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The effect of changes in nutritional state on the lipolytic activity of rat adipose tissue

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SUMMARY

When intact epididymal fat bodies, obtained from rats given excess carbohydrate, are incubated *in vitro* in the presence of albumin and chyle, free fatty acids (FFA) appear in the incubation medium. These FFA are produced following hydrolysis of the chyle triglyceride by clearing factor lipase present in the fat tissue. When fat tissue obtained from fasted rats is incubated under the same conditions, the amount of FFA appearing in the incubation medium is much less, and it is derived from the tissue rather than from the chyle triglyceride. The function of the clearing factor lipase in adipose tissue is discussed in relation to these findings, and some of the limitations of the study are defined.

It has been reported recently that when intact epididymal fat bodies are incubated with triglyceride in an albumin solution *in vitro,* free fatty acids (FFA) appear in the incubation medium. The production of FFA is highest when the fat tissue is obtained from animals that have been fed carbohydrate, and appears to be due to the action of the clearing factor lipase present in the fat tissue (1, 2). The results of a similar study, in progress in this laboratory, confirm and extend the above findings.

METHODS

Wistar strain male albino rats, weighing between 150 and 200 g., were used. Two groups of rats were studied. The rats in one group were usually fasted for 18 hours, although in certain experiments the period of fasting was increased to 48 hours. Those in the other group were fed their normal diet and were given, at intervals of 1 hour, 3 injections (2 ml. per injection) of glucose (25 per cent) in 0.16 M sodium chloride solution. The injections of glucose were given via the tail vein, with the animals restrained under light ether anesthesia.

After fasting in the one group, or 1 hour after the final injection of glucose in the other, the rats were anesthetized with ether, and from each rat the two epididymal fat bodies were removed. Each body was rinsed in 0.16 M sodium chloride solution, dried lightly on filter paper, and placed in a 25-ml. Erlenmeyer flask containing the incubation medium. The flasks were incubated at 37°C without agitation. The tissue spread out as a thin sheet and presented a large surface area to the medium. Preliminary experiments showed that shaking the flasks did not affect the rate of appearance of FFA in the incubation medium.

In most experiments the incubation medium contained albumin and chyle. Usually 6 ml. of 6 per cent bovine albumin (Armour Fraction V) in 0.16 M sodium chloride solution was used, but in certain experiments the volume, concentration, and pH of the albumin solution were varied. These variations are indicated in the descriptions of individual experiments. The chyle was collected from olive oil-fed rats by the thoracic duct cannulation technique described by Bollman *et al.* (3). The quantity of total esterified fatty acids in the chyle was determined by the method of Stern and Shapiro (4), and the amount of chyle added to the incubation medium was adjusted to give a final concentration of esterified fatty acids in the medium of approximately 6 μmoles per ml. The volume of chyle required was 3 to 5 per cent of the volume of albumin solution.

In experiments in which the nature of the enzyme responsible for the FFA release was investigated, other substances were added to the incubation medium. These included heparin (Pularin-Evans, 100 units per mg.), protamine sulfate,[1] and tetrasodium pyrophosphate.

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[1] Light and Co., Ltd., Colnbrook, Slough.

In most experiments the pH of the incubation medium was adjusted to 8.1 with sodium hydroxide before introducing the fat tissue. Some experiments were carried out at pH 6.0. The albumin in the medium had considerable buffering capacity, and reduction in pH during incubation was small (0.1 to 0.4 units).

When the effect of different agents and conditions on the production of FFA was investigated, one epididymal fat body from each rat was incubated in the test medium and the contralateral body was used as a control. This procedure was necessary since the FFA produced during incubation varied considerably from animal to animal within a group, even though all the animals in the group had received the same preliminary treatment (Table 1). On the other hand, when the two epididymal fat bodies from the same animal were incubated separately under similar conditions, the increases in the FFA content of the medium were in good agreement (Table 1).

In certain experiments the production of FFA was followed when chyle triglyceride was hydrolyzed by the clearing factor lipase of plasma. Plasma containing the clearing factor lipase was obtained from rats injected with heparin (5). The rats were bled 3 minutes after the intravenous injection of 10 units of heparin, and 0.25 ml. of the plasma was added to the incubation medium.

After the fat tissue had been introduced into the incubation medium, the incubation flask was agitated gently for 30 seconds and then a 0.5 to 1.0 ml. sample of the medium was removed. Usually the next sample was taken after 180 minutes' incubation; but in some experiments samples were also taken at intermediate times; and, in one experiment (to show that lipase appeared in the medium during incubation), the tissue was removed after a period and samples of the medium were taken then and also after further incubation.

The FFA were determined in the samples by the method of Dole (6) but phenolphthalein was used as the indicator. To expel all the carbon dioxide from the sample, nitrogen was bubbled through the titration vessel for 30 seconds before beginning the titration. Preliminary experiments showed that observer bias could affect the titration values. Consequently the titrations were carried out in such a manner that the observer was unaware of the nature of the sample being assayed.

Preliminary experiments also showed that under the present experimental conditions the production of FFA was linear over the incubation period but that it was not related in any simple way to the weight of tissue used (Table 1). This last observation was unexpected since in some other studies (1, 7) the production of FFA by intact adipose tissue *in vitro* has been expressed in terms of the wet weight of the tissue. In this study the production of FFA has been expressed as μmoles FFA per hour per epididymal fat body.

In certain experiments the change in the esterified fatty acid content of the medium was determined using the method of Stern and Shapiro (4). Samples of the medium were removed immediately after introducing the fat tissue and 180 minutes later.

RESULTS

The Effects of Fasting and of Glucose Injection on Free Fatty Acid Production. The production of FFA

TABLE 1. PRODUCTION OF FFA WHEN EPIDIDYMAL FAT BODIES ARE INCUBATED *in Vitro* IN THE PRESENCE OF CHYLE AND ALBUMIN

Fasted		Injected with Glucose	
Weight of Tissue	FFA*	Weight of Tissue	FFA*
g.		*g.*	
0.49	0.48	0.52	1.92
0.85	0.40	0.56	2.08
0.42	0.56	0.78	2.00
0.94	0.70	1.25	1.35
0.36	0.59	1.02	2.70
1.13	0.62	0.81	2.35
0.66	0.19	0.50	2.38
0.95	0.38	0.87	4.40
0.64	0.12	0.81	3.92
0.75	0.54	1.05	0.67
0.58	0.49	0.90	1.32
1.10	0.48	0.86	2.30
Mean ± standard deviation	0.46 ± 0.16		2.28 ± 1.1
Left Body*	Right Body*	Left Body*	Right Body*
0.50	0.53	2.56	2.67
0.59	0.61	3.89	3.52
0.40	0.36	2.25	2.70
0.45	0.46	3.56	3.50

* μmoles/fat body/hr.
Upper table: One fat body was used from each animal.
Lower table: Both fat bodies were used.

when intact fat bodies were incubated at 37°C and pH 8.1 in an albumin solution containing chyle is shown in Table 1. The mean rate of appearance of FFA was 0.46 ± 0.16 μmoles per fat body per hour with tissue from fasted rats, and 2.28 ± 1.1 μmoles per fat body per hour with tissue from rats injected with glucose.

The effect on the production of FFA when chyle was omitted from the incubation medium is shown in Table 2. One fat body from each animal was incubated with chyle and the contralateral body was incubated without chyle. In the absence of chyle the production of FFA was markedly reduced when the fat tissue was obtained from animals injected with glucose. On the other hand, with fat tissue from fasted animals, the production of FFA was unchanged. Thus it appeared that in fasted animals the FFA were derived from the fat tissue, whereas in glucose-injected animals the FFA were derived from the chyle triglycerides in the medium.

