Histochemical Studies on the Nervous and Humoral Regulation of Lipids and Carbohydrate Metabolism

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Abstract

The purpose of the present study is to reveal the precise mechanism of nervous and humoral regulations of lipid and carbohydrate metabolisms in the adipose tissues. Histochemical and biochemical observations were made on the innervated and denervated interscapular brown adipose tissues and partly on the liver and adrenal cortex of male mice during starvation with or without carbohydrate introduction with special consideration to the changes of the lipid and glycogen contents and to the activities of several important enzymes as well as to pH values in the tissues. In a state of absolute starvation, the animals died in a few days showing a gradual discharge of stored lipids from the innervated brown adipose tissues, while in the denervated tissues the stored lipids increased gradually even in a state of slight or moderate starvation as well as in the cases of normally fed animals. The increase of lipids continued before the stage of severe starvation and the stored lipids being rapidly discharged became nil at the terminal stage of life. Introduction of glucose into starved animals caused also a more marked deposition of glycogen in the denervated than in the innervated tissues in proportion to the degree of starvation, although it did not cause the deposition in both tissues at the terminal stage of life. These facts represent that the nervous regulation is essential for the mobilization of lipids and carbohydrates from this tissue. Adrenalectomy also caused the death of animals within a few days with a gradual decrease of depot lipids. In this case denervation likewise caused a marked depositon of lipids in the brown adipose tissues, showing a sudden escape of lipids at the end of life. Experiments on transplanted adipose tissues taken from the animals at the terminal stage of starvation, proved that the tissue cells retain the ability to deposit lipids until the end of life. Chemical estimation elucidated that the serum glucose and lipids fall markedly at the terminal stage of life. The innervated tissues showed increased activities of succinic dehydrogenase, alkaline phosphatase, ATPase and lipase during starvation with a gradual discharge of lipids. Glucose injection increased the degree of the activities of all these enzymes, though in the terminal stage of starvation the ATPase activity declined again. The activity of total cholinesterase declined slightly in severe starvation. The pH value fell gradually with the progress of starvation. On the other hand, in the denervated tissues the activity of succinic dehydrogenase fell with an increased deposition of lipids, though in the final stage of starvation the activity rose with the discharge of lipids; while the activities of phosphatase, ATPase and lipase rose during starvation and total, unspecific and specific cholinesterase activities declined slightly. The pH value in the denervated tissues rose slightly during mild starvation and fell markedly in

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severe starvation. Observations proved that the activities Df these enzymes and pH, which are under the control of the autonomic nervous system, have close relationships to the deposition and the discharge of lipids and glycogen from the adipose tissues, and that the rapid discharge of lipids from the denervated tissue at the terminal stage of life is an expression of the onesided progress of oxidative process which may mean a complete loss of regulation of metabolism.

HISTOCHEMICAL STUDIES ON THE NERVOUS AND HUMORAL REGULATIONS OF LIPID AND CARBOHYDRATE METABOLISMS

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A considerable literature has accumulated on the humoral regulation of fat and carbohydrate metabolisms in the adipose tissues[20,26,35,38], but comparatively little information is available concerning the nervous regulation of them. Through the clinical and patho-anatomical observations of the various diseases associated with the lesions of the central and peripheral nerves as well as some experimental investigations on animals it has been found that these metabolisms are under the control of the autonomic nervous system[2,11,16,24,42,43]. Little is known, however, of the precise mechanism by which the nervous stimulus or its inhibition causes the discharge or deposition of fat in the adipose tissues. The purpose of the present study is to reveal the precise mechanism of the nervous and humoral regulation of lipid and carbohydrate metabolisms in the adipose tissues. Histochemical and partly biochemical observations were made on the innervated and denervated interscapular brown adipose tissues of male mice during starvation with special consideration to the changes of the lipid and glycogen contents and to the activities of several important enzymes such as succinic dehydrogenase, phosphatase, adenosintriphosphatase, lipase and cholinesterase, as well as pH values in the tissues. The same observations were also made after carbohydrate introduction during the starvation, and the relationships of the changes of the lipid contents in the adipose tissues to those in the other organs such as the liver and adrenal cortex as well as to those of blood sugar and serum lipids during starvation were investigated. Moreover, the effects of adrenalectomy on the lipid and glycogen contents in the innervated and denervated brown fats in starvation and after carbohydrate introduction were observed.

