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The mobilization of free fatty acids from rabbit adipose tissue *in situ*

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1. The epigastric adipose tissue of rabbits has been prepared so that the effects of close arterial injections and infusions on blood flow and release of free fatty acids (FFA) can be studied. The effects of pharmacologically active agents and hormone preparations have been investigated.

2. Release of FFA was stimulated by synthetic adrenocorticotrophic hormone (ACTH), α and β melanophore stimulating hormone (MSH), porcine growth hormone, glucagon, thyrotropic hormone (TSH) and luteotropic hormone (LTH). Single injections of fat-mobilizing agents produce a sustained rise in the release of FFA.

3. Although pitressin caused release of FFA, synthetic vasopressin and oxytocin failed to do so. The FFA releasing activity of pitressin has therefore been attributed to a contaminant.

4. Catecholamines were found not to stimulate release of FFA from this fat depot, but were found to increase plasma FFA when infused intravenously.

5. Injections of acetylcholine, histamine, bradykinin, 5-hydroxytryptamine, synthetic arginine vasopressin, and lysine vasopressin, oxytocin, angiotensin and FSH did not stimulate release of FFA although marked effects on blood flow were produced.

6. Injections of prostaglandin E_1 gave sustained increases in blood flow, and inhibited FFA release when stimulated by growth hormone.

7. The mobilization of FFA is sometimes associated with an increased rate of blood flow.

The mobilization of free fatty acids (FFA) from adipose tissue has been extensively studied in recent years and a number of substances are known to stimulate the process. In the main, fat mobilizing activity has been assessed by the ability of a substance to stimulate FFA release from slices of adipose tissue incubated *in vitro* or to increase the FFA concentration in the circulating blood. While these techniques emphasize the importance of lipolysis in fat mobilization, neither takes account of the role of the blood stream in transporting the FFA produced away from the fat depot. The importance of the transport function of the blood stream is suggested by the observation that the blood content of adipose tissue is increased during fasting when increased fat mobilization occurs (Stoner & Matthews, 1966).

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The present investigation with adipose tissue *in situ* was undertaken to study further the relationship between blood flow and lipolysis. Blood flow through the epigastric adipose tissue of dogs has been measured by Oro, Wallenberg & Rosell (1965). This tissue is usually well developed in adult rabbits and was selected for these experiments.

Methods

New Zealand White rabbits, mainly females, 3.0 to 5.5 kg were anaesthetized with urethane (6 ml./kg of 25% urethane in saline, 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 and retrograde arterial injections were made as described previously (Lewis & Matthews, 1968).

In some experiments, the fat pad was not isolated and changes in the plasma FFA concentration were estimated on samples drawn from a cannula placed in the femoral artery. Injections and infusions were made through a cannula in the femoral vein.

Free fatty acid estimation

Blood samples, approximately 1 ml., were collected in cold 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. of plasma using 3.0 ml. of copper reagent and 10 ml. chloroform.

Plasma lipase activity

Plasma lipase activity was estimated by modification of the method of Boberg & Carlson (1964). Samples of 2–3 ml. of arterial blood were collected as described for FFA estimation, or in tubes calibrated at 2.0 ml. capacity containing 0.2 ml. 0.1M sodium citrate. In order to prevent blood clotting in the cannula it was filled with 1% heparin or 0.1M sodium citrate (0.125 ml.) after each sample was taken. Three volumes of assay mixture containing " Intralipid " as substrate were used to one of plasma in most experiments, though in others the plasma was first diluted 1 : 1 with the ammonia buffer.

The FFA concentration of the complete assay mixture was estimated by Duncombe's method on 0.5 ml. samples taken from the incubation mixture after 15, 30 and 45 min. The rate of FFA production was estimated from the slope of the graph drawn through these three points.

Materials

The following substances were used: ACTH (synacthen, CIBA); α MSH and β MSH (melanophore stimulating hormone, CIBA); porcine growth hormone (somatotropin, STH, Sigma); glucagon (Eli Lilly); TSH (thyrotropic hormone, Sigma); LTH (luteotropic hormone, Sigma); FSH (follicle stimulating hormone, Sigma); Pitressin (Parke-Davis); synthetic arginine- and lysine-vasopressin (Sigma);

Syntocinon (Sandoz); synthetic oxytocin (kindly supplied by Dr. G. Bisset); bradykinin (Sigma); angiotensin (Hypertensin CIBA); prostaglandin E_1 (kindly supplied by Dr. Jane Shaw); acetylcholine (Light & Co.); theophylline (aminophyllin, Sigma); phentolamine (Rogitine, CIBA); hydrocortisone sodium succinate (Upjohn); heparin (Evans Medicals Ltd.); L-thyroxine (Na salt, grade II, Sigma); porcine thyroglobulin (type II, Sigma); Substance P (crude from hog intestine, Sigma); the concentrations of the following substances refer to the base: adrenaline hydrogen tartrate; noradrenaline hydrogen tartrate; isoprenaline sulphate; 5-hydroxytryptamine creatinine sulphate; histamine acid phosphate.

Results

Mobilization of free fatty acids

The effects of injections or infusions on lipolysis in the tissue were estimated from the FFA concentration in samples of arterial and venous blood taken at suitable intervals. An increase in lipolysis is indicated by a rise in FFA concentration of the blood leaving the pad.

The concentrations of FFA in the initial samples of arterial and venous blood were found to vary widely between different experiments. For arterial blood the range in sixty-nine experiments extended from 0.092 to 2.19 m-equiv/l. with a mean of 0.908 ± 0.118 (S.E.). The venous concentration was 1.061 ± 0.185 (S.E.) m-equiv/l. During the resting period after the onset of anaesthesia and before

TABLE 1. *Effect of injections of fat-mobilizing agents on the FFA concentration in arterial blood and blood draining the fat pad*

Agent	Dose (μg)	No. expts.	Venous conc. Initial	Max.	Diff.	Arterial conc. Init.	Max.	Diff.
ACTH	1	3	0·79	1·30	0·51	0·71	0·66	−0·05
	3	2	0·47	1·31	0·85	0·45	0·80	0·35
	10	8*	0·71	2·29	1·57	0·54	0·87	0·33
			±0·10	±0·19	±0·19	±0·14	±0·14	±0·10
β MSH	1	1	2·66	2·83	0·17	1·13	1·06	−0·07
	3	3	2·35	3·07	0·72	1·59	1·97	0·38
	10	2	1·56	3·43	1·92	0·72	1·50	0·78
α MSH	10	1	0·25	2·19	1·84	0·17	1·57	1·40
GH	1	1	1·59	1·71	0·12	2·42	2·30	−0·12
	3	2	1·58	1·70	0·12	1·06	1·20	0·04
	10	3	1·80	1·97	0·17	0·97	1·06	0·09
	100	6†	0·73	2·19	1·46	0·67	0·77	0·10
			±0·14	±0·39	±0·26	±0·11	±0·10	±0·15
Glucagon	50	3	0·88	1·53	0·65	0·56	0·80	0·24
	100	2	1·28	2·64	1·37	1·07	1·51	0·44
TSH	0·5U	1	1·33	2·09	0·76	0·73	0·58	−0·15
	1·0U	2	1·61	2·68	1·09	1·80	2·02	0·22
LTH	10	3	1·31	2·37	1·05	0·70	1·03	0·32

* Six values for arterial concentrations. † Five values for arterial concentrations. TSH dose—units of activity.
Initial samples were obtained just before the injection was given, maximum values were reached within 1 hr. Results are expressed as the mean of the number of experiments shown, with standard errors for the larger groups. FFA concentration in m-equiv/l. plasma.

injection of the pharmacologically active substances, there was no significant change in the mean concentrations of FFA in arterial and venous blood. In twenty experiments the mean venous concentration changed from 1.168 to 1.263 m-equiv/l. plasma ($P<0.5$) after 14–18 min while the arterial concentration changed from 1.004 to 0.943 m-equiv/l. ($P<0.6$).

Anterior pituitary hormones. In the present experiments, only preparations of anterior pituitary hormones and glucagon stimulated lipolysis (Table 1). In each case an increase in the venous FFA concentration was detected within 5 to 7 min of the injection. The concentration continued to rise in successive samples until a peak was reached 20 to 30 min after the injection. This was followed by a slow decline towards the unstimulated levels of FFA which took 1 to 2 hr. The most effective substance was synthetic adrenocorticotrophic hormone (ACTH), of which injections of 1 μg were usually effective while 10 μg gave large responses. Melanophore stimulating hormone (β-MSH) was effective in doses of 3–10 μg and in one experiment 10 μg α-MSH produced a similar response. Porcine growth hormone (GH) and the pancreatic hormone glucagon gave large responses although larger amounts (10–100 μg) were usually required. Preparations of thyroid-stimulating hormone (TSH) and luteotropic hormone (LTH) were also found to stimulate lipolysis. Of the anterior pituitary hormones tested, only follicle stimulating hormone (FSH) failed to stimulate lipolysis.

When an injection is followed by a very large increase in the FFA concentration of the venous blood, there is usually a smaller and slower rise in the FFA concentration of the arterial blood. This is commonly seen after larger doses of ACTH, MSH and glucagon (Table 1) (Fig. 1), and may be due to the hormone passing through the pad into the general circulation and stimulating other fat depots. When comparable increases in the venous concentration have been produced by GH, however, this arterial rise is either very small, or completely absent (Fig. 2, Table 1).

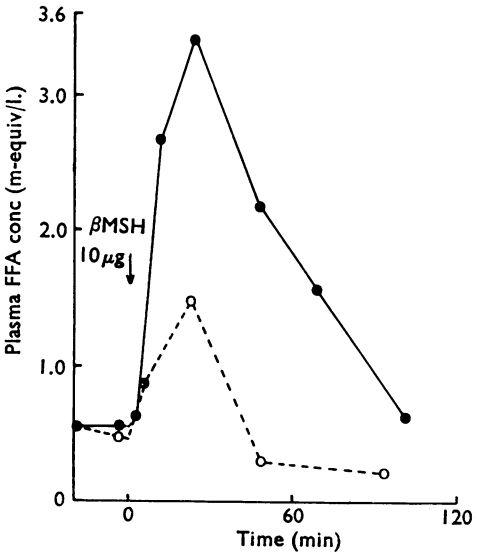

FIG. 1. Effect of β MSH 10 μg injected close arterially to the epigastric adipose tissue on the concentration of FFA in venous (●——●) and arterial (○- - -○) blood.

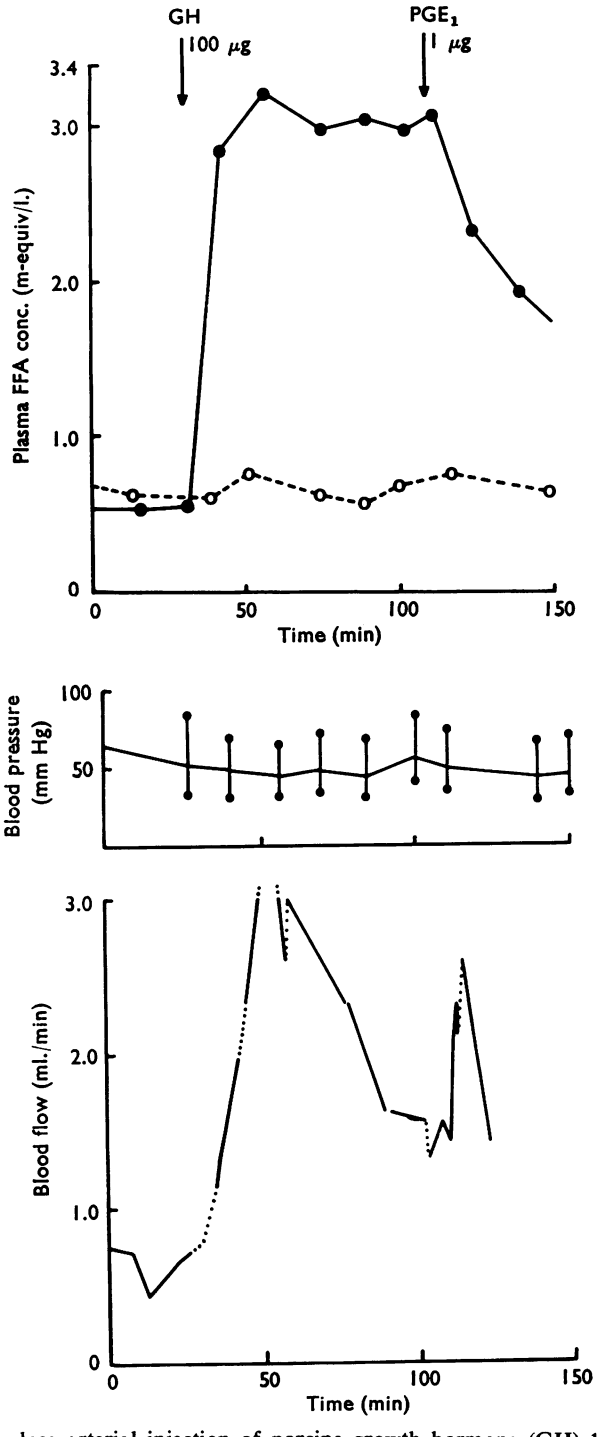

FIG. 2. Effect of close arterial injection of porcine growth hormone (GH) 100 μg and prostaglandin E_1 (PGE_1) 1 μg on the concentration of FFA in venous (●——●) and arterial (○- - -○) blood in the upper record, and on the blood flow through the epigastric adipose tissue in the lower record. Systolic and diastolic blood pressures are plotted in the middle record. The dotted regions of the blood flow plots represent periods where no record was obtained through interference with flow when blood samples were being taken.

Posterior pituitary hormones. Although preparations of vasopressin extracted from the posterior pituitary gland (Pitressin) were found to stimulate FFA release, synthetic arginine and lysine vasopressin in the same dose were ineffective (Table 2, Fig. 3). The fat-mobilizing activity of Pitressin could not be attributed to a small amount of oxytocin which might be present, since synthetic oxytocin was also found to be inactive.

Catecholamines. Although catecholamines are powerful mobilizers of FFA from the adipose tissue of other species, the results of the present *in vivo* experiments agree with the finding of Rudman, Brown & Malkin (1963) that isolated rabbit adipose tissue does not respond to adrenaline or noradrenaline.

Injections of up to 10 μg adrenaline and noradrenaline failed to increase the FFA concentration in the venous blood (Table 3). At this dose, these amines

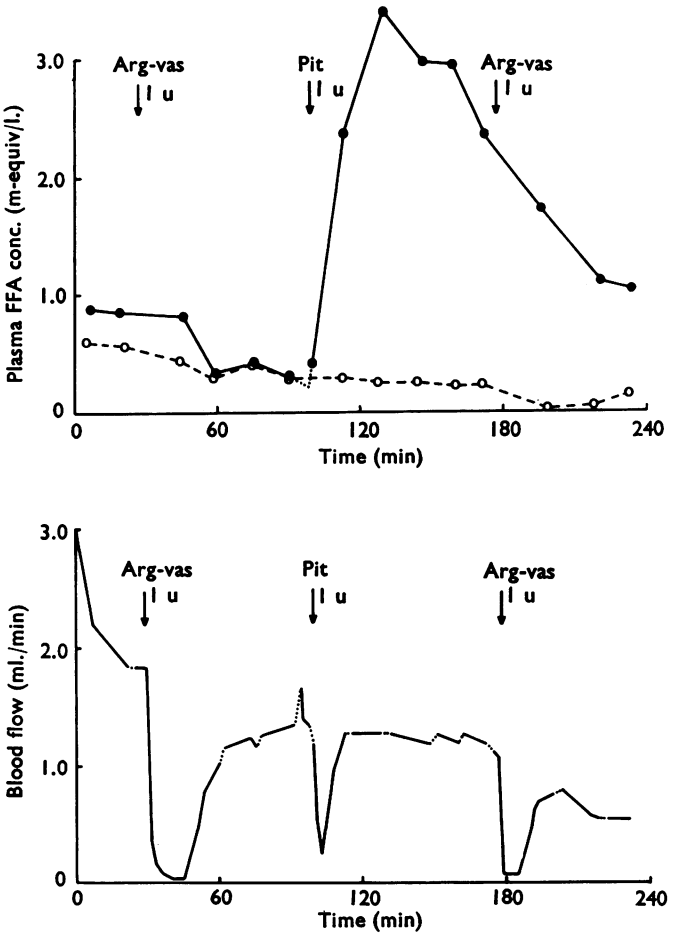

FIG. 3. Effect of close arterial injection of synthetic arginine vasopressin (Arg-vas) 1 unit and Pitressin (Pit) 1 unit on the FFA concentration of venous (●——●) and arterial (○- - -○) blood in the upper record and on blood flow through the epigastric adipose tissue in the lower record. The dotted regions of the blood flow plots represent periods where no record was obtained through interference with flow when blood samples were being taken.

produce a powerful vasoconstriction, but it does not seem likely that absence of a lipolytic action was due simply to the constriction preventing the amines reaching the adipose cells. Infusions of noradrenaline at rates which did not stop the flow completely did not stimulate FFA release. Neither adrenaline nor noradrenaline in 10 μg doses stimulated release when their vasoconstrictor effect had been blocked by 100 μg phentolamine. Finally isoprenaline, which usually dilated the vessels of the fat pad, was ineffective both when injected or infused.

In contrast to these results, Hagen & Hagen (1962) found that intravenous infusions of noradrenaline into unanaesthetized rabbits increased the concentration of FFA and glycerol in the arterial plasma. This finding has been confirmed in the

TABLE 2. *Effect of natural and synthetic vasopressin on the venous FFA concentration*

		Venous FFA			
Vasopressin	Dose units	Before response	During response I	During Response II	Increase
Natural grade I	0·1	0·63	0·63	0·57	−0·06
(Sigma)	0·3	1·78	1·61	1·14	−0·64
Natural pitressin	0·3	0·25	0·50	0·38	0·13
(Parke Davis)	0·3	1·08	1·24	2·07	0·99
	0·3	0·70	1·11	1·50	0·80
	1·0	0·29	2·36	3·39	3·10
	2·0	0·75	1·00	2·85	2·10
	2·0	1·21	1·74	2·28	1·07
Synthetic lysine	0·3	2·36	2·36	2·11	−0·25
Vasopressin	2·0	0·44	0·35	0·40	−0·04
Synthetic arginine	1·0	0·50	0·62	0·46	−0·04
Vasopressin	1·0	0·85	0·82	0·33	−0·52
	2·0	2·03	1·62	1·61	−0·42

The first sample (I) during the response was taken after blood flow had reached its minimum, and the second (II) before recovery was complete. Results are shown for individual tests. FFA concentration in m-equiv/l. plasma.

TABLE 3. *Effect of catecholamines on the venous FFA concentration*

			FFA concentration		
Substance	Dose (μg)	No. tests	Before response	During response I	During response II
Adrenaline	*0·03	1	1·08	1·27	1·70
	*0·1	1	1·29	0·55	1·10
	10	2	2·00	1·86	1·50
Noradrenaline	*0·03	2	1·30	1·83	1·73
	*0·1	3	1·36	0·72†	1·82†
	*0·3	2	0·53	0·61†	0·83
	1	1	0·77	0·77	0·67
	3	3	0·92	0·97	0·63
	10	4	1·34	1·31	1·25
Isoprenaline	*1	3	1·55	1·22	0·87
	1	1	1·53	1·47	1·33
	3	2	2·20	1·99	1·95
	10	3	1·58	1·45	1·72

The samples during the response were taken (I) after the change in blood flow had become maximal and (II) before recovery was complete or just after the end of the infusion.
Mean FFA concentration in m-equiv/l. plasma. * Indicates the catecholamine was given by infusion and the dose is μg/min. † One sample missing.

present experiments using anaesthetized animals and Fig. 4 illustrates the effect of the intravenous infusion of noradrenaline 0.1 μg/kg/min—the same rate as that used by Hagen & Hagen.

These experiments suggest that although the subcutaneous adipose tissue does not respond to catecholamines, the release of FFA is stimulated from other sites in the body. This conclusion is supported by experiments in which theophylline was injected. This substance potentiates the effect of catecholamines in rats by preventing the breakdown of cyclic 3′,5′,adenosine monophosphate (3′,5′,AMP), an agent through which catecholamines are thought to act (Butcher, 1966). An injection of 10 mg/kg intravenously increased the concentration of FFA in the arterial blood by 0.45 m-equiv/l., but injections of 200 μg into the arterial supply of isolated fat pads were without effect on FFA release. Infusions of theophylline 10 or 25 μg/min did not increase FFA release. In one experiment 100 μg/min caused a small increase but injection of noradrenaline 10 μg given 3 min later caused no further rise.

Blood flow

The initial rate of blood flow through the tissue was very variable and was not closely correlated with the weight of the isolated tissue. In thirty-seven experiments in which the weight of the tissue was determined at the end of the experiments, the initial rate of flow ranged from 1.37 to 22.0 ml./100 g/min with a mean of 8.07 ± 1.99 (S.E.).