This was confirmed by determining the change in the esterified fatty acid content of the medium when fat tissue was incubated in the presence of chyle. When tissue from rats injected with glucose was used, the increase in FFA was accompanied by a fall in esterified fatty acid content of the medium, but with tissue from fasted rats, the esterified fatty acid content did not change during the incubation period (Fig. 1).

Also shown in Figure 1 are the changes in the concentrations of free and esterified fatty acids in the medium when clearing factor lipase, in the form of plasma obtained from rats injected with heparin, was incubated with chyle in albumin solution *in vitro*. As expected, the increase in FFA concentration was accompanied by a fall in the esterified fatty acid concentration.

TABLE 2. Effect of Chyle on the Production of FFA* When Epididymal Fat Bodies Are Incubated *in Vitro*

Fasted		Injected with Glucose	
With Chyle	Without Chyle	With Chyle	Without Chyle
0.47	0.53	1.32	0.35
0.57	0.56	2.00	0.10
0.70	0.95	1.46	0.20
0.50	0.49	1.36	0.18

* μmoles/fat body/hr.

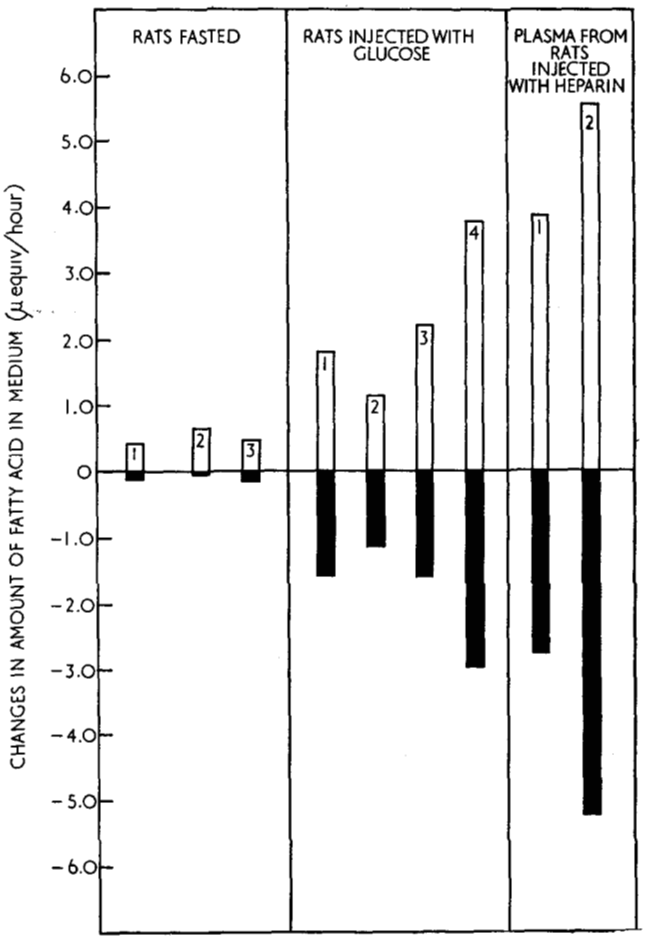

Fig. 1. Changes in the esterified (■) and FFA (□) content of the incubation medium when (*a*) epididymal fat bodies from fasted rats, (*b*) epididymal fat bodies from rats fed and injected with glucose, and (*c*) plasma from rats injected with heparin were incubated with chyle and albumin *in vitro*. Conditions of incubation as described in the Methods.

The Effect of pH on Free Fatty Acid Production. The results set out in Figure 2 show that when fat bodies from rats injected with glucose were incubated with chyle and albumin, the production of FFA at pH 6.0 was much lower than at pH 8.1. When fat bodies from fasted rats were used, the production of FFA was only slightly reduced at pH 6.0.

The Effect of Inhibitors of the Clearing Factor Lipase on Free Fatty Acid Production. In the presence of protamine sulfate (5 mg. per ml.), sodium pyrophosphate (0.01 M), and sodium chloride solutions (M), the clearing factor lipase in either heparinized plasma or in extracts of adipose tissue is inhibited (8 to 11). These substances also considerably reduce the production of FFA when epididymal fat tissue from rats that have been injected with glucose is incubated in the presence of chyle and albumin (Fig. 3).

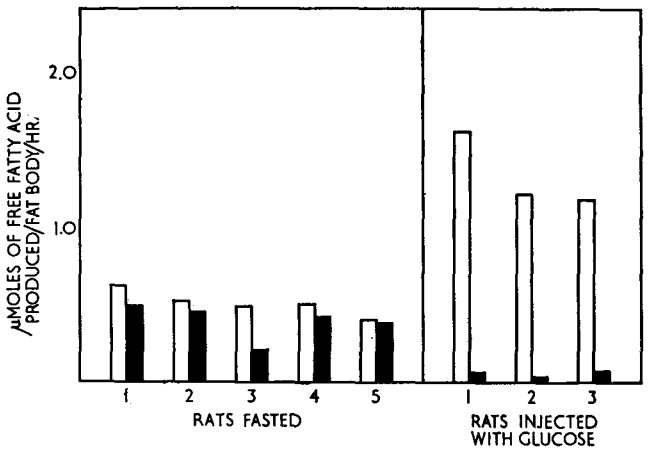

FIG. 2. The production of FFA in the medium when epididymal fat bodies from rats either (*a*) fasted or (*b*) fed and injected with glucose were incubated with chyle and albumin *in vitro* at pH 8.1 (□) and at pH 6.0 (■). Conditions of incubation as described in the Methods.

Their effect on the production of FFA when epididymal fat tissue from fasted rats was incubated under the same conditions was much less.

The Effect of Heparin on Free Fatty Acid Production. Clearing factor lipase appears in the blood following the intravenous injection of heparin (12), and heparin increases the clearing factor lipase activity in aqueous extracts of rat adipose tissue (13). Heparin appears to be an essential component of the complete enzyme system (14) and may be responsible for its stability (15).

The effect of heparin on the production of FFA when epididymal fat tissue from rats, either fasted or injected with glucose, was incubated in the presence of chyle and albumin is shown in Figure 4. In both cases the production of FFA was increased in the presence of heparin. However, the percentage increase was less with fat tissue from fasted fats; and, after 48 hours of fasting, the percentage increase was less than after 18 hours of fasting.

The Effect of Removal of the Fat Tissue from the Medium on Free Fatty Acid Production. Experiments were carried out to determine whether after a preliminary incubation of the fat tissue, FFA continued to be formed in the incubation medium when the fat tissue was removed. The results are shown in Table 3.

When the fat tissue was obtained from rats injected with glucose, the rate of increase of the FFA content of the medium after removal of the fat tissue was similar to the rate of increase in the presence of the tissue. If heparin was present in the medium, the rate of formation of FFA was greater initially and was maintained at a higher level when the fat tissue was removed.

When the fat tissue was obtained from fasted rats, FFA continued to be formed in the medium after removal of the tissue, but the rate of formation was reduced. Heparin had little effect on the rate of formation of FFA in these circumstances.

DISCUSSION

Activity of Fat Tissue from Animals Injected with Glucose. The experiments reported here show that when intact epididymal fat bodies from rats injected with glucose are incubated with chyle *in vitro*, the FFA formed in the incubation medium are derived from the chyle triglyceride by the action of an enzyme closely resembling the clearing factor lipase. Thus production of FFA is high at pH 8.1 but low at pH 6.0; and production is reduced in the presence of protamine sulfate, sodium pyrophosphate, and high concentrations of sodium chloride. Heparin, which increases the

TABLE 3. RATE OF APPEARANCE OF FFA IN THE MEDIUM FOLLOWING THE REMOVAL OF EPIDIDYMAL FAT TISSUE*

Fat Body Present (1st incubation period)			Fat Body Absent (2d incubation period)	
−Heparin	+Heparin		−Heparin	+Heparin
Glucose Injected				
2.5	6.0		1.82	6.85
4.5	7.8		4.8	8.1
Fasted 48 Hours				
0.70	0.75		0.38	0.43
0.47	0.60		0.37	0.20

* Rate of appearance expressed as μmoles/fat body/hr.

The fat tissue was incubated in either 6 ml. (glucose-injected rats) or 4 ml. (fasted rats) of a bovine albumin solution (100 mg./ml.) containing 7 μmoles of esterfied fatty acids per ml. as chyle. Heparin, if present, was at a final concentration of 0.25 units/ml. Samples of the medium (1 ml.) were taken (*a*) immediately after the introduction of the fat tissue, (*b*) following its removal 90 minutes later, and (*c*) after incubation of the medium for a further 90 minutes.

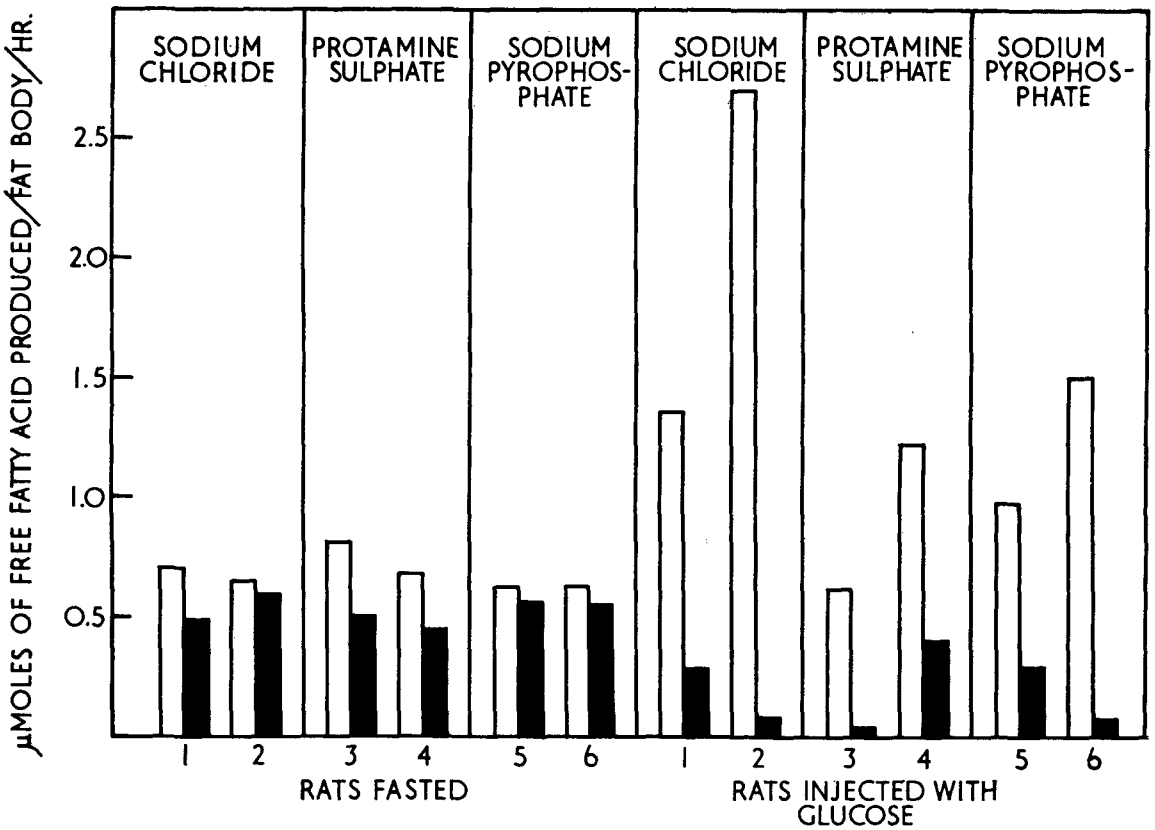

FIG. 3. The effect of various inhibitors on the appearance of FFA in the medium when epididymal fat bodies from rats either (*a*) fasted or (*b*) fed and injected with glucose were incubated with chyle and albumin *in vitro*. The final concentrations of the inhibitors were: sodium chloride, M; protamine sulfate, 5 mg./ml.; sodium pyrophosphate, 0.01 M. Production of FFA without inhibitor present (□); production of FFA with inhibitor present (■). Conditions of incubation as described in the Methods.

activity of the clearing factor lipase in extracts of adipose tissue, also increases the amount of FFA formed in these experiments.

Lipase is released from the tissue into the medium, both in the absence and presence of heparin, though the activity released is greatest when heparin is present. It seems probable that both the ability of heparin to release the clearing factor lipase from the tissues (16) and the affinity of the enzyme for its substrate (17) are factors controlling the appearance of enzyme activity in the medium.

These observations confirm and extend those reported recently by Hollenberg (1) and by Cherkes and Gordon (2). Hollenberg has shown that hydrolysis of the triglyceride component of a coconut oil emulsion by rat epididymal adipose tissue occurs *in vitro* when the fat tissue is obtained from rats fed their normal diet. He showed that heparin increased the lipolytic activity of the tissue and that lipase was released into the incubation medium. The activating effect of heparin could also be demonstrated with tissue from fasted rats, but only if glucose and insulin were present in the incubation medium. Hollenberg identified the lipase as the clearing factor lipase on the basis of the activating effect of heparin. Cherkes and Gordon (2), using the same fat tissue and a similar triglyceride substrate, demonstrated the ability of heparin to liberate lipase *in vitro* when the tissue was obtained from rats fed carbohydrate. They identified the lipase as the clearing factor lipase by the inhibitory effect of protamine sulfate and M sodium chloride solutions.

The demonstration of the activity of the clearing factor lipase in the adipose tissue of animals fed their normal diet or given carbohydrate in excess of their immediate nutritional requirements raises the question of the role of the clearing factor lipase in such circumstances. Elsewhere it has been suggested that the enzyme may be present normally in association with the walls of the blood vessels (18, 19) and that it may

act at this site to facilitate the passage of triglycerides from the blood to the tissues (16). It seems reasonable to suggest, therefore, that its activity in adipose tissue, under conditions that favor the deposition of triglyceride in the fat depots, may be concerned with the transport of triglyceride into this tissue.

Activity of Fat Tissue from Fasted Animals. During fasting the concentration of FFA in the blood rises (6, 20, 21, 22); and when epididymal fat tissue from fasted rats is incubated in an albumin medium *in vitro* in the absence of triglyceride substrate, FFA are released into the medium (7, 23). It has also been shown that when fat is mobilized following the injection of epinephrine, the proportion of mono- and diglycerides in adipose tissue is increased (24).

These observations have led to the view that under conditions in which the depot fat is mobilized, as, for example, during fasting, the depot triglyceride is hydrolyzed *in situ* and the FFA produced are carried in the blood from the depots to the tissues where they are utilized (25). In the present investigation, and in that of Hollenberg (1), however, triglycerides in the incubation medium were not hydrolyzed by epididymal fat tissue from fasted rats. Free fatty acids did appear in the medium in the present study, at a slow rate similar to that observed by Gordon and Cherkes (7) and by Reshef *et al.* (23), but their appearance was uninfluenced by the presence of chyle and was only slightly reduced when inhibitors of the clearing factor lipase were present and when the pH was reduced from 8.0 to 6.0. The rate of appearance of FFA was increased in the presence of heparin, but this effect was reduced when the period of fasting was raised from 18 to 48 hours. Thus no evidence has been obtained for a role of the clearing factor lipase in the release of FFA by epididymal fat tissue from fasted rats. If lipolysis of the depot triglyceride does occur *in situ* during fasting, either it is due to a lipase that is distinct from the clearing factor lipase (26, 27) or, if the clearing factor lipase is responsible, then this enzyme is no longer demonstrable by methods which can detect it in intact adipose tissue obtained from rats that have received glucose.

Limitations of the Method. In conclusion, certain limitations of the present type of study must be mentioned. First, changes in the composition of the incubation medium only are detectable. Changes in the composition of the fat tissue, that may or may not be reflected in changes in the medium, may also occur. Again, only net changes in the FFA content of the medium are determined. Both uptake and release of FFA by the fat tissue may take place under certain

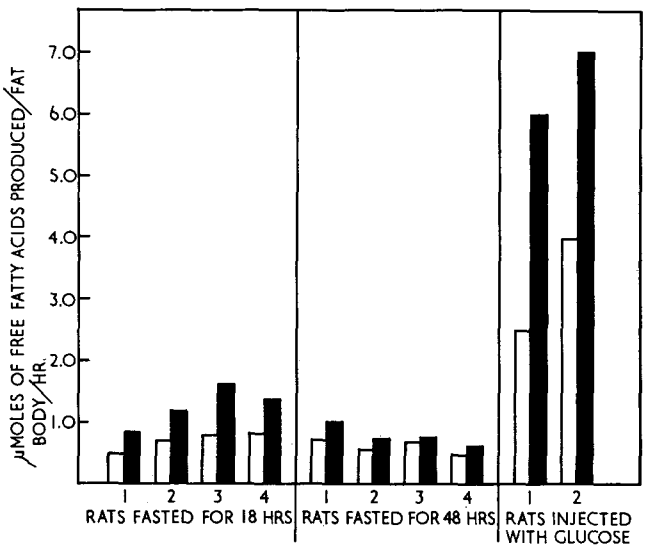

FIG. 4. The effect of heparin on the appearance of FFA in the medium when epididymal fat bodies from rats either (*a*) fasted or (*b*) fed and injected with glucose were incubated with chyle and albumin *in vitro*. A quantity of chyle containing 24 μmoles of esterfied fatty acid was added to 4 ml. of a solution containing 100 mg. of albumin/ml. (pH 8.1). Heparin was either absent (□) or present (■) at a final concentration of 0.25 units/ml. Samples (1 ml.) of the medium were taken immediately after the introduction of the fat tissue and after incubation for either 1 or 3 hours. The shorter period of incubation was used with fat bodies from the rats injected with glucose.

circumstances. Stern and Shapiro (28), for instance, using mesenteric fat tissue from rats starved for 5 days, reported an uptake of both triglyceride and FFA by the tissue *in vitro*.

Further, the experiments relate only to the two extreme nutritional states of complete fasting and of the intake of carbohydrate in excess. Some similar experiments were carried out on epididymal fat tissue obtained from rats fed their normal diet. The results suggested that hydrolysis of chylomicron triglyceride in the incubation medium occurred as a result of the action of the clearing factor lipase, but the extent of hydrolysis was less than that occurring with tissue obtained from animals injected with glucose, and the results were less clear cut. Glucose was not administered in the drinking water in this study because it was felt that the absorption of food might be thereby inhibited (29) and because the amount of glucose taken by an animal over a given time interval could not easily be controlled.

The present study only relates to intact epididymal fat tissue. Zemplényi and Grafnetter (30) have reported that when minced mesenteric fat is incubated with lipemic serum, the amount of FFA appearing in the medium is independent of whether the animals

from which the tissue was derived were fed their normal diet or were fasted for 60 hours.

Finally, it is evident that results obtained with systems similar to that studied here, in which the tissue is exposed only on its external surface to substances in the incubation medium, cannot be related directly to the situation in the intact animal. Adipose tissue *in vivo* has a rich blood supply, and the surface available for interaction and interchange between substances present in the blood and components in the tissue is very much greater than that in the system *in vitro*. For the same reason, neither triglycerides nor FFA are likely to accumulate *in vivo* in the blood in the concentrations studied here. The effect of such concentrations on the tissue and its metabolism is unknown.

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