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MATERIALS AND METHODS

Seventy five healthy male mice, weighing 15 to 25 grams, were used in these investigations. The nerves distributed in the right lobes of the interscapular brown adipose tissues, five in all, were cut under the lower margin of the scapula. After the operation sixty five animals were fasted absolutely and ten were fed normally. Thus one to four days later, the animals in a mild, moderate, or severe starvation were decapitated and histological as well as histochemical observations were made on the innervated, left, and the denervated, right, tissues, comparatively. Histologic observation was made on the preparations stained with hematoxylin-eosin. Lipids were stained with Sudan III, Sudan black B, or osmium tetraoxide. On the normally fed animals the observations were made one to fifteen days after the operation. About one half of the animals, fed normally or fasted absolutely during one or three days after the operation, were given a carbohydrate diet and injected with 2 c.c. of 20 per cent glucose solution subcutaneously. The diet was prepared by cooling after boiling the mixture of 10 g. of starch, 5 g. of sugar and 20 c.c. of water. Two hours later histochemical observations were made on the innervated and the denervated brown fats. Glycogen were examined by means of Best carmine stain and PAS reaction with the control tests for digestion by saliva. Biochemical estimation of blood sugar was made by Hagedorn-Jensen's method[14] and that of plasma-lipids by W. R. Bloor's method[3]. Seligman and Rutenburg's method[37] for histochemical demonstration of succinic dehydrogenase, Ohtani's modifications[30] of Gomori's method[12] for alkaline phosphatase and adenosinetriphosphatase, Gomori's modification method[12] for lipase, and Koelle's improved methods[22,23] for total, unspecific and specific cholinesterase were employed. ATP used was extracted by ourselves from rabbit muscle by K. Lohmann's method[25]. Biochemical estimation of succinic dehydrogenase activity were made by Oda's method[28]. pH values were measured by using bromethymol blue as an indicator and glass electrode pH meter. The other methods for special purposes will be described in each chapter.

EXPERIMENTS AND RESULTS

The effects of denervation and starvation on the lipid contents in the brown adipose tissue :

The results are shown in Fig. 1 by diagrams. The "brown fat" is one of the important storage organs of lipids, glycogen and others[36]. The fat cells in this tissue of normally fed animals are gorged with numerous
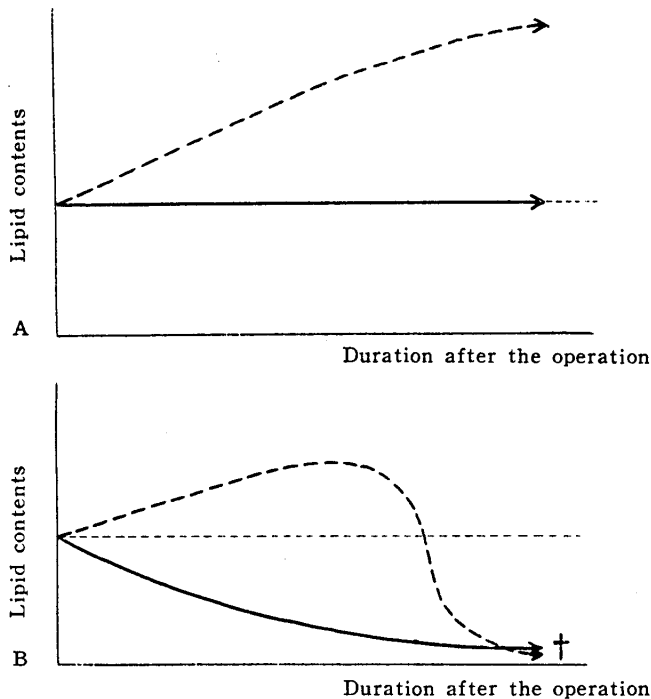
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Fig. 1.   Diagrams of the changes of the lipid contents in the innervated (——→) and the denervated (---→) brown adipose tissues of normal (A) and fasted (B) mice.

small lipid-droplets among which the nucleus is imbedded, and they are richly supplied with blood capillaries (Fig. 9). In a state of absolute starvation the animals died in a few days with the decrease of body weight showing a gradual discharge of stored lipids in the innervated tissues (Figs. 10 and 11). In the denervated tissues, however, the lipid-droplets become large in size gradually showing the increase of the lipid contents not only in a state of normal feeding (Fig. 12) but also in that of a mild or moderate starvation (Fig. 13). The increase continued before the stage of severe starvation, in which the animals were severely exhausted and the loss of body weight was marked, and then the stored lipids were rapidly discharged thus being depleted almost to nil at the terminal stage of life (Fig. 14). These results show that the nervous stimulus plays an important rôle in the regulation of lipid contents in the brown fat.

    The effects of denervation and starvation on the glycogen deposition in the "brown fat" after carbohydrate introduction :

    The results are shown diagrammatically in Fig. 8. In the animals of

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normal condition the tissues contained generally only a little or no glyco-
gen. In the normally fed animals the glycogen contents hardly increased
in the innervated tissues even by introduction of sufficient amount of
carbohydrates, while it did slightly in the denervated tissues. By starva-
tion the glycogen disappeared, and in the fasted animals no trace of gly-
cogen granules was found both in the innervated and in the denervated
tissues, though in the latter the lipids were markedly increased in con-
tents. These facts may suggest that glycogenesis is not essential for
lipogenesis in the fat cells. The introduction of sufficient amount of car-
bohydrates to the starved animals caused the deposition of glycogen,
which was more marked in the stage of moderate or severe starvation,
except at the terminal stage of life, than in that of slight starvation.
Moreover it was far marked in the denervated (Fig. 16) than in the in-
nervated tissues (Fig. 15). These facts may suggest that the permeability
of the cell membrane or catabolic and anabolic processes in the cells are
under the control of the vegetable nerves. The introduction of carbohy-
drates to the severely starved animals in the terminal stage of life caused
no appearance of glycogen granules both in the innervated (Fig. 17) and
in the denervated tissues (Fig. 18). The loss of ability of glycogenesis in
this stage may be an expression of the one-sided progress of catabolism in
the whole body which may mean a complete breaking of regulation of
the metabolism in the terminal stage of life.

