbohydrates to fat was not the liver but probably the adipose tissue itself. Even though we cannot agree completely with his reasoning, it nevertheless shows that people were beginning to ascribe a metabolic role to adipose tissue. At the same time, von Gierke (1906) found that adipose tissue accumulated glycogen if animals were refed after starvation and postulated that adipose tissue was the site of transformation of carbohydrate into fat. In Germany the hypothesis of the "lipomatoese Tendenz" is often mentioned when speaking about the special activity of adipose tissue. Von Bergmann postulated that the primary cause of obesity was not a lower basal metabolic rate, as was commonly assumed, but a greater capacity in certain individuals to store excessive amounts of fats in the adipose tissue; this process being controlled by hormonal and neural factors. Von Bergmann himself apparently believed that trophoneurotic disturbances were responsible for the greater fat affinity of adipose tissue in obese individuals but that this fat could presumably be taken over quite passively from the extracellular fluids, as, for instance, uric acid can be removed selectively by mesenchymal tissues under certain pathological conditions (Von Bergmann & Stroebe, 1927). The examples cited above represent opinions on the physiological role of adipose tissue which were quite different from the commonly accepted ideas of the time. Some of these opinions were expressed in leading articles, but the ideas were never really pursued. The case of Wassermann and Hausberger is quite different. Wassermann, on the basis of histological and embryologic studies, came to the conclusion that adipose tissue was unique in its capacity to accumulate and mobilize fat

(Chapter 10). His basic work on this subject bears the programatic title "Die Fettorgane des Menschen" (Wassermann, 1926). Further histological studies as well as transplantation experiments and subsequently metabolic and endocrine research by Hausberger reinforced this first postulation and led to the present state of knowledge (Chapter 52).

There is no doubt that the classical work of Schoenheimer and Rittenberg, summarized by Schoenheimer (1942) in his book *The Dynamic State of Body Constituents* greatly influenced the general attitude regarding the role of adipose tissue. For the first time the rapid turnover of body fat was clearly demonstrated; there was no question that these lipids were being continually synthesized and degraded in the animal organism. As their ideas penetrated our thoughts, it became gradually clear that adipose tissue, like all other active tissues, participates actively in the dynamic process of the body. But at that time the liver was still regarded as the predominant site of energy metabolism of lipids and adipose tissue was allotted only a minor role.

While Wassermann was studying the histological and embryological aspects of adipose tissue, we became interested in its physiology through studies on the metabolism of starvation. After a few hours of starvation the limited glycogen reserves become depleted and the body passes over almost entirely to fat metabolism, as evidenced from the RQ of approximately 0.70. In addition, some gluconeogenesis from proteins has to be taken into account, which is a separate problem in itself.

A man can survive a state of complete starvation, provided that an ample amount of water is supplied, for approximately 30 days. Of his 70 kg of total body weight, approximately 7 kg are fat. This would provide enough calories, in the form of stored fat, to last for a month provided that a regular minimum quantity of calories is mobilized daily from adipose tissue. Facts such as these helped to indicate that the belief about the inertness of adipose tissue was probably not correct. Certain clinical observations pointed in such a direction too. Goering, for example, came to the conclusion that, in special cases, localized fat deposition and depletion is governed by nervous factors and it is possible to see an accumulation or a depletion of fat in the area of certain nerve fibers (Goering, 1922). The most interesting case was that of a patient, described by Müller, who had suffered a spinal injury 13 years previously which caused a lesion of the upper lumbar regions. The patient was in a satisfactory nutritional condition until 2 years prior to his death, which was caused by tuberculosis. He had lost weight very rapidly during the disease and on post-mortem examination, a rather remarkable observation was made. Müller found that the part of the body with intact innervation had no adipose tissue while in the paralyzed lower extremities, where innervation had been destroyed, normal deposits of adipose tissue were found. This suggested that mobilization of fat from adipose tissue requires an intact nerve supply (Müller, 1921). We tried now to show experimentally that adipose tissue is really under nervous control. Scission of the spinal cord in the vicinity of the first thoracic segment prevents the transport of fat from adipose tissue to the liver and the formation of a fatty liver in a phloridzin-treated fasting dog (Wertheimer, 1926). If the scission is made at the sixth thoracic segment or lower, this difference is considerably smaller. It was further shown that scission of the liver nerves alone does not prevent fatty infiltration of the liver in hunger and phloridzin diabetes. Subsequently, many other experiments conducted along these lines showed with certainty the existence of sympathetic nervous regulation in adipose tissue. Hausberger (1935) has shown this in an especially elegant manner. He was able to cut the nerve connection supplying one side of the symmetrical intrascapular brown fat body of the mouse and thereby set up conditions by which the intact side of the body could serve as an ideal control. It was observed that the fat content of the denervated side exceeded that of the control side, especially during starvation.

Apart from the regulation of fat mobilization, of no lesser interest was the regulation of fat deposition in adipose tissue. We first tried to find out the initial steps involved in the replenishment of fat in adipose tissue after prolonged starvation. The first metabolic change which we were able to record was the accumulation of glycogen particularly after refeeding a carbohydrate-rich diet. This glycogen, as we have recently learned through the experiments of Dr. Shafrir in our Department, has a much higher rate of turnover in adipose tissue than in liver. That glycogen is present in adipose tissue is not so important per se, but that it is always found in significantly high amounts concomitant with the formation of new fat is especially significant. During periods of equilibrium when net fat formation is not taking place, only small amounts of glycogen can be detected. Mirski (1942), in our laboratory, found that adipose tissue of rats refed after a period of starvation contained considerable amounts of glycogen and its RQ rises to much more than one. All these experiments were so convincing that they left little doubt that carbohydrate was actually converted into fat in the adipose tissue itself. Pertinent in this connection was the observation that after injection of small amounts of insulin to the rat, the glycogen content of adipose tissue begins to rise after only ½–1 hr, a maximum is reached after 8–12 hr and glycogen continues to be found even after a whole day. This effect is especially marked in brown adipose tissue. These experiments demonstrated for the first time a direct effect of insulin on adipose tissue and perhaps an effect on the transformation of glucose into fatty acids by this tissue. It was not until 1948 that lipogenesis from glucose in adipose tissue was proved unequivocally and directly through the demonstration that deuterium was incorporated into the fatty acids of adipose tissue incubated in serum containing deuterium oxide (Shapiro & Wertheimer, 1948). These facts suggested that adipose tissue itself must be very active metabolically and warranted further investigations in vitro. The first tissue preparation in which such metabolic activity could be demonstrated was rat mesenterium (Shapiro et al., 1948, 1952).

It was at this stage that an invitation came from Dr. C. N. H. Long to write an article for *Physiological Reviews* on the general theme, "The Physiology of Adipose Tissue." This was a great stimulus for us since we believed that the time was ripe not only for evaluating our own results but also for reviewing the work of others in this field. Moreover, this was the first official recognition that the whole subject of adipose tissue physiology was no longer a subject which could be ignored. Accordingly, Shapiro and I wrote this review with great enthusiasm. It seems pertinent at this point to quote its conclusions:

> Adipose tissue is a tissue with a special structure and a special type of cell. It is supplied by a comparatively dense capillary net and innervated by sympathetic nerve fibers. Deposition and mobilization of fat in adipose tissue is an active process, involving the metabolism of the tissue. Under conditions favoring fat deposition, adipose tissue accumulates glycogen, which is presumably built in the tissue cells themselves. Synthesis of new fatty acids from carbohydrates as well as transformation of one fatty acid into another proceed continuously in this tissue. All these metabolic activities are regulated by nervous and endocrine factors (Wertheimer & Shapiro, 1948).

Many scientists who previously had not been acquainted with the field of adipose tissue became interested in the subject. In the ensuing period, it could be felt that this tissue became accepted in the official field of physiology. On the other hand, our research work had slowed down particularly because of lack of success to demonstrate in vitro the physiological removal of fat from the depot tissue.

At this time there was in general no doubt that liver played the major role in maintaining the caloric homeostasis of the body. Although it was accepted that adipose tissue participated in energy metabolism, its function was considered to be only of secondary importance as it always seemed to be capable only of sluggish types of reactions.

3. THE EXPLOSIVE DEVELOPMENT AFTER 1948

In the last 15 years however, a rapid succession of experimental developments necessitated a revolutionary change of opinion. We should recall that some 30% of the dietary carbohydrate is converted to fat before it is further metabolized. The amount converted can be greatly increased by excessive caloric intake. This, together with the finding that fatty acids derived from dietary lipids can also be actively incorporated into adipose tissue fat should not obscure the fact that the synthesis of fatty acids from carbohydrates proceeds rapidly in the animal maintaining a constant body weight. The in vitro experiments of Hausberger et al. (1954) and Feller (1954) which were later confirmed in vivo by Favarger & Gerlach (1954) present clear-cut evidence that adipose tissue itself is the major site of conversion of carbohydrates into fat and not the liver.

In view of the great importance of this process in adipose tissue, it is not surprising that mechanisms must exist which regulate the rate at which carbohydrates can be converted into fat. This process is essentially unidirectional. The other crucial problem still unresolved at that time was: What substance is actually mobilized from adipose tissue and how is it transported to and oxidized by the various organs? Only in recent years did Dole at The Rockefeller Institute and, at the same time, Gordon at the National Institutes of Health recognize the great physiological importance of the free fatty acid fraction of blood plasma. Although it comprises only about 5% of the total fatty acids, it has an extremely rapid turnover (Dole, 1956; Gordon & Cherkes, 1956). These free fatty acids, transported mostly on albumin molecules, are by virtue of their rapid turnover rate an important link between the release and the transport from various fat depots. It is now recognized that they provide a source of energy for the working cell and are in fat metabolism what glucose is in carbohydrate metabolism. This further confirms the major physiological role of adipose tissue since this tissue has been shown to be the main, if not the only, source of free fatty acids in serum.

Subsequent developments in this field were then really explosive due, in great part, to the contributions of a number of gifted young scientists. In Boston, Renold and Cahill, together with their colleagues, entered the field of adipose tissue metabolism with energy and great enthusiasm while at the same time Fawcett performed his experiments by histological methods. In Jerusalem, Shapiro and his co-workers and in Bethesda, Steinberg and others accelerated the pace. The late Dr. Frank Engel and his team at Duke University, Ball at Harvard and countless others produced a steady stream of new and fascinating information. No doubt many other research teams were influenced by this development and the present state of knowledge was attained in an unbelievably short time. Perhaps the best proof is the large number of review articles on this subject, which have appeared during the last 12 years—most of them, in fact, during the last 5–6 years. The articles to be listed do not include those reviews on the transport of free fatty acids and its regulation and on lipids in general despite the fact that adipose tissue is a central theme in most of them. These reviews, when compiled into one comprehensive treatise, give us not only an idea of the tempo of research in this field, but present us with an up-to-date evaluation of existing knowledge.

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4. HAS THE RAPID DEVELOPMENT A SOUND BASIS TODAY?

One cannot help but question whether the rapid development as described above is really justified or whether we are merely witnessing an exaggerated peak of enthusiasm about the importance of adipose tissue. The best indication that we are now entering a more stable period is that during the last 3–4 years we have seen relatively less of the exciting work which was produced during the "explosive period" but instead, good "routine" work similar to that found in many other fields. Today the study of adipose tissue is still a popular subject, the interest in it is as great as ever, and everyone believes that the development of this field will continue. What may well be an exaggeration is the overemphasis placed on studies of rat adipose tissues. There is enough experimental evidence today to suggest that conclusions based on rat adipose tissue must be interpreted with caution because such results do not necessarily apply to other mammals. Moreover, epididymal fat, which is used for so many experiments, may have a unique metabolic pattern and cannot be considered as a "typical" adipose tissue. It is known today that there are considerable quantitative differences between the various adipose tissues which are, in addition, differentiated according to their functions, whether they are more metabolic, mechanical or both. Considering all, a cautious generalization seems possible today.

5. FUTURE INVESTIGATIONS

What then are the possible lines of investigation for the future? One important aspect now being studied in a number of laboratories concerns such dynamic processes as lipolysis and re-esterification of free fatty acids by adipose tissue. In addition, many studies have been carried out on the various enzyme systems of adipose tissue in order to understand better the pathways of mobilization and accumulation of fat, specific for adipose tissue. Much work has been done on the interrelationships between adipose tissue, serum, liver and intestine. Nevertheless, all these phases of investigation need further development. A very promising approach would be a detailed study of the pharmacological aspects of adipose tissue. The importance of the sympathico-adrenal system in the mobilization of fat is well known and would warrant further investigations of pharmacological influences on lipogenesis and related processes in adipose tissue. It has been shown that while anti-adrenergic substances such as dibenamine can accumulate in adipose tissues and depress the mobilization of fat, administration of adrenergic substances accumulating in adipose tissue can accelerate fat mobilization and cause a depletion of fat in various adipose tissues (Brodie et al., 1954). Lipid-soluble toxins may be selectively accumulated by various adipose tissues and thereby exert an especially strong effect in that particular tissue. For instance, it is well known that certain barbiturates accumulate mainly in adipose tissue and by selecting the particularly fat soluble barbiturates, it may perhaps be possible to selectively paralyze it without damaging other tissues (Hermann & Wood, 1952). The effect could actually be one of chemical extirpation of all the adipose tissues. It is interesting to note that certain toxins exert different quantitative effects on the various types of adipose tissue from which it follows also that considerable variations must exist in the metabolic patterns of the different adipose tissues.

Little is known about the pathological aspects of adipose tissue metabolism. Studies on experimental obesity and especially diabetes have shown that the adipose tissue itself is clearly implicated and further work along these lines should be rewarding. Moreover, adipose tissue should be investigated in other pathological conditions without overt adipose malfunction, since in diseases such as nephrosis or liver damage, certain irregularities may be discovered (Gutman & Shafrir, 1963).