Studies on Lipolysis in Human Adipose Cells *

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Summary. Epinephrine stimulated lipolysis and the uptake of oxygen by subcutaneous adipose cells of man. When glucose-¹⁴C was present in the medium, its utilization was not increased by epinephrine, although lipolysis was accelerated. Insulin did not reduce the production of fatty acids that had been stimulated by epinephrine.

The combination of human growth hormone and cortisol stimulated the production of fatty acids by isolated human adipose cells to a lesser extent than epinephrine. When human growth hormone or cortisol was used singly, or when bovine growth hormone was added in combination with cortisol, no effect on fatty acid production was observed. Furthermore, an acetone-dried preparation of human pituitary glands, which was shown to stimulate lipolysis in rat adipose cells, had no effect on fatty acid formation in human adipose cells. This suggested that the human pituitary gland contained no more potent lipolytic agents than growth hormone and was supported by the lack of response of human adipose cells to purified corticotropin.

Introduction

Starvation in man and other mammals produces an increase in the concentration of free fatty acids in the plasma (1). It has been claimed that the sympathetic nervous system (2) or growth hormone (3), possibly in combination with a glucocorticoid (4) or a state of insulin deficiency (5), is responsible for this phenomenon. Since plasma fatty acids mainly originate from adipose tissue, the first two hypotheses imply that epinephrine or growth hormone (with a glucocorticoid) is capable of stimulating lipolysis in this tissue.

A possible way to assess the relative importance of these hormones in raising the plasma levels of fatty acids in man is to examine their

Address requests for reprints to Dr. David J. Galton, New England Medical Center Hospitals, Boston, Mass. 02111. effects on lipolysis in human adipose tissue *in* vitro. This is not to suggest that the findings from such simplified experiments can be directly applied to the complex physiological state of fasting, but experiments such as those reported here provide some guide for the analysis of the complex conditions existing in the whole animal.

Methods

Source of tissue. Human subcutaneous adipose tissue was obtained during abdominal operations from a total of 41 patients (Tables I and II). Patients with diabetes and jaundice were excluded. Preoperative treatment involved a fast of 8 hours and premedication with sodium pentobarbital and general anesthesia induced by thiopental sodium and maintained with the agents listed in the Tables. Twice it was possible to obtain adipose tissue from the abdominal wall of patients undergoing spinal anesthesia, and the activity of their adipose cells is compared with that of the tissue of patients adjacent in the series who had undergone general anesthesia (Table III). It appears from this that general anesthesia does not markedly inhibit the metabolism of adipose tissue. The adipose tissue was removed most frequently at the time of the first incision, though on some occasions the tissue was obtained just before closure of the wound. After removal, the tissue was washed and placed on a gauze soaked in saline (0.9%). Dermis and epidermis were dis-

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Clinical details of the patients employed in this study

Patie	ent Age	Sex	Wt	Anesthetic agent	Operation
	years		pounds		
R.B	. 52	F	150	Cyclopropane	Vaginal repair
M.N	A . 31	F	122	Halothane, nitrous oxide	Herniorrhaphy
N.V	V. 26	М	238	Halothane, nitrous oxide	Laminectomy
D.C	. 60	М	188	Cyclopropane	Repair gastrocolic fistula
Ĩ.C.	70	М	166	Halothane	Laminectomy
H.R	. 66	Μ	138	Pontocaine	Prostatectomy
B.W	<i>.</i> 66	F	72	Halothane	Adrenalectomy
Ī.A.	34	F	140	Halothane	Laminectomy
N.L	. 44	F		Cyclopropane	Colostomy
A.C	. 41	Μ		Cyclopropane	Gastrojejunostomy
E.B	. 46	М	179	Halothane, nitrous oxide	Sympathectomy, Jumbar
$\overline{0.1}$	44	F		Cyclopropane	Gastrectomy
Č.T	. 56	М	188	Halothane	Repair aneurysm (abdominal)
W.N	A. 32	F	167	Cyclopropane	Cholecystectomy
B.G	. 44	F	127	Cyclopropane	Cholecystectomy
Ē.Ē	. 41	М	174	Halothane	Lumbar sympathectomy
C.N	i. 54	F		Cyclopropane	Hysterectomy
Y.I.	42	F	145	Cyclopropane	Hysterectomy
W.F	C. 45	М	150	Penthrane	Spinal fusion
C.R	. 48	М	186	Halothane, nitrous oxide	Cholecystectomy
S.T.	17	F	173	Penthrane, nitrous oxide	Appendectomy
E.M	I. 31	F	182	Cyclopropane	Hysterectomy
M.N	И. 64	F	115	Nupercaine	L. nephrectomy
R.F	. 64	Μ	175	Pontocaine	Suprapubic prostatectomy
Ĥ.Ē	. 65	Μ	144	Halothane, nitrous oxide	Bilateral sympathectomy
R.C	. 76	Μ	154	Halothane, nitrous oxide	Gastrectomy
R.L	. 16	М	113	Cyclopropane	Abdominal perineal resection
Ĥ.I	. 57	F	185	Cyclopropane	Polypectomy
P.C	. 51	F	165	Cyclopropane	Repair of hernia
0.0	. 24	F	121	Cyclopropane	Repair of hernia