*The changes of the lipid and glycogen contents in the liver during
starvation :*

In order to investigate the relationship between the variation of the
lipid contents in the brown fat and that in the other organs, histochemical
observation was made of the lipid and glycogen contents in the liver dur-
ing starvation in the same experiments. The experimental results are
shown in Fig. 2. The lipid contents in the liver increased gradually during
the mild or moderate starvation and then showed a tendency to diminish
in advanced starvation. The glycogen reserves in the liver fell during
starvation, and carbohydrate introduction after starvation showed almost
the same results as those in the brown fat.

The changes of blood-sugar and plasma-lipids during starvation :

In order to investigate the relationships between the changes of the
lipid contents during starvation in the innervated as well as denervated
brown fats and those of blood-sugar and plasma-lipids, the same experi-
ments as those previously described were carried out by using rabbits.
The results are shown in Fig. 3. In the animals receiving no operation,
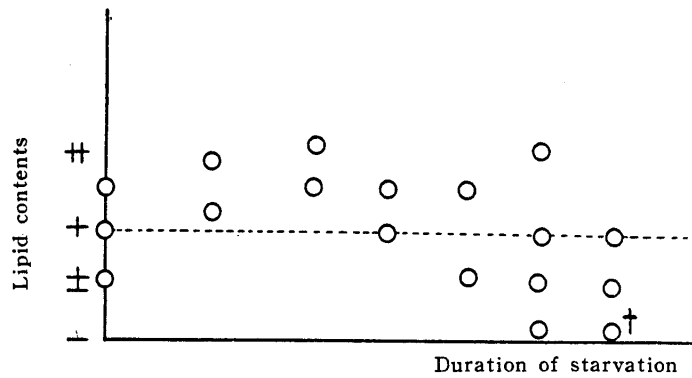
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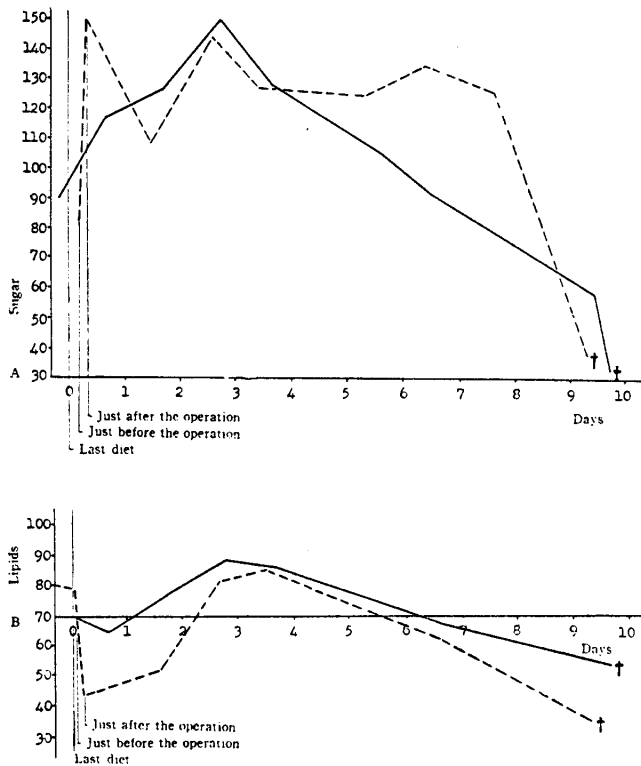Fig. 2. A diagram of the changes of the lipid contents during starvation in the liver of mice.

Fig. 3. Blood-sugar changes (expressed in mg%) (A) and blood-lipid changes (expressed in absorption range) (B) during starvation in rabbits with (--->) and without (——>) denervation of the brown adipose tissues.

the content of blood sugar rose gradually after taking diet and during the first stage of starvation resulting in hyperglycemia, which was gradually followed by hypoglycemia with the progress of starvation, and blood sugar reached extremely low levels at the terminal stage of life. In the animals receiving the operation, the blood sugar rose suddenly immediately after the operation, but it returned to normal soon afterwards and increased again showing hyperglycemia followed by hypoglycemia in the stage of severe starvation, and the blood sugar reached also extremely low levels at the terminal stage of life. In the animals receiving no operation, serum lipids rose gradually showing hyerlipemia during starvation, which was followed by hypolipemea with the progress of starvation. In the animals receiving the operation, hypolipemia was caused immediately after the operation and then the content of serum-lipids rose gradually during starvation, reaching hyperlipemic levels, but it declined again approaching gradually marked hypolipemic levels with the progress of starvation.

From the data of this and the previous chapters the following deductions may be drawn : During starvation the stored glycogen in the liver is diminished and the stored lipids in the innervated brown fat are discharged into blood causing hyperlipemia and transfered to the liver, where they are utilized as energy source. With the progress of starvation the depot lipids decrease in amount resulting in hypolipemia and then the liver lipids decrease in amount causing the intense deficiency of energy source. In the denervated brown fat, however, lipids increase in amount not only in the hyperlipemic stage during the mild starvation but also in the not so-marked hypolipemic stage during the moderate starvation. Reaching the severe starvation, in which blood-sugar and serum-lipids show extremely low levels, the lipids are suddenly discharged from the denervated brown fat, making the residual content almost to nil. The sudden increase of blood-sugar immediately after the operation may be considered to be caused by adrenalin secreted in the adrenal medullae against the stress.

The effects of nutrition of the recipient animals upon the transplanted "brown fat" from normally fed and fasted mice.

The purpose of this experiment is to clarify definitely the mechanism by which the lipids in the denervated brown fat are discharged at the stage of severe starvation whether it is due to the irreversible, complete loss of function of the fat cell itself or due to the influence of extracellular humoral factors as shown in the previous chapter.

The brown fats of healthy mice were transplanted into the subcu-

tanous tissues of the other healthy mice. The lipid contents of the transplanted tissues were increased in the state of normal nutrition of the recipient animals and were decreased in the terminal stage of their starvation. The brown fats of mice in the terminal stage of starvation were transplanted in the same way into the other healthy mice. The lipid contents of the transplanted tissues were increased in the state of normal nutrition of the recipient animals and were as little as those before transplantation in the terminal stage of their starvation. These results may prove that the brown adipose tissue cells retain until the end of life the ability to take or form lipids when they are placed in the optimal milieu of nutritions.

The changes of the lipid contents in the adrenal cortex during starvation :

An attempt was made in this and the next chapters to investigate the hormonal regulation of lipid metabolism in the adipose tissues. In this chapter, observations were made of the changes of the lipid contents in the adrenal cortex of mice during starvation, since its hormones are considered to be one of the most important factors in the hormonal regulation of lipid and carbohydrate metabolisms against the stress.

The results are shown in Fig. 4 by a diagram. During starvation,
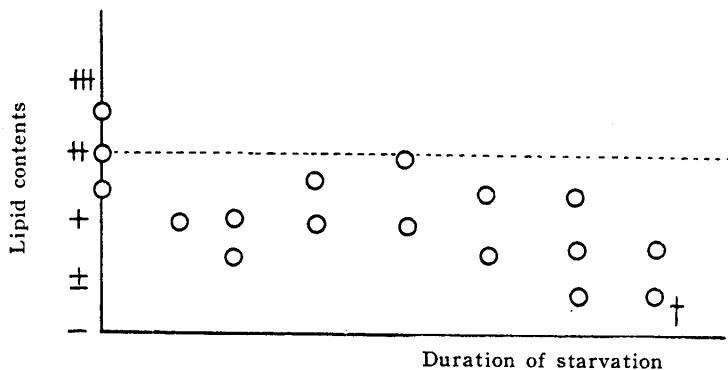

Fig. 4.   A diagram of the changes of the lipid contents during starvation in the adrenal cortex.

the adrenal cortex showed hypertrophy with multiple small lipid-granule formation and discharge of the lipid granules, resulting in the decrease of lipid contents in the tissues, the contents fluctuating somewhat upward then down again with advances in starvation.

The effects of adrenalectomy on the lipid and glycogen contents in the innervated and denervated "brown fats" :

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Bilateral adrenalectomy was performed on mice at the same time as of the operation described previously. After the operation the animals were fed normally (Group A) or fasted absolutely (Group B), and histo-chemical observations were made of the lipid contents in the innervated and denervated "brown fat". Glycogenesis in the innervated and de-nervated "brown fats" of the adrenalectomized mice were observed by the same method as that described previously.

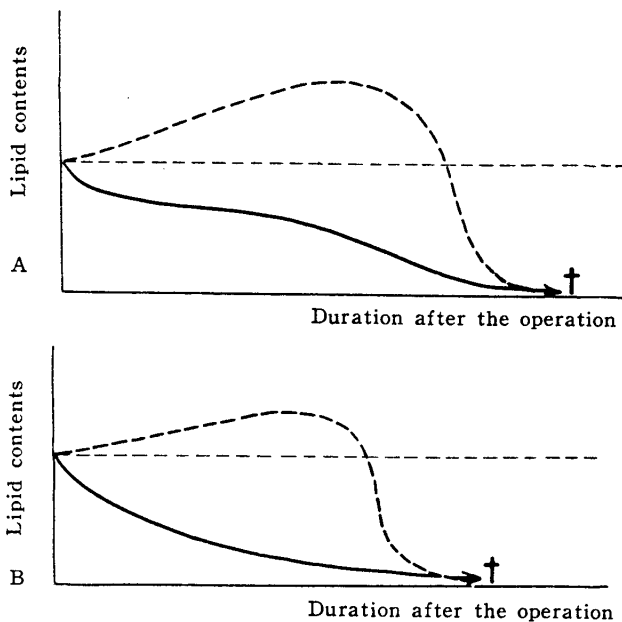The results are shown in Fig. 5 by diagrams. Adrenalectomy caused

Fig. 5. Diagrams of the lipid contents during normal feeding (A) and absolute fasting (B) in the innervated (⟶) and the dener-vated (---⟶) brown adipose tissues of mice after receiving bilateral adrenalectomy.

the death of animals within a few days with a gradual discharge of stored lipids in the innervated tissues, irrespective of feeding or fasting. De-nervation caused, however, a deposition of lipids in the "brown fat" of the adrenalectomized mice as markedly as that of the non-adrenalectomi-zed mice, though a sudden discharge of lipids was seen at the end of life in both groups. Glycogenesis in adrenalectomized mice showed also almost the same results as those in the animals receiving no adrenalec-tomy.

These results may signify that adrenal hormones exert influences on

the lipid and glycogen metabolisms in the innervated "brown fat" but hardly in the denervated tissue, suggesting that nervous regulation plays more important rôle than the hormonal regulalion on those metabolisms in the "brown fat".

The effects of denervation and starvation as well as carbohydrate administration on the enzyme activities in the "brown fats" :

The results of the histochemical observations are shown by a diagram en bloc in Fig. 8.

Succinic dehydrogenase : — After the enzyme reaction was carried out with neotetrazolium chloride on the fresh tissue specimens from the mice, they were fixed with 10 per cent formol solution, and then frozen or carbowax-embedded sections were prepared. In the preparations reduced neotetrazolium, diformazan, were demonstrated as bluish violet granules corresponding to mitochondriae[28,29]. Unspecific stain of lipids due to the oil solubility of the diformazon was not so marked. The unfavorable phenomenon was avoided by Takamatsu's method[33] using potassium tellurite as a hydrogen acceptor. The normal "brown fats" showed moderate activity of the enzyme (Fig. 19). During starvation the activity rose in the innervated tissues (Fig. 20), while it fell in the denervated tissues (Fig. 21). Carbohydrate introduction during starvation caused the rising of the activity more markedly in the innervated than in the denervated tissues. In the terminal stage of starvation the activity rose in the both tissues. Absorption spectrum of the reduced neotetrazolium by Beckmann's spectrophotometer is shown in Fig. 6. The absorption maximum is seen in the wave length of 520 mμ. Biochemical estimations of the reduced neotetrazolium in the innervated and denervated "brown fat" of the normal and fasted mice are presented in Fig. 7, which indicate the same changes of activities of succinic dehydrogenase as those described previously in the histochemical data.

The fall of the activity of succinic dehydrogenase by denervation suggests a drop in terminal respiration, the disturbances of oxidative process of carbohydrates and lipids in the TCA cycle.

Phosphatase : — Alkaline phosphatase activity demonstrated by modification of Gomori's method with sodium glycerophosphate as a substrate was very slight in the normal "brown fat" (Fig. 22). During starvation the activity rose in both the innervated (Fig. 23) and the denervated tissues (Fig. 24), which was accelerated by carbohydrate introduction during starvation. The degree of the rise was slightly higher in the innervated than in the denervated tissues.

Adenosinetriphosphatase : — In the normal "brown fat" the enzyme

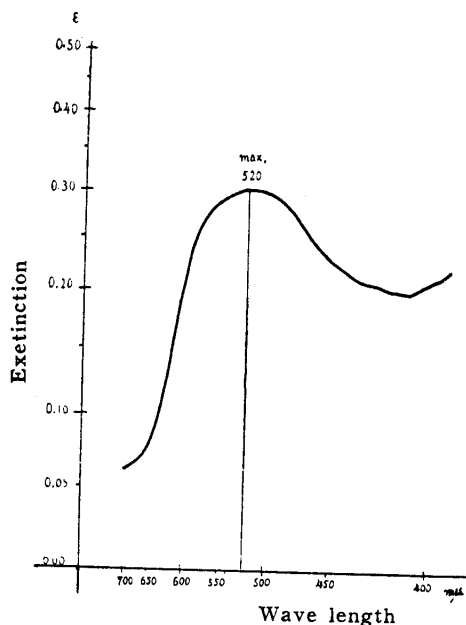166   T. ODA, K. OHTANI, M. AWAI and A. SAKAI

Fig. 6.   Absorption spictrum of reduced Neo-tetrazolium by succinc-dehydrogenase (Beckmann's autorecording spectrophotometer)
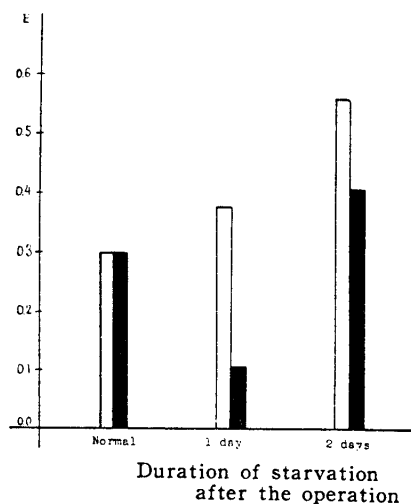
Fig. 7.   Biochemical estimations of succinic-dehydrogenase activity in the innervated (white column) and denervated (black column) "Brown fat" of fasted mice.

activity was very slight (Fig. 25). During a mild or moderate starvation it rose moderately in the innervated tissues (Fig. 26) and slightly in the denervated tissues, which was accelerated also by carbohydrate introduction during starvation in the both tissues (Fig. 27). At the stage of a severe starvation it declined in the innervated tissues (Fig. 28).

These facts show that the activities of phosphatase and adenosine-triphosphatase are invigorated when lipids and glucose are discharged from fat cells as well as taken or assimilated in them, suggesting that phosphorylation and energy supply are accelerated in these metabolic processes.

Lipase : — The normal "brown fat" showed mild degree of lipase activity (Fig. 29). The activity rose moderately during starvation both in the innervated tissues in which lipids decreased in amount (Fig. 30) and in the denervated tissues in which lipids increased (Fig. 31). In the terminal stage of starvation the activity was moderately invigorated in the innervated as in the denervated tissues, in which discharge of lipids was marked. Glucose administration in the fasted mice caused a marked rise in the activity both in the innervated and denervated tissues.

These data indicate that lipase activity rises both in the anabolic and in the catabolic stages of lipids.

Cholinesterase : — In the normal "brown fat" the activities of total (Fig. 32), unspecific and specific cholinesterase were demonstrated only slightly. Denervation and starvation did not caused so significant and definite changes of the activities. However, in the innervated tissues total cholinesterase activity generally showed a tendency to decline slightly during severe starvation, and in the denervated tissues total, unspecific and specific cholinesterase activities showed also a tendency to decline slightly during moderate and severe starvation.
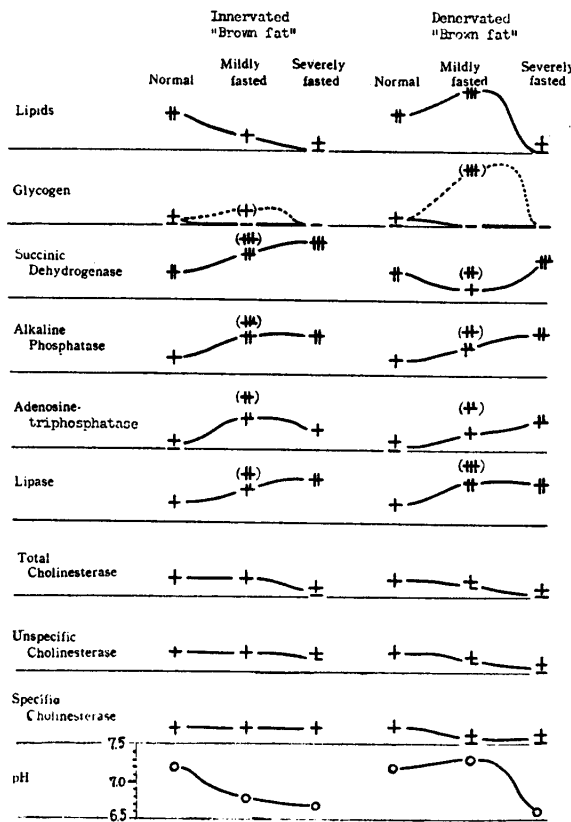
Fig. 8. Diagrams of the histochemical observations of enzyme activities and pH values as well as the lipid and glycogen contents during starvation in the innervated and denervated brown adipose tissues of mice. The glycogen contents and each enzyme activity after receiving glucose injection during starvation are shown as dotted lines and in parentheses, respectively, but the parentheses on the dotted lines represent the degree of the glycogen contents.

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The effect of denervation and starvation on pH in the "brown fat" :

The results are shown in Fig. 8 by diagrams. The normal tissues presented pH 7.2 or 7.3, the innervated tissues pH 6.8 or 6.9 in a stage of moderate starvation and pH 6.7 or 6.8 in that of severe starvation, while the denervated tissues pH 7.3 or 7.4 in the moderate starvation and pH 6.6 or 6.7 in the svere starvation. These facts show that pH value rises or falls in proportion to an increase or a decrease of the lipid contents in the tissues.

GENERAL DISCUSSION

As the comments on each experimental results were already presented in each chapter, some supplemental discussions will be made here, summarizing all the results together with some additional clitiques of literatures.

Since the famous discription of CLAUDE BERNALD (1854) it has been well known that carbohydrate metabolism is under the control of the autonomic nervous system with the inter-brain as its center[2]. After insulin was found, however, the hormonal regulation has been regarded as the more important on the metabolism. Nevertheless, the rôle of the nervous regulation can not be ignored[2], and there still remain many unsolved problems to be studied on the relationship between the both regulation mechanisms. It has also been reported that lipid metabolism is regulated by the autonomic nervous system ; DOGIELS (1898)[8], STÖHR (1930)[41], and HAUSBERGER (1934)[15] et al. demonstrated the innervation of the adipose tissue. TOLDT (1870)[42], WERTHEIMER (1932)[43], HAUSBERGER (1935)[16], and KURÉ, ŌI and OKINAKA (1937)[24] reported experimental investigations on the nervous regulation of fat matabolism. GOERING (1922)[11] elucidated the regulation on the basis of clinical and patho-anatomical observations. Especially KURÉ et al.[24] presented some interesting data by experimental study on the subcutanous adipose tissue in the upper thigh of the dog, in which they stated that the sympaticus acts inhibitively on fat deposition, and the parasympaticus accerelatively, though WERTHEIMER[43] indicated that the vagus takes no part in regulation of fat metabolism. Through many literatures until the present, little is known, however, of the precise biochemical mechanism by which the nervous stimulus or its inhibition causes the discharge or deposition of fats in the adipose tissues.

Concerning the hormonal regulation of glycogen metabolism in the adipose tissue, SANO (1934)[35] reported on the basis of obsevations of the effects of various hormones on glycogeneses in the supravital "brown fat"

that insulin acts acceleratively, and adrenalin, anteglandol, thyroprotein, orphorin and spermatin inhibitively. For the hormonal regulation of lipid metabolism, MORII et al.[6] investigated the actions of steroid hormones on the intermediate metabolism of lipids, and stated that introductions of insulin and corpus lutein hormone cause an increase of lipid contents in the adipose tissues and male sex hormones prevent or inhibit a pathologic fat deposition, but hormonal action on the mechanism of fat mobilization and deposition is generally complex and not uniform.

Recently, SELYE concluded in his monograph[38] that discharge of lipids from the "brown fat" represents a characteristic response during exposure to various types of systemic stresses but the exact mechanism by which the "brown fat" responds to stress is not yet clear. On the other hand, DALE[7], HODGKIN[19], and FATT and KATZ[10] et al. have recently placed a great importance on the rôle of achethylcholine or its allied substance, having a close relationship to cholinesterase, in an impulse transmission in endplates. Especially, FATT et al.[10] asserted that nervous stimuli cause an increase of permeability against various ions unselectively in the end-plates, resulting in an electrical potential changes in the cells. It is also a clearly established fact that adrenalin acts on the endplates of the autonomic nerves. Following the recent study by NAKANE (1952)[27], the actions of adrenalin in the muscle are to be carried out by influencing the degradation process of acethylcholine by cholinesterase and diminishing acethylcholine in amount. In additions, the actions of adrenalin are inhibited by a rise in the pH value in tissues, and are accelerated by its fall.

Judging from the available literatures and our experimental results mentioned previously, the mechanism of the nervous regulation of lipid and carbohydrate metabolisms in the adipose tissues are to be considered from two points of view; the one is a change in permeability of the cell membranes by nervous stimulus itself, which causes mobilization of ions and changes of electrical potential and pH value, and as the results it changes enzyme activities and metabolic processes. The other is the rôle of nerves on the maintenance of balance of hormonal actions, especially between those of insulin as well as corpus lutein hormone and of adrenalin as well as male sex hormone, which act antagonistically on the catabolism and anabolism, respectively. Now, although the mechanism of impulse transmission in the nervous terminals in the adipose tissue has been quite obscure, following deduction may be drawn: from our experimental results that during starvation lipids are dischaged from the innervated "brown fat" with the rising

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of activities of lipase, succinic dehydrogenase, alkaline phosphatase and adenosinetriphosphatase and with the falling of pH value, it may be considered that the nervous stimulus causes an increase of the permeability, electrical potential changes of various ions and changes of these enzyme activities, which accelerate the hydrolysis as well as phosphorylation of lipids, and thus the lipids are discharged from the "brown fat" into blood stream. That is to say, the nervous stimulus acts acceleratively on lipid metabolism, especially on its anabolic processes, resulting in discharge of lipids from the adipose tissues. The rise in the activities of these enzymes may also have close relationships to the processes of permeation of glucose into the fat cells and to glycogenesis as well as lipogenesis after the introduction of carbohydrates during starvation. Above all, the rise in the succinic dehydrogenase activity means the acceleration of the terminal respiration after glucolysis, and at the same time the turnover of the TCA cycle yields ATP, which supplies energy necessary to the anabolic processes by the action of adenosinetriphosphatase. The fact that lipase activity rose both during the stage of increasing and decreasing of lipids is quite significant because lipase catalyzes both the anabolic (hydrolysing) and catabolic (esterizing) processes of lipids[21,32].

On the contrary a fall in the succinic dehydrogenase activity in the denervated tissues suggests the disturbances of oxidative process in the TCA cycle, as the result terminal degradations of lipids and glucose are inhibited, and lipids are increased in amount by lipogenesis from glucose in the fat cells and or by the permeation of lipids into the fat cells. At the same time, a relative increase of insulin action against the inhibition of adrenergic action may play an important rôle in lipogenesis and glycogenesis in the denervated tissues[4,20]. That is to say, an emphasis may be placed on the fact that innervation of the tissues are essential for the maintenance of balance of actions between adrenaline and insulin, which act antagonistically with each other in the regulation of the lipid and carbohydrate contents in the tissues.

In addition, as mentioned previously the fat cell without innervation posesses the characteristic function to take or synthesize lipids always under the optimal condition in the body. Discharge of lipids from the denervated tissues in severe starvation, as mentioned previously in comment, has close relationships to the extreme decreases of lipids and glucose in the blood and to the marked falling of pH in the tissues as well as to the rising of succinic dehydrogenase activity, showing the monolateral progress of catabolic processes in the body at the terminal stage of life.

SUMMARY

The purpose of the present study is to reveal the precise mechanism of nervous and humoral regulations of lipid and carbohydrate metabolisms in the adipose tissues. Histochemical and biochemical observations were made on the innervated and denervated interscapular brown adipose tissues and partly on the liver and adrenal cortex of male mice during starvation with or without carbohydrate introduction with special consideration to the changes of the lipid and glycogen contents and to the activities of several important enzymes as well as to pH values in the tissues.

In a state of absolute starvation, the animals died in a few days showing a gradual discharge of stored lipids from the innervated brown adipose tissues, while in the denervated tissues the stored lipids increased gradually even in a state of slight or moderate starvation as well as in the cases of normally fed animals. The increase of lipids continued before the stage of severe starvation and the stored lipids being rapidly discharged became nil at the terminal stage of life. Introduction of glucose into starved animals caused also a more marked deposition of glycogen in the denervated than in the innervated tissues in proportion to the degree of starvation, although it did not cause the deposition in both tissues at the terminal stage of life. These facts represent that the nervous regulation is essential for the mobilization of lipids and carbohydrates from this tissue.

Adrenalectomy also caused the death of animals within a few days with a gradual decrease of depot lipids. In this case denervation likewise caused a marked depositon of lipids in the brown adipose tissues, showing a sudden escape of lipids at the end of life.

Experiments on transplanted adipose tissues taken from the animals at the terminal stage of starvation, proved that the tissue cells retain the ability to deposit lipids until the end of life.

Chemical estimation elucidated that the serum glucose and lipids fall markedly at the terminal stage of life.

The innervated tissues showed increased activities of succinic dehydrogenase, alkaline phosphatase, ATPase and lipase during starvation with a gradual discharge of lipids. Glucose injection increased the degree of the activities of all these enzymes, though in the terminal stage of starvation the ATPase activity declined again. The activity of total cholinesterase declined slightly in severe starvation. The pH value fell gradually with the progress of starvation. On the other hand, in the de-

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nervated tissues the activity of succinic dehydrogenase fell with an in-
creased deposition of lipids, though in the final stage of starvation the
activity rose with the discharge of lipids; while the activities of phos-
phatase, ATPase and lipase rose during starvation and total, unspecific
and specific cholinesterase activities declined slightly. The pH value
in the denervated tissues rose slightly during mild starvation and fell
markedly in severe starvation.

Observations proved that the activities of these enzymes and pH,
which are under the control of the autonomic nervous system, have close
relationships to the deposition and the discharge of lipids and glycogen
from the adipose tissues, and that the rapid discharge of lipids from the
denervated tissue at the terminal stage of life is an expression of the one-
sided progress of oxidative process which may mean a complete loss of
regulation of metabolism.

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bruary 1957.

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EXPLANATION OF PLATES

Fig. 9. The interscapular "brown fat" of a normal mouse. (Paraffin section, hematoxylin-eosin stain)

Fig. 10. The innervated "brown fat" of a mouse fasted 1 day. (Freezing-section, Sudan III stain)

Fig. 11. The innervated "brown fat" of a mouse fasted absolutely for 3 days. (Paraffine section, hematoxylin-eosin stain)

Fig. 12. The denervated "brown fat" of a mouse fed normally for 15 days after operation. (Paraffin section, hematoxylin-eosin stain)

Fig. 13. The denervated "brown fat" of a mouse fasted absolutely for 2 days after operation. (Paraffin section, hematoxylin-eosin stain)

Fig. 14. The denervated "brown fat" of a mouse fasted absolutely for 3 days after operation. (Paraffin section, hematoxylin-eosin stain)

Fig. 15. Histochemical demonstrations of glycogen in the innervated "brown fat" of a mouse, 2 hours after the subcutanous injection of 2 cc of 20 % glucose solution at the stage of 2 days' starvation. (Alcohol fixation, Best carmine stain)

Fig. 16. Histochemical demonstrations of glycogen in the denervated "brown fat" of the same mouse as in Figure 15.

Fig. 17. The innervated "brown fat" of a mouse, 2 hours after the subcutanous injection of 2 cc of 20 % glucose solution at the terminal stage of life after 3 days' starvation. No glycogen granule appears.

Fig. 18.   The denervated "brown fat" of the same mouse as in Fig. 17. No glycogen granule appears.

Fig. 19.   Histochemical demonstrations of succinic-dehydrogenase in the "brown fat" of a normal mouse. (Seligman et Rutenburg's method, using neotetrazolium as a hydrogen acceptor)

Fig. 20.   Histochemical demonstrations of succinic-dehydrogenase in the innervated "brown fat" of a mouse fasted absolutedly for 2 days.

Fig. 21.   Histochemical demonstrations of succinic-dehydrogenase in the denervated "brown fat" of the same mouse as in Figure 20.

Fig. 22.   Histochemical demonstrations of alkaline phosphatase in the "brown fat" of a normal mouse. (Modificatin of Gomori's method)

Fig. 23.   Histochemical demonstrations of alkaline phosphatase in the innervated "brown fat" of a mouse fasted 1 day.

Fig. 24.   Histochemical demonstrations of alkaline phosphatase in the denervated "brown fat" of a mouse fasted for 3 days.

Fig. 25.   Histochemical demonstrations of adenosinetriphosphatase of the "brown fat" of a normal mouse. (Modification of Gomori's method)

Fig. 26.   Histochemical demonstrations of adenosinetriphosphatase of the innervated "brown fat" of a mouse fasted absolutely 1 day.

Fig. 27.   Histochemical demonstrations of adenosinetriphosphatase of the denervated "brown fat" of a mouse, 2 hours after glucose injection at the stage of 1 day starvation.

Fig. 28.   Histochemical demonstrations of adenosinetriphosphatase of the innervated "brown fat" of a mouse fasted absolutely for 3 days.

Fig. 29.   Histochemical demonstrations of lipase in the "brown fat" of a normal mouse. (Modification of Gomori's method)

Fig. 30.   Histochemical demonstrations of lipase in the innervated "brown fat" of a mouse fasted absolutely 1 day.

Fig. 31.   Histochemical demonstrations of lipase in the denervated "brown fat" of a mouse fasted absolutely 1 day.

Fig. 32.   Histochemical demonstrations of total cholinesterase in the "brown fat" of a mouse fasted for 2 days. (Koelle's improved method)

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