Blood flow was most rapid at the start of the experiment, perhaps partly because of the stimulation the pad received during its isolation. At first, blood flow fell rapidly. In twenty experiments the rate fell from 1.55 ± 0.16 (S.E.) to 0.98 ± 0.11 (S.E.) ml./min in 14–18 min. This high rate of change was not maintained, so that experiments could often be continued for more than 3 hr. A factor contributing to this slowing of blood flow was the progressive fall in blood pressure which was observed in all experiments. This fall was accentuated after injection of large doses of fat mobilizing agents, which made it difficult to assess the effect of such agents on local blood flow. In some experiments, however (that of Fig. 2, for example), mobilization of free fatty acids was accompanied by a marked vasodilation.

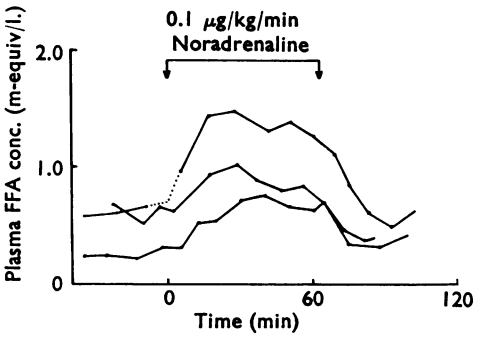

FIG. 4. Effect of intravenous infusions of noradrenaline 0.1 μg/kg/min on plasma FFA concentration. Each curve represents a separate experiment.

Pharmacologically active substances capable of influencing blood flow have been tested for their effects on FFA release. An experiment illustrating the effects of some of them is shown in Fig. 5. In the main, these substances had little effect on FFA release. These experiments are summarized in Table 4, which includes tests with other substances which did not affect blood flow.

Bradykinin, acetylcholine, and isoprenaline at concentrations which increased blood flow did not stimulate FFA release. In contrast to these substances which caused a dilation lasting for a few minutes, prostaglandin E_1 produced an increase in blood flow which persisted for up to half an hour. Prostaglandin had no effect on the basal rate of FFA release. When 1 μg prostaglandin was injected while

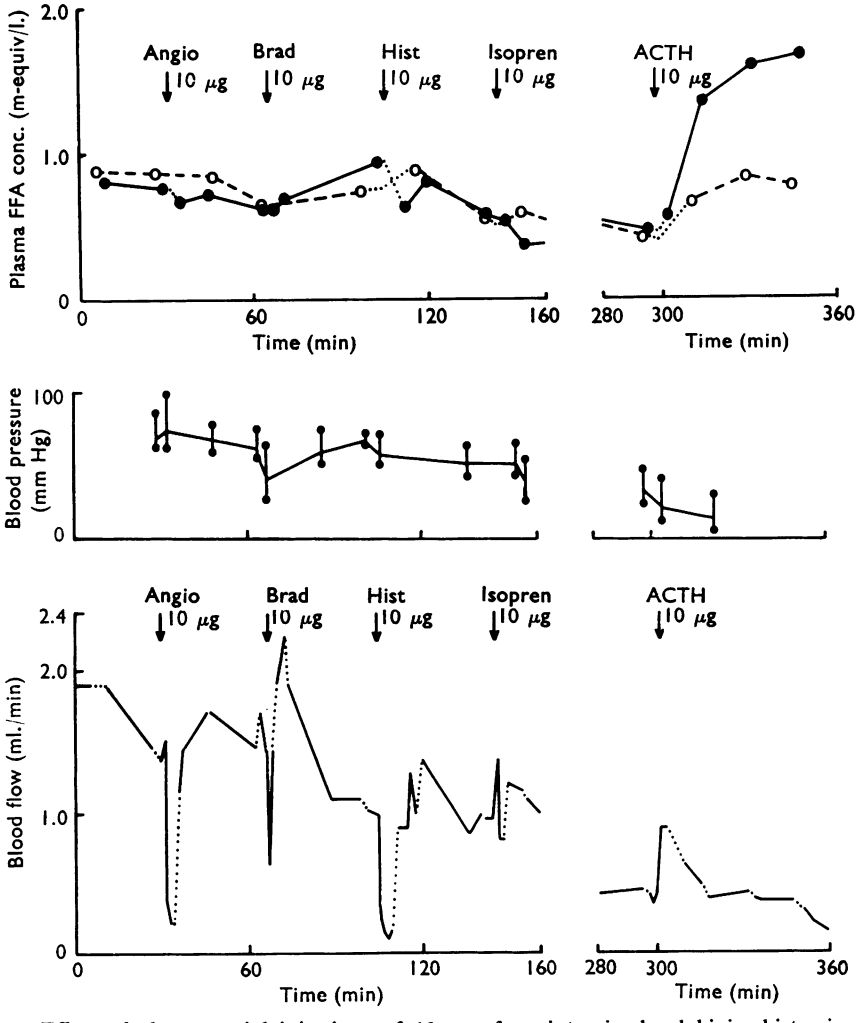

FIG. 5. Effect of close arterial injections of 10 μg of angiotensin, bradykinin, histamine, isoprenaline and ACTH on FFA concentration of venous (●——●) and arterial (○- - -○) blood in the upper record; on the systolic and diastolic arterial blood pressure in the middle record; and on the blood flow through the epigastric adipose tissue in the lower record. The dotted regions of the blood flow plots represent periods where no record was obtained through interference with flow when blood samples were being taken.

FFA release was at a maximum because of an earlier injection of 100 μg growth hormone, however, the venous concentration fell rapidly (Fig. 2).

Angiotensin, oxytocin, synthetic vasopressin, 5-hydroxytryptamine, histamine, adrenaline and noradrenaline all reduced blood flow. So far as can be judged from the small number of experiments with each substance, oxytocin, vasopressin and the catecholamines were particularly effective and in some experiments, especially with low rates of blood flow, produced prolonged constrictions from which recovery was incomplete. Venous blood samples taken during constriction and the recovery phase showed no rise in the FFA concentration.

In some experiments the constriction produced by histamine and noradrenaline was followed by a short phase of vasodilatation. In one experiment a constriction gave way to dilation during the course of noradrenaline infusion, and a cyclic pattern of blood flow became established. Samples of venous blood taken when flow was rapid had higher FFA concentrations than samples from the control period. This was the only experiment in which there was any indication that noradrenaline could stimulate FFA clearance from the subcutaneous adipose tissue.

Plasma lipase activity

As a result of using heparin as an anticoagulant, lipoprotein lipase (LPL) is released into the plasma. Because the plasma FFA levels might be raised by this enzyme, experiments were performed to show that the activity of LPL was not a serious complication in the interpretation of our results.

Six experiments were performed in which the fat pad was not isolated. Intravenous injections of heparin and infusions of MSH were given and LPL activity and FFA concentration were estimated in blood from the femoral artery. It was found that MSH 0.4 μg/min produced a rise in the arterial FFA concentration

TABLE 4. *Summary of tests with substances which do not stimulate FFA release after single injections*

Substance	Dose (μg)	Tests made	Effect on blood flow
Acetylcholine	10	3	Increase
Bradykinin	1–10	5	Increase
Prostaglandin E_1	0·5	2	Increase†
	1·0	3	Increase†
	2·0	1	Increase†
Isoprenaline	1–10	5	Increase
Follicle stimulating hormone	1–3*	3	Decrease
Oxytocin	1–10*	5	Decrease†
Vasopressin-arginine	1–2*	3	Decrease†
Vasopressin-lysine	0·3–2*	2	Decrease†
Vasopressin (Grade I)	0·1–0·3*	2	Decrease†
Angiotensin	1–10	3	Decrease†
Histamine	1–10	4	Decrease
5-Hydroxytryptamine	10	3	Decrease
Adrenaline	10	2	Decrease
Noradrenaline	1–10	6	Decrease
Thyroglobulin	50	2	None
D-Thyroxine	50	2	None
Theophylline	200	2	None
Substance P	10	2	None
Heparin	5 mg	2	None
Hydrocortisone	10	2	None

* Doses in units of activity. † Prolonged effect on blood flow.

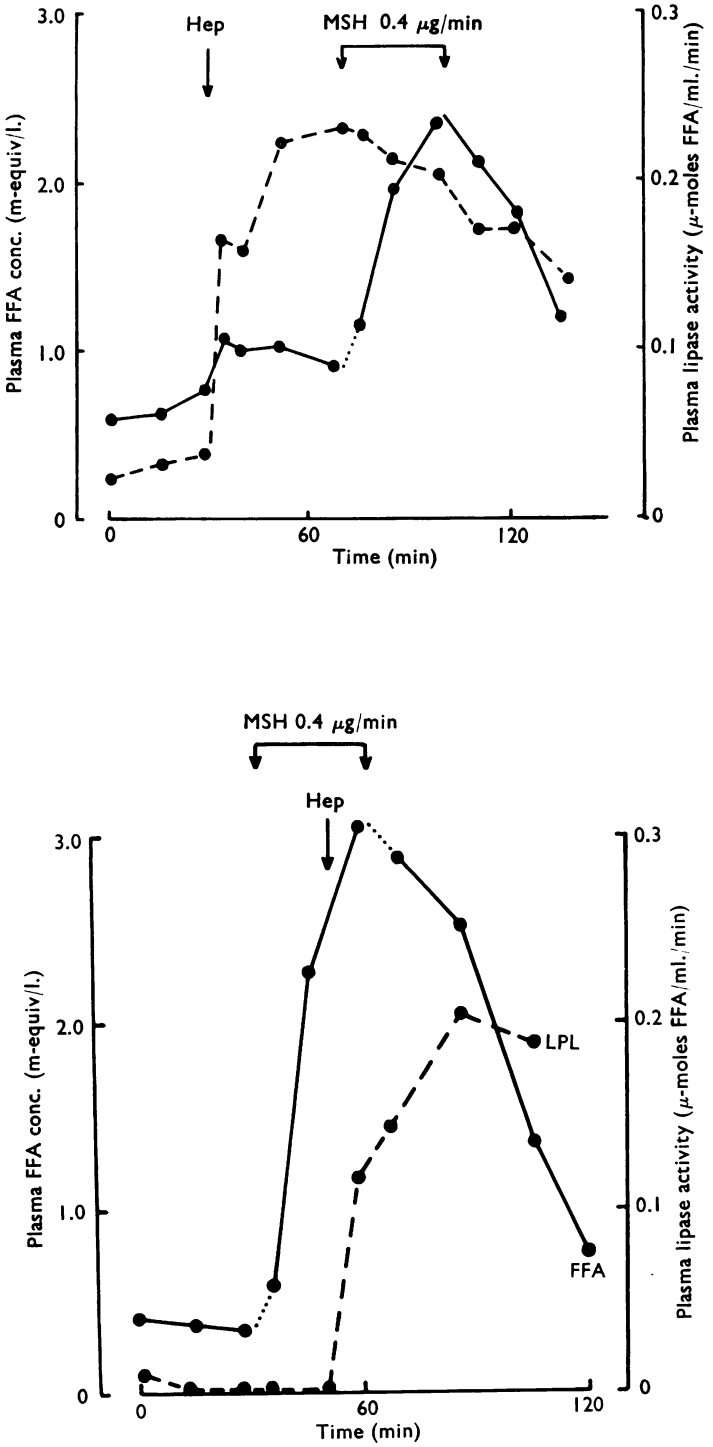

FIG. 6. Effect of intravenous injection of heparin 10 mg/kg and infusion of β MSH 0.4 μg/min on the plasma concentration of FFA (●——●) and plasma lipase activity (●– –●). The two records represent two separate experiments in which the heparin was injected before (upper record) or during (lower record) infusion of β MSH.

detectable within 5 min. The rise continued throughout the infusion, which was followed by a slow decline. In response to the injection of heparin 10 mg/kg, the dose normally used as anticoagulant, plasma lipase activity rose rapidly, an increase being detected 3 min later. Maximum activity was observed about 40 min after the injection, after which activity declined slowly (Fig. 6). No difference in the response to MSH was detected whether the infusion was made before or after heparin had been injected, nor did LPL activity change as the result of MSH infusion. In two further experiments in which LPL was estimated in blood draining the fat pad, no increased activity was observed as a result of the infusion of growth hormone.

Although heparinization of the whole animal was usually followed by an increase in plasma FFA (Fig. 6), the largest rise being 0.80 m-equiv/l., injections of 5 mg heparin into the arterial supply to the fat pad did not stimulate FFA release. Consequently, it may be concluded that although heparin may influence the initial level of FFA, it does not directly influence the response to fat-mobilizing agents.

Discussion

Rabbits showed wide variation in all parameters measured: resting arterial and venous FFA concentrations, blood flow through the epigastric fat pad, blood pressure and the size of the fat pad. Furthermore, the magnitude of response to a given dose of an agent was also variable. Nevertheless, the technique gave qualitatively consistent results in response to a wide variety of substances affecting lipolysis and blood flow.

The persistence of the response to the injection or infusion of a fat-mobilizing agent suggests that either fat-mobilizing agents are rapidly bound by the tissue and slowly inactivated or that they have a trigger action on lipase activity. In some experiments the maximum rate of FFA release was not reached until a few minutes after the injection had been given. This effect has been observed in isolated perfused adipose tissue from rats and it has been suggested that activation of the lipase and transport of FFA out of the cells may be responsible for the delay in attaining maximum FFA release (Ho, Ho & Meng, 1967).

In order to prevent blood clotting in the cannulae, rabbits were given heparin as an anticoagulant. It is, however, well established that heparin stimulates the release of lipoprotein lipase from adipose and other tissues, and there is therefore a possibility that the action of this enzyme on substrates in the plasma would interfere with the responses to fat-mobilizing agents.

We have shown that changes in FFA induced by fat-mobilizing agents are not correlated with, or influenced by, changes in LPL activity. These findings therefore agree with those of Ho *et al.* (1967), who showed that heparin does not stimulate FFA release from adipose tissue and that lipoprotein lipase and hormone sensitive lipase are separate enzymes.

Table 5 shows those substances that possess fat mobilizing activity in the rabbit epigastric fat pad. The table also shows, for comparison, the results in the same species from the isolated adipose tissue test (Rudman *et al.*, 1963 ; Vaughan *et al.*, 1964), and *in vivo* tests in which the increase in the venous FFA concentration was estimated after single subcutaneous injections (di Girolamo *et al.*, 1961). The

three sets of results differ somewhat and several factors can be distinguished to account for this disagreement.

The species from which natural hormones are prepared is important in some cases. *In vivo* tests show that porcine growth hormone is active, whereas bovine growth hormone is inactive both *in vivo* and *in vitro*.

In addition, with commercial preparations of pituitary glands it is not certain whether fat-mobilizing activity reflects the inherent activity of the main component, or is the result of the presence of some other material. For example, we have shown that whereas Pitressin (extract of posterior pituitary lobe) caused release of FFA, neither synthetic arginine vasopressin nor lysine vasopressin was active in our preparation. Oxytocin was also inactive, so we conclude that the lipolytic activity of Pitressin is due to a third unidentified component. This finding, however, is not in agreement with that of Rudman *et al.* (1963), who found synthetic arginine vasopressin to be active *in vitro* at a concentration as low as 0.000175 μg/ml.

Preparations of TSH and LTH were found to stimulate FFA release, although unlike other peptide hormones they reduced blood flow. Other workers have not found them to be active. We have not, however, established whether the fat-mobilizing activity is a true property of the hormone or is the result of a contaminant.

Hagen & Hagen (1962) observed an increase in the plasma FFA in response to intravenous infusions of noradrenaline, and we have confirmed this finding. No increase in the FFA content of perirenal adipose tissue from rabbits was observed after incubation in adrenaline or noradrenaline 100 μg/ml. (Rudman *et al.*, 1963), however, and no FFA mobilization from epigastric tissue was observed after close arterial injections in the present experiments. The small increases sometimes

TABLE 5. *Ability of pituitary and other hormones to stimulate FFA release in the rabbit as shown by different techniques*

	This study	*In vivo*	*In vitro* (a)	*In vitro* (b)
ACTH	+	+	+	+
β MSH	+		+	
α MSH	+		+	
Glucagon	+		−	+
Growth hormone, porcine	+	+		
Growth hormone, bovine		−	−	
TSH	+	−	−	−
LTH (Prolactin)	+	−	−	
FSH	−	−	−	
ICSH		+	+	
Oxytocin	−	−	−	
Vasopressin, arginine	−		+	
Vasopressin, lysine	−		−	
Vasopressin (Pitressin)	+	+	+	
Angiotensin	−		−	
Adrenaline	−		−	+
Noradrenaline	−	+*	−	

+, Activity shown; −, no activity found.
This study: Adipose tissue *in vivo*—activity detected by increase in FFA release after close-arterial injection.
In vivo : Ability to increase FFA concentration in venous blood after subcutaneous injection (di Girolamo *et al.*, 1961).
In vitro : Ability to mobilize FFA in perirenal adipose tissue incubated (a) without albumin (Rudman *et al.*, 1963) or (b) with albumin (Vaughan *et al.*, 1964).
* Hagen & Hagen (1962)—infusion.

observed after infusion of noradrenaline do not resemble the striking effects of peptide fat mobilizers. Such small changes might be attributed not to mobilization but to the clearance of the FFA which had accumulated during the period of vasoconstriction. These observations suggest that catecholamines do not release FFA from rabbit adipose tissue, although the results from intravenous infusions indicate that FFA can be released in response to noradrenaline, from other sites in the body. Vaughan *et al.* (1964) found that retroperitoneal (perirenal) adipose tissue did respond to adrenaline *in vitro*, but only if ascorbic acid was present in the incubation medium, and suggest that rabbit adipose tissue has a much greater capacity than that of the species to inactivate adrenaline.

The anatomical position of the noradrenaline-sensitive tissue in rabbits has not been established. Dawkins & Hull (1964) showed that the release of glycerol and FFA from slices of interscapular adipose tissue incubated *in vitro* was increased by noradrenaline. They were principally interested in the physiological role of brown adipose tissue in newborn rabbits. Large masses of this tissue are present in the interscapular region of the newborn rabbit, but are replaced later by white fat. Although brown fat is metabolically very active, and shows a marked response to noradrenaline, most of the FFA produced by lipolysis is oxidized within the tissue. Brown fat actually releases less FFA than white fat in response to noradrenaline. It is therefore unlikely that the response of adult white fat is caused by the presence of some brown adipose tissue. Thus it appears either that depots of white fat differ greatly in their sensitivity to noradrenaline, or that the FFA are released from some other site.

FFA can be mobilized from the epigastric adipose tissue of dogs by electrical stimulation of the sympathetic nerve to the tissue (Oro, Wallenberg & Rosell, 1965). Our attempts to perform similar experiments in rabbits have been unsuccessful. No nerve could be observed visually and stimulation of the perivascular tissue was followed only by vasoconstriction without the subsequent vasodilatation and FFA release described by Oro *et al.* in dogs.

Several difficulties were encountered in assessing the effects of substances on blood flow in the epigastric fat pad. First, blood flow responses were generally short-lasting in comparison to the sustained increase in FFA release. Small changes in blood flow were not easily seen when the resting blood flow was irregular, which often occurred as a result of fluctuations of blood pressure. Second, the rate of blood flow declined throughout the course of all experiments, probably partly because of a slow fall in blood pressure, and partly because of recovery of the fat pad from the stimulation it received during the operative procedure.

In experiments in progress, however, we find that when fat-mobilizing agents are given by infusion instead of by injection, the blood flow responses are more consistent.

Growth hormone was found to differ from other fat-mobilizing agents in one respect. Whereas a rise in the arterial FFA concentration was observed after all but the smallest doses of ACTH, MSH and glucagon, there was no such rise after injections or infusions of growth hormone.

The difference may be due to different rates of inactivation. It is possible that growth hormone is inactivated or taken up during its passage through the adipose tissue, whereas the other lipolytic agents reach the general circulation and stimulate

FFA release from other sites in the body. An alternative explanation would be that in addition to its fat-mobilizing action in adipose tissue growth hormone possesses the property of promoting FFA uptake and utilization in other tissues of the body.

The question of whether growth hormone stimulates fatty acid oxidation is discussed by Knobil & Hotchkiss (1964). Although there is considerable evidence that fatty acid oxidation is increased by growth hormone it may be that this is partly an indirect effect brought about by increased availability of FFA. It would not account for the apparent differences between growth hormone and other fat-mobilizing agents in the present experiments, because all increase the availability of FFA. The experiments of Rabinowitz and Zierler (1962) support the view that growth hormone has a dual action on fatty acid metabolism. Infusions of human growth hormone into the brachial artery of man produced a rise in the FFA concentration of the blood from a superficial vein draining predominantly adipose tissue, indicating that FFA release was stimulated. On the other hand, the concentration fell in a vein draining muscle, suggesting that FFA was extracted. A fall in forearm R.Q. indicated that FFA oxidation by the muscle was increased.

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