sected away with a pair of fine scissors. The adipose tissue was then cut with scissors into small pieces (approximately 5 to 10 mm in diameter), and any thick strands of connective tissue were removed. The tissue pieces (up to 2 g per vessel) were placed in 3 ml of an albumin-bicarbonate buffer without glucose in conical flasks that had been siliconized, and adipose cells were isolated by the method of Rodbell (6). Approximately 30 to 50 mg of collagenase was in the flasks, and the tissue was incubated in a water bath at 37° under a gas phase of $CO_2 + O_2$ (5:95). At the end of incubation, which varied from 30 to 60 minutes depending on the activity of the collagenase preparation, the contents of the conical flasks were transferred to polyethylene tubes. The remaining tissue was gently ground with a piece of polyethylene tubing, and the tubes were then centrifuged at $300 \times g$ for approximately 1 minute. The liquid-fat layer (derived from broken cells), the sediment, and the infranatant were discarded; the supernatant, which contained free adipose cells and small clumps of adipose cells, was washed two to three times with fresh albuminbicarbonate buffer. After the final washing the infranatant was discarded, and the lightly packed cells were transferred to the experimental vials or Warburg flasks.

Materials. All reagents were analytical grade. The bicarbonate buffer was composed of NaCl (127 mM), KCl (2.7 mM), CaCl₂ (1.4 mM), MgCl₂ (0.5 mM), NaHCO₃ (12 mM), and NaH₂PO₄ (4 mM). The albu-

min-bicarbonate buffer was freshly prepared for each experiment and contained Fraction V bovine albumin,¹ 1 g per 100 ml (wt/vol) for the collagenase digestion and 4 g per 100 ml (wt/vol) for the experiments on lipolysis. The buffer was gassed with a $CO_2 + O_2$ mixture (5:95) and its pH adjusted to 7.3. The Krebs-Ringer phosphate buffer was modified to contain NaCl (150 mM), KCl (6.2 mM), CaCl₂ (0.8 mM), KH₂PO₄ (0.1 mM), MgSO₄ (0.1 mM), and phosphate buffer (12 mM). It was prepared daily before use and contained 4 g per 100 ml (wt/vol) of Fraction V bovine albumin (lot 21908) at 7.2 pH. The agents used were the following: soluble insulin,² diluted in albumin-bicarbonate buffer to contain 0.1 U per ml; epinephrine hydrochloride,³ diluted in 0.15 M sodium chloride; human growth hormone,⁴ dissolved in 0.01 N HCl and stored at 4°; Oxycel-purified corticotropin,⁵ dissolved in 0.01 N HCl; hvdrocortisone,⁶ dissolved in absolute ethyl alcohol and then diluted with saline to give a concentration of 0.2 mg per ml; crude human pituitary powder,4 suspended in albumin buffer be-

¹ Armour Pharmaceutical Co., Kankakee, Ill.

² Iletin-U-40, Eli Lilly and Co., Indianapolis, Ind.

³ Adrenaline, Parke, Davis and Co., Detroit, Mich.

⁴ Kindly supplied by Dr. M. S. Raben.

⁵ Corticotropin, Wilson Labs., Chicago, Ill.

⁶ Hydrocortisone alcohol, Sigma Chemical Co., St. Louis, Mo.

LIPOLYSIS IN HUMAN ADIPOSE CELLS

						Fat	tty acid producti	on
						Human grow	th hormone	
Name Age Sex	Sex	Wt	Anesthetic agent	Operation	Control	5 μg/ml H Cortisol,	2.5 μg/ml 1 μg/ml	
	years		pounds			μmo	les/mmole TG/4	hr†
E.L.	53	F	152	Halothane, nitrous oxide	Oophorectomy	0.00	0.79	0.45
G.V.	54	F	136	Cyclopropane, oxygen	Vaginal repair	-0.16	1.9	0.38
C.W.	70	М	160	Cyclopropane	Cholecystectomy	0.32	0.78	0.59
M.A.	51	м	159	Cyclopropane	Pancreatic biopsy	0.69	1.05	0.67
S.E.	48	F	89	Cyclopropane	Hysterectomy	-0.43	-0.20	-0.30
C.M.	48	F	178	Cyclopropane	Appendectomy	0.12	0.29	0.33
E.M.	21	F	134	Halothane, nitrous oxide	Splenectomy	-0.56	-0.31	
L.F.	54	F	148	Halothane, nitrous oxide	Bilateral lumbar sympathectomy	0.05	0.10	
L.L.	32	F		Halothane, nitrous oxide	Renal arterial explant	0.16	-0.29	
W.C.	34	Μ	176	Pontocaine	Left inguinal herniorrhaphy	0.40	0.44	
E. B .	32	F	224	Cyclopropane	Appendectomy	-0.53	-0.24	
					Mean:	0.005	0.39	0.35
					p versus control:		0.005	

 TABLE II

 Effects of human growth hormone and cortisol on production of fatty acids by isolated human fat cells*

* Isolated fat cells (approximately 200 μ moles of triglyceride) were incubated for 4 hours in 2 ml of albumin-bicarbonate buffer containing glucose (5.6 mM). The results are values of individual experiments. The significance of the difference p was calculated by a nonparametric sign test (11).

 $\dagger TG = triglyceride.$

fore use; porcine pituitary glands (lyophilized),⁷ treated in a similar manner; and glucose-¹⁴C,⁸ added to incubation flasks to make a final specific activity of 0.02 μ c per μ mole.

Experimental design. Adipose cells were distributed among 3 to 20 flasks, depending on the amount of tissue available. Each flask contained 1 or 2 ml of albumin buffer (as stated in the Tables) with the appropriate substrates and hormones added. Zero time samples were taken after 5 minutes' equilibration, but when small amounts of tissue were available a medium blank was used. We incubated the flasks under air when using a phosphate buffer or under a gas mixture of $CO_2 + O_2$ (5:95) when bicarbonate buffer was used. Controls without special additions were carried out. At the end of incubation, the contents of the flasks were centrifuged at $300 \times g$, and a 200-µl sample was removed from the infranatant for assay of glycerol. The reaction was then stopped by addition of 5 ml of an extraction mixture for fatty acids.

Assay procedures. Oxygen consumption was mea-

⁸ New England Nuclear Corp., Boston, Mass.

sured in conventional Warburg vessels as previously described (7). Fatty acids were extracted by a modification of the procedure of Dole and Meinertz (8), and titrations were carried out in 95% (vol/vol) ethyl alcohol with Nile blue as indicator.

Glycerol was estimated by a modification of the method of Wieland (9).⁹ The reaction system contained the following: 200 to 500 μ l of the incubation medium, which was not deproteinized; glycerokinase, 1 μ l (5 mg per ml); glycerophosphate dehydrogenase, 5 μ l (10 mg per ml); NAD, 0.3 mM; ATP, 0.6 mM; and hydrazine-glycine buffer (0.8, 0.16 M, pH 9) containing 2 mM MgCl₂. We added enzymes last in 1% (vol/vol) mercaptoethanol to make a final volume of 3 ml. Readings were performed in a spectrophotometer (Spectronic 20) at 340 m μ 30 minutes after adding the enzymes. Standard curves were prepared for each experiment, and 0.1 μ mole of glycerol gave an absorbency of approximately 0.18.

Radioactivity. In some experiments, we added glucose-¹⁴C to the incubation medium to make a final specific activity of 0.02 μ c per μ mole glucose. At the end of the in-

⁷ Kindly given by Dr. E. B. Astwood.

⁹ Reagents purchased from C. F. Boehringer and Sons, Mannheim, Germany.

				Fatty acid production	
Experi- ment no.	Patient, age, and sex	Anesthesia	Oