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THE MECHANISM OF FUNCTIONAL VASODILATATION IN RABBIT EPIGASTRIC ADIPOSE TISSUE

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SUMMARY

1. The effect of close-arterial infusions of fat-mobilizing substances has been examined on the release of free fatty acids and blood flow in the epigastric adipose tissue of rabbits.

2. All the fat mobilizers in addition to causing the release of free fatty acids also caused an increased blood flow in the fat tissue.

3. Both the fat mobilization and the vasodilatation continued for an hour or so after the end of infusion.

4. Although no vasodilator substance could be detected in the venous effluent from the activated adipose tissue, a vasodilator could be detected in acid–ether extracts of adipose tissue excised during a period of fat mobilization.

5. It is suggested that a vasodilator substance is released or formed in adipose tissue during fat mobilization and that this substance accounts for the vasodilatation accompanying activity in the tissue.

INTRODUCTION

The intimate relationship between fat cells and capillaries has been a point of interest ever since investigators have examined adipose tissue. Their concurrent development was first described by Flemming (1876, 1879). As the fat cells develop, take up and store fat globules, the conspicuous capillaries become less apparent than they are in less developed or depleted fat depots. A relationship between blood flow and activity was indicated as early as 1895 when Hammar observed that in obese individuals the fat is much lighter in colour than in starved animals. He attributed part of this coloration to increased blood content and, in fact, observed histologically that in starved individuals in which there would presumably be a high rate of fat mobilization, the tissue was rich in blood supply and the vessels appeared to be dilated.

Gersh & Still (1945) estimated the surface area of the capillaries in

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adipose tissue by microscopy and found that the area per unit volume of tissue from lean animals was about four times that estimated for well developed fat tissue. They also showed that the colour of the fat reflected this difference. More recently, Stoner & Matthews (1966) injected rats with radioactively labelled red cells and showed that the amount of blood in fat tissue of fasting rats was over twice that in the fat of fed animals.

Ngai, Rosell & Wallenberg (1966) examined the nervous control of blood flow through subcutaneous adipose tissue of dogs and found that, although during sympathetic nerve stimulation the blood vessels were constricted, after a period of stimulation there was a release of free fatty acids (FFA) which was sometimes accompanied by a vasodilatation.

Measuring blood flow directly in the epigastric adipose tissue of rabbits, Lewis & Matthews (1968*a*, *b*) found that in some experiments there was an increase of blood flow during FFA release, although it was not a consistent finding. The reason for this was probably due to the fact that after injection of large doses of mobilizing agent, there was usually a fall in blood pressure, thus lowering perfusion pressure.

In the present experiments, several fat-mobilizing agents have been administered by slow close-arterial infusion to the rabbit epigastric fat tissue. It has been observed that all these substances increase blood flow not only during the infusion but for the considerable time after the end of the infusion during which fat mobilization continued to take place. Furthermore, we have shown that the vasodilatation which accompanies fat mobilization is probably brought about by a vasodilator substance produced in the tissue during activation.

METHODS

New Zealand white rabbits, 3·0–5·5 kg, were anaesthetized with urethane (25% urethane in saline 6 ml./kg body wt.) intravenously. The trachea and external jugular vein were cannulated and blood pressure was measured by a Statham strain gauge through a cannula in the carotid artery.

The right epigastric adipose tissue was separated from the surrounding tissues. The femoral blood vessels were prepared so that venous outflow could be recorded and retrograde arterial injections and infusions made as described previously (Lewis & Matthews, 1968*a*). The blood was returned to the animal through a cannula in the contralateral femoral vein. When recording venous outflow, polyethylene tubing, PP 100, was used initially but later PP 220 was used as this was found to offer a lower resistance to flow.

Fatty acid estimation. Blood samples were collected from the epigastric vessels into glass tubes containing a little solid heparin and kept in ice until required. FFA was estimated colorimetrically by Duncombe's method (1964) on 0·25 ml. plasma, using 3·0 ml. copper reagent and 10 ml. chloroform.

Materials. The following substances were used: ACTH (Synacthen, CIBA); β-MSH (melanophore-stimulating hormone, CIBA); PGH (porcine growth hormone); somatotropin (STH, Sigma); glucagon (Eli Lilly); LTH (luteotropic hormone,

Sigma); noradrenaline hydrogen tartrate; bovine serum albumin (fraction V, Armour); rabbit serum albumin (fraction V, Koch-Light).

Extraction procedures. Extraction procedures of blood samples and adipose tissue are based on that described by Samuelsson (1963) for prostaglandins. Blood samples were collected as described for FFA estimation. The plasma was separated by centrifugation and stored in the deep freeze until required. For extraction, the plasma was adjusted to pH 3·0, and 2 volumes of ethyl alcohol added. This solution was extracted three times with an equal volume of petroleum ether. The alcohol was removed from the aqueous phase by means of a rotary evaporator, and the remaining solution extracted three times with an equal volume of diethyl ether. The pooled ether extracts were dried by evaporation and the residue dissolved in saline. Samples of perfusate were also extracted by the same procedure.

In a few experiments whole blood was collected in cold ethyl alcohol, which was adjusted to 80 % (v/v) before proceeding with the extraction.

Adipose tissue was extracted by first homogenizing in ice-cold ethanol (5 ml./g) using an Ultra-Turrax homogenizer. After centrifugation at 3000 rev/min for 15 min the residue was re-suspended in 95 % alcohol and centrifuged again. The combined supernatants were dried below 40° C using a rotary evaporator. The residue was then taken up in water acidified to pH 2 and extracted with diethyl ether 3×2 volumes. The ether phases were then combined and washed with water until the pH was neutral. In some experiments the tissue was homogenized in Krebs bicarbonate solution (3:1), and a preliminary extraction with petroleum ether was carried out. The diethyl ether phases were dried and traces of moisture removed using a cold-finger rotary evaporator.

Albumin-FFA complexes. Plasma lipids were extracted by the method of Folch, Lees & Sloane Stanley (1957). The dry residue from up to 10 ml. plasma was heated with a slight excess of 0·1 N sodium hydroxide. The volume was made up to 0·5 ml. by the addition of 0·9 % NaCl (w/v), and then 2·0 ml. 25 % serum albumin in 0·9 % NaCl at pH 7·4 was added to the warm solution. Bovine and rabbit albumins were used as purchased, and bovine albumin was also used after FFA had been removed by the methods of Garland, Newsholme & Randle (1962) and Campbell, Martucci & Green (1964).

RESULTS

Relationship between increased blood flow and FFA release

We have previously shown that close arterial injections of synthetic ACTH $^{1-24}$, MSH, PGH, glucagon and LTH to the epigastric fat pad of rabbits caused the mobilization of FFA (Lewis & Matthews, 1968*b*). However, although mobilization was sometimes associated with an increased rate of blood flow, clear-cut effects could not be distinguished in most experiments, partly due to influence of changes of blood pressure. In the present experiments we have found that the use of infusions instead of single injections gave much more consistent results and the effects of transient or gradual changes in blood pressure were not so evident.

The release of FFA following infusions was similar to that after injections reported in the earlier paper. The FFA concentration in the venous blood rose slowly to reach a maximum at the end of the infusion or shortly afterwards, and subsequently declined slowly. In the experiment of Fig. 1,

close-arterial infusion of synthetic ACTH (1 μg/min) produced a marked increase in the rate of FFA mobilization. The FFA concentration of the venous effluent blood from the fat pad rose from about 1·2 to 3·5 m-equiv/l.

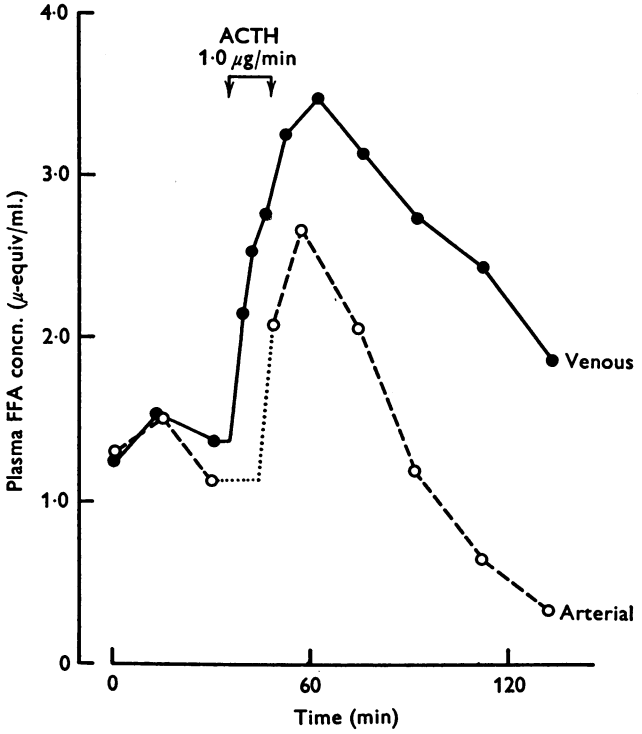

Fig. 1. Effect of infusion of ACTH 1 μg/min to the epigastric fat depot on arterial (O—O) and venous (●—●) FFA concentrations.

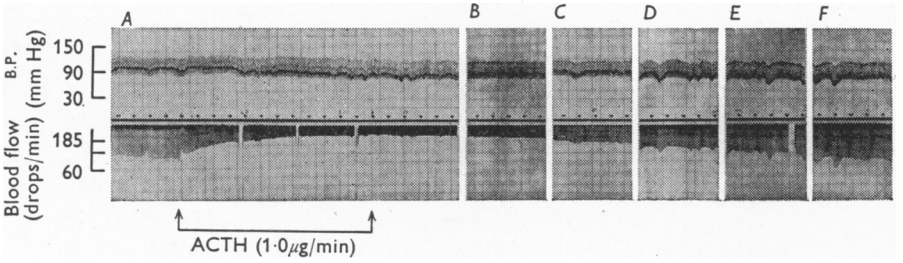

Fig. 2. Rabbit, ♀, 5 kg. The upper record is of arterial blood pressure, the lower record of venous outflow from the epigastric adipose tissue, in drops/min. The latter was measured with a Gaddum drop counter with the base at the top of the record. Between the arrows ACTH, 1 μg/min, was infused close-arterially to the epigastric fat pad. Time marks, 1 min; between each section, 10 min.

At the same time, the arterial concentration of FFA rose, indicating either a recirculation of the released FFA or a release from other fat depots as a result of the recirculation of the ACTH. An increase in the arterial FFA concentration was observed in nearly all experiments with ACTH, MSH and sometimes with glucagon, but not with growth hormone (GH).

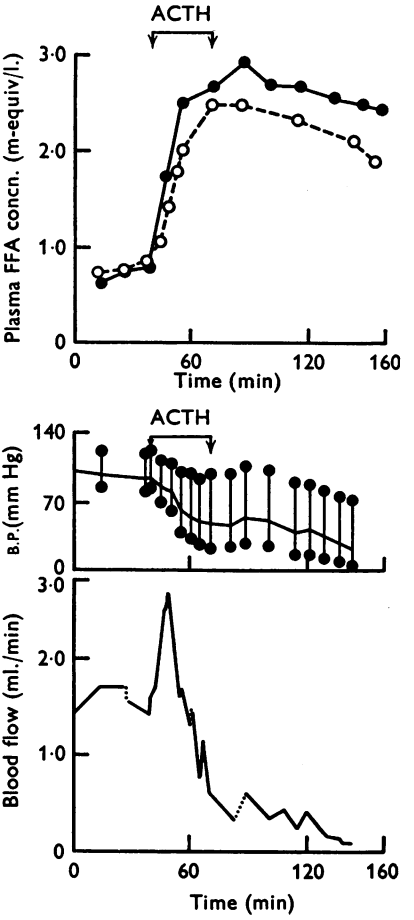

Fig. 3. Effect of ACTH, 3 μg/min (between arrows), infused close-arterially to the epigastric fat depot, on arterial and venous FFA concentrations (● venous, ○ arterial; upper record), on arterial blood pressure (middle record) and on venous outflow from the epigastric fat depot (lower record).

Fig. 2 is a record of the arterial blood pressure and venous outflow from the fat pad taken from the same experiment as Fig. 1. The blood flow through the adipose tissue, like the fat mobilization, increased soon after the beginning of the infusion, reached a maximum towards or just after

the end of the infusion and continued for an hour or more after the infusion had ceased.

In some experiments the infusion of the lipolytic substance caused a fall in blood pressure which was probably responsible for the consequent reduction in blood flow through the fat pad masking the vasodilatation. The effect is illustrated in the experiment of Fig. 3. In this experiment a higher concentration, 3 μg/min, was infused and this resulted in a marked increase in the arterial FFA concentration. Although mobilization was initially accompanied by an increase in blood flow, this increase was not sustained because the blood pressure fell to about 40 mm Hg during the infusion and continued to fall after the infusion had stopped.

TABLE 1. The effect of fat-mobilizing agents on FFA mobilization and blood flow. Initial values apply to the period immediately before the infusion started, and final values to the end of the infusion period. GH, growth hormone; Gluc, glucagon

Mobilizer (μg/min)	Pad weight (g)	Blood flow (ml./min)		Venous FFA (μ-equiv/ml.)		FFA output (μ-equiv/min)	
		Initial	Final	Initial	Final	Initial	Final
ACTH 0·1	28·3	0·45	1·80	0·58	2·36	−0·02	1·21
ACTH 0·1	32·8	3·20	3·60	1·70	3·14	0·38	2·21
ACTH 0·1	28·8	3·30	3·70	3·17	5·01	1·40	4·92
ACTH 0·1	10·6	0·64	1·48	0·49	1·63	−0·05	0·37
ACTH 1·0	8·3	0·40	1·38	0·63	0·83	0·07	0·41
ACTH 1·0	21·3	0·91	2·70	1·07	2·36	0·11	1·05
ACTH 1·0	38·7	1·60	4·75	1·37	2·76	0·24	1·93
ACTH 1·0	22·7	1·38	1·80	0·56	1·96	0·06	0·35
GH 5·0	8·9	0·42	0·72	0·61	2·15	0·06	0·81
GH 5·0	15·1	1·20	1·79	0·52	1·18	—	—
Gluc 5·0	19·2	0·58	1·51	0·75	1·79	0·04	0·29
Gluc 5·0	24·6	0·59	2·29	0·88	2·45	0·13	1·83

In a series of twenty experiments, nine showed persisting dilations like that of Fig. 2, whilst in six more an initial dilation passed off during the infusion. Narrow tubing was used for the venous cannulae in these experiments and frequently rapid FFA mobilization was followed by the appearance of numerous petechial haemorrhages throughout the pad, suggesting that it had become congested with blood. This effect was never seen when the wider tubing was used. Some experiments with the wider tubing are summarized in Table 1. They show that each infusion stimulated FFA release as indicated by the rise in the concentration in the blood leaving the tissue. P values of $< 0{\cdot}01$ were obtained by a paired t test in the experiments with ACTH 0·1 and 1·0 μg/min. In each case, as shown in Fig. 4, increased fat mobilization was accompanied by an increase in blood flow. A P value of $< 0{\cdot}05$ was obtained with infusion of ACTH 0·1 μg/min

but with ACTH 1·0 μg/min the increase in blood flow just failed the paired t test for statistical significance ($P < 0{\cdot}10$). This finding might mean that with infusion of higher doses of ACTH additional factors such as the formation of local oedema might come into play. The output values shown in Table 1 were calculated as the product of the FFA concentration difference between the venous and arterial plasma and the rate of plasma flow. The latter was derived from the rate of blood flow, assuming a haematocrit of 40 %.

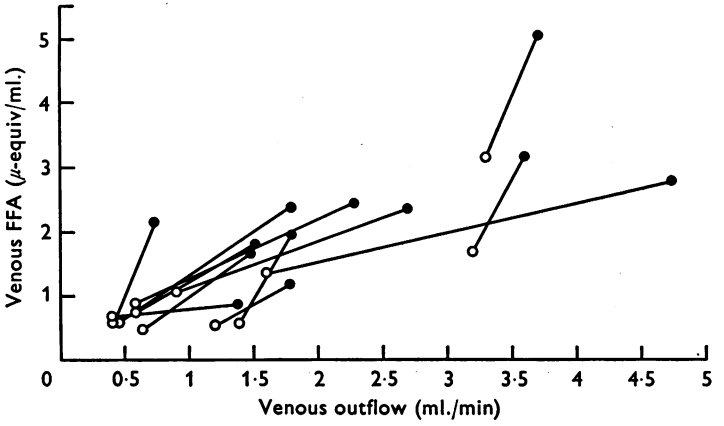

Fig. 4. The relationship between the blood flow and venous FFA concentration taken immediately before the infusion started (○) and at the end of the infusion of mobilizing substance (●). The values are taken from Table 1.

There is no statistically significant correlation between vasodilatation as measured by increase in venous outflow and FFA release as indicated by increase in the A–V difference of FFA. But as there are probably other factors involved in determining the blood flow through the adipose tissue this is not surprising. However, there is a positive correlation between the blood flow and the venous concentration of FFA before ($r = 0{\cdot}85$, $P < 0{\cdot}001$) and after ($r = 0{\cdot}67$, $P < 0{\cdot}02$) activation of the tissue.

In some experiments, particularly those in which growth hormone was used, there was no increase in the FFA of the arterial blood although there was a marked increase in venous blood FFA. The reason for this difference is not clear but has been discussed in the earlier paper. The experiment of Fig. 5 shows the FFA release together with the accompanying vasodilatation which occurs during and after infusion of porcine growth hormone, 5 μg/min, and glucagon, 4 μg/min. There was no increase in arterial FFA after growth hormone and only a slight increase after glucagon. In this experiment the venous concentration of FFA after the mobilizing was less

than in the experiments of Figs. 1 or 3, but even in experiments where growth hormone caused the venous FFA to exceed 3 m-equiv/l. there was still no rise in arterial FFA. It was also found in these experiments that the blood pressure did not fall to the same extent as that after infusion of ACTH.

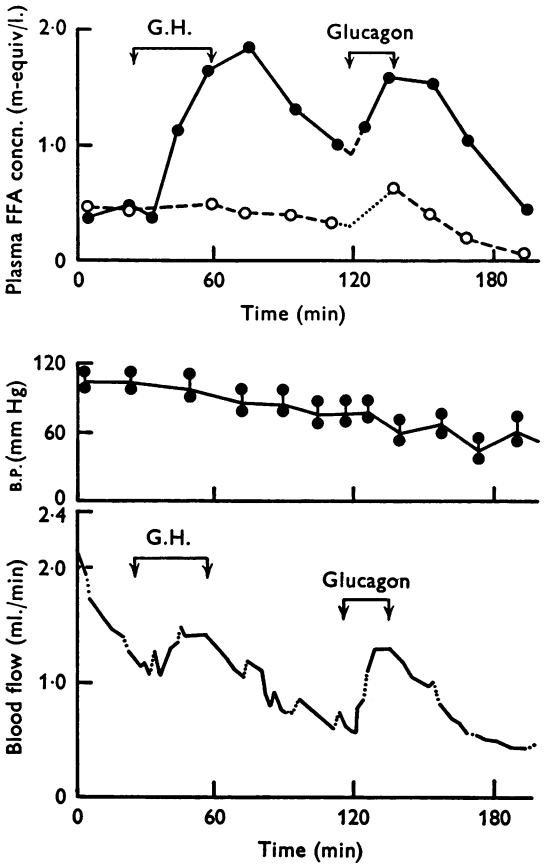

Fig. 5. Effect of close arterial infusions of porcine growth hormone (5 μg/min) and glucagon (4 μg/min) to the epigastric fat depot on arterial and venous FFA concentrations (● venous, ○ arterial; upper record), arterial blood pressure (middle record) and venous outflow (lower record).

The relationship between fat mobilization and blood flow is further emphasized by experiments with LTH and noradrenaline. Both substances reduce blood flow but only LTH stimulates FFA release from this fat depot (Lewis & Matthews, 1968*b*). When LTH was infused, there was first a reduction in flow but later, when FFA release had been stimulated, constriction gave way to a vasodilatation which persisted after the in-

fusion period as illustrated in Fig. 6. The vasodilatation was not a reactive hyperaemia in response to a period of vasoconstriction such as that produced by noradrenaline and illustrated in Fig. 7. In contrast, the catecholamine induced vasoconstriction throughout its infusion, after which there was only a transient increase in flow.

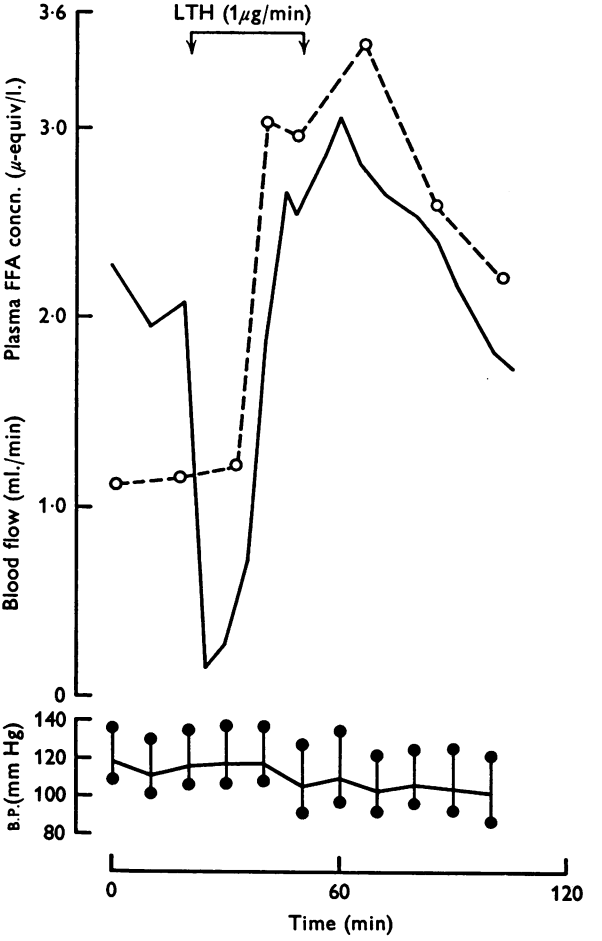

Fig. 6. Effect of close-arterial infusion of luteotropic hormone (LTH), 1 μg/min, on venous FFA concentration (O - - - O), venous outflow (——) and arterial blood pressure (lower record).

There was a small increase in venous FFA after the end of the infusion of noradrenaline in the experiment of Fig. 7. This might be the result of a small mobilization by the noradrenaline as described by Oro, Wallenberg & Rosell (1965) in dogs or alternatively it could be that the FFA accumula-

tion during the period of vasoconstriction was being washed out during the short-lasting vasodilatation which followed.

Evidence for the production of a vasodilator substance during fat mobilization

As the time course of FFA release and increased blood flow correspond, it seemed likely that the fat mobilization was accompanied by the formation or release of a vasodilator substance. It was therefore hoped that such

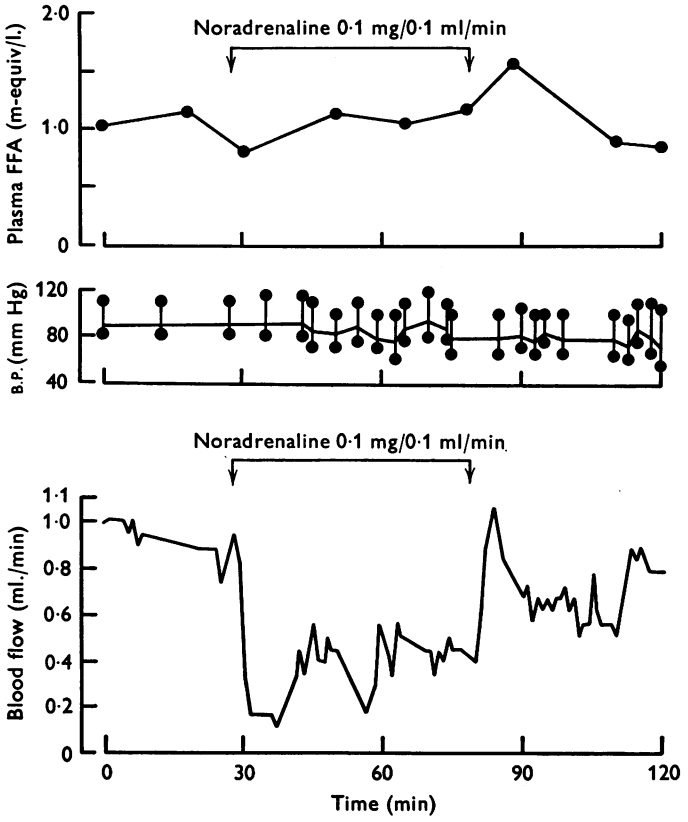

Fig. 7. Close-arterial injection of noradrenaline (0·1 μg/min) to the epigastric fat depot. Upper record is venous FFA concentration, middle record is arterial blood pressure and lower record is venous outflow.

a substance might be detected in the venous blood draining the activated fat pad. However, no substance could be detected in the effluent blood or perfusate from the pad, but extracts of the activated fat pad itself did contain a vasodilator substance.

Extracts of venous effluent. The first attempts to demonstrate the presence

of a vasodilator in the blood draining the pad were made by re-infusing whole blood. Venous blood was collected in a cold heparinized syringe either during or soon after an infusion of ACTH (1 μg/min), when mobilization was at its peak. The preparation was then left for about 1 hour, to allow the effects of stimulation to pass away, and the venous FFA concentration to fall. The syringe was warmed in water and the blood re-infused through the arterial cannula. Although the infusion raised the FFA concentration in the venous blood considerably, there was little or no change in blood flow. Only two experiments of this type were performed satisfactorily because stored blood usually developed powerful vasoconstrictor activity.

In order to test whether the vasodilatation observed during fat mobilization was due to the high plasma concentration of FFA or other lipids, experiments were carried out using the total lipid fraction of plasma. The FFA present were made soluble by conversion into neutralized complexes with albumin. The amounts of FFA infused arterially in these albumin complexes were sufficient to raise the FFA concentration in the venous outflow. However, no consistent effects on blood flow were obtained, nor could the effects of arterial plasma extracts be distinguished from those of venous plasma obtained at the height of fat mobilization. The main difficulty was that albumin solutions were themselves vasoactive. In addition, acid–ether extracts of venous and arterial plasma and also of whole blood collected in alcohol were injected close-arterially but no vasodilator activity was found.

Perfusion of the fat pad with either Locke solution or 5% bovine albumin in Krebs bicarbonate saline also gave inconclusive results. Acid–ether extracts of perfusates obtained before and after infusing ACTH to stimulate FFA mobilization were tested for vasodilator activity by injecting into the artery supplying a fat pad, and for smooth muscle-stimulating activity on the guinea-pig ileum and rat uterus. No activity was found with Locke solution and no conclusions could be reached when albumin was used because the albumin itself interfered with the bio-assays.

Extracts of fat pads. The epigastric fat pad was stimulated by close arterial infusion of ACTH (1 μg/min) for 15 min. At this time both the activated and the contralateral resting pads were excised. When the ether extract of the activated fat pad was injected close-arterially into a fat pad there followed a pronounced and prolonged vasodilatation. On the other hand, similar extracts from the contralateral resting fat pad produced only a smaller vasodilatation which was short-lived compared to that from the stimulated pad. A typical experiment is illustrated in Fig. 8. In nine experiments extracts of stimulated fat pads contained a strong vasodilator activity, while in five of these in which extracts of the resting

fat pads were also examined, they were found to contain a smaller vasodilator activity like that shown in Fig. 8.

It seemed, therefore, that when fat mobilization was stimulated, the activated fat pad contained an acid–ether soluble vasodilator substance which might be responsible for the accompanying functional vasodilatation.

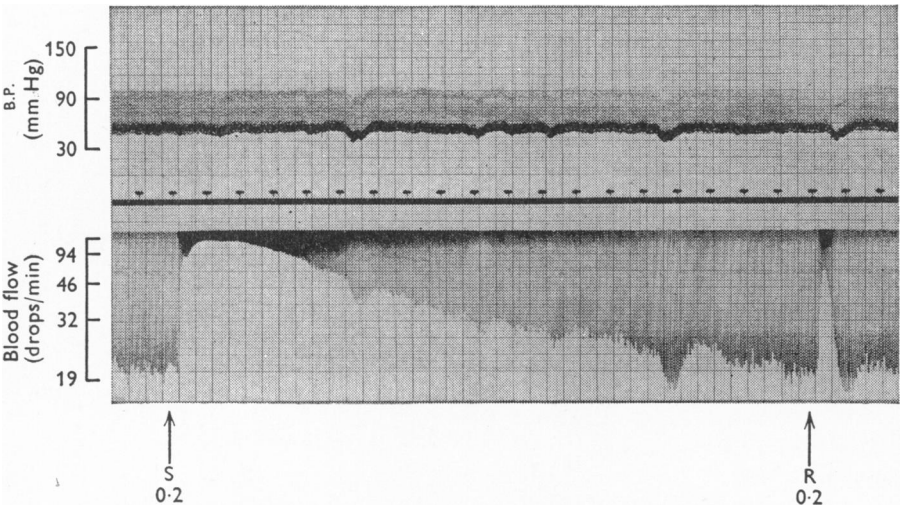

Fig. 8. Rabbit, ♀, 5 kg. The upper record is arterial blood pressure and the lower record venous outflow from the epigastric fat depot. At the first arrow injection of 0·2 ml. of extract of a stimulated fat pad, S, and at the second arrow injection of 0·2 ml. of extract of the contralateral resting fat pad, R. Time marks 1 min.

DISCUSSION

The present finding that there is an increased blood flow in adipose tissue during active lipolysis is in agreement with several other current studies. Herd, Goodman & Grose (1968) estimated blood flow in five different fat depots in rats, using [^{3}H]DDT as the indicator, and showed a correlation between blood flow and lipolysis. The highest blood flows were found in brown fat and the lowest in epigastric and epididymal fat. The values of 10–27 ml./100 g.min which they found in rats are in the higher range of the blood flows of 1·37–22·0 ml./100 g.min we found earlier in rabbits (Lewis & Matthews, 1968*b*). On the other hand, Nielsen, Bitsch, Larsen, Lassen & Quaade (1968) found lower rates of flow in human subcutaneous adipose tissue 2–7 ml./100 g.min using locally injected ^{133}Xe. They found that infusions of noradrenaline as well as injections of glucagon increased blood flow in addition to increasing FFA release. Further, they showed that 4 days of fasting led to increases in both blood flow and blood

FFA by about 100 %. These authors also observed that the vasodilatation in adipose tissue was maintained during the period of active lipolysis. This similarity in the time course of FFA release and vasodilatation was particularly well illustrated in the present experiments in rabbits and highlights the close association of the two effects.

In our earlier experiments and in those reported here we have found that catecholamines do not cause a release of FFA from the epigastric adipose tissue of rabbits. It was not surprising, therefore, that we did not achieve FFA release or vasodilatation, but only vasoconstriction when we stimulated either the perivascular tissue leading to the epigastric fat pad or the lumbar sympathetic chain. In dogs, however, Oro *et al.* (1965) found that during a 10 min period of epigastric nerve stimulation, there was a strong constriction with no FFA release but that during the 20–30 min which followed there was a release of FFA accompanied by an increased blood flow. Ngai *et al.* (1966) and Rosell (1966) concluded that the vasoconstrictor nerves are sympathetic in origin and adrenergic in nature and are similar to those innervating the blood vessels of skeletal muscle and skin. They observed, however, that the vasodilator response was not brought about by activation of the sympathetic cholinergic vasodilator outflow in the hypothalamus, thus excluding the presence of cholinergic vasodilator fibres.

The present finding that a vasodilator substance is produced or released in adipose tissue during active lipolysis provides an alternative explanation. Although activation of the adipose tissue was not brought about by nerve stimulation in the present experiments, it might well be that regulation of FFA release is under the control mainly of humoral factors and not the sympathetic nervous system.

Wirsén (1965) has shown with fluorescence microscopy that, although there are numerous sympathetic fibres around the blood vessels in white adipose tissue, the majority of fat cells are, however, apparently not in contact with terminals containing noradrenaline. Nor is there any correlation between the innervation pattern in white adipose tissue of different species and their responsiveness to noradrenaline as shown by Carlson, Liljedahl, Verdy & Wirsén (1964). Wirsén suggests that in responsive species noradrenaline may be one of the humoral factors regulating FFA release and the importance of local adrenergic innervation as a specific means of stimulating FFA release is doubtful.

All species, however, seem to depend on adrenergic mechanisms for stimulation of lipolysis in brown adipose tissue. Although Rudman, Brown & Malkin (1963) showed that white adipose tissue from rabbits did not respond to catecholamines, Dawkins & Hull (1964) demonstrated the release of glycerol and FFA from slices of brown interscapular adipose

tissue of rabbits. Brown fat is metabolically very active and most of the FFA produced by lipolysis is oxidized within the tissue. The adrenergic terminals in the brown fat therefore appear to have a special function not related to the mobilization of FFA into plasma but to the production of heat.

Adipose tissue of the rabbit, guinea-pig, pig, fowl and sheep (Rudman *et al.* 1963; Wirsén, 1965; Astwood, 1965; Carlson *et al.* 1964; Lewis & Matthews, 1968*a*) are unresponsive to catecholamines and it therefore seems unlikely that the sympathetic nervous system plays a significant role in the mobilization of FFA in these tissues.

It seems possible that there may exist two mechanisms of FFA release. The first, mediated by the sympathetic nervous system, would mobilize FFA rapidly and act for a comparatively short time, in response to a sudden demand for energy. Secondly, a humoral control of FFA release mediated by hormones from the anterior pituitary gland would provide a prolonged release of FFA and may be utilized when there is a sustained demand.

The relative contributions of these two mechanisms appear to differ in various fat depots. The present experiments show that in the rabbit the former is not important in subcutaneous adipose tissue. But the previous finding that intravenous noradrenaline causes a rise in circulating FFA suggests that it might be present in other depots.

The present experiments have shown that when rabbit adipose tissue is activated by humoral agents, there is an increased blood flow in the tissue brought about by a vasodilator which is produced locally. It appears that both effects, FFA release and vasodilatation, are consequences of the action of the fat-mobilizing hormones. It is not yet clear whether the products of FFA release are responsible for causing the vasodilatation or if the two effects are brought about by different mechanisms simultaneously activated by the hormones. Vasodilatation *per se* does not give rise to the FFA release. But with this local regulatory system, the tissue is assured of a blood supply necessary to cope with its functional needs. In particular, the supply of carrier albumin necessary to transport the newly formed FFA away from the tissue is increased.

The fall in blood pressure observed in many experiments could be the result of a leakage of this vasodilator substance into the general circulation. However, since we could not show the presence of the vasodilator in effluent blood from the fat pad, this explanation seems unlikely. Alternatively, if the fat mobilizer recirculates to cause fat mobilization in many fat depots about the body, the consequent vasodilatation may lower peripheral resistance sufficiently to cause a fall in blood pressure. However, there is not always a linear relationship between the fall in blood

pressure and reduced blood flow during the experiment, and other effects brought about either by the vasodilator itself or by the excess fatty acids liberated might be involved. Woods & Kellner (1964) have shown not only that prolonged infusions of ACTH or extracts of pituitary gland are lethal to rabbits but that obese animals are more susceptible than non-obese animals. They attributed the effect to the inability of the animal to dispose of the excessive amounts of FFA mobilized from the fat depots. However, the findings with growth hormone are not consistent with this view. Although growth hormone does not cause a rise in arterial FFA, probably due to its action in increasing FFA uptake and subsequent oxidation in tissues (Rabinowitz & Zierler, 1962; Goodman & Bressler, 1967), nevertheless, it still caused the blood pressure to fall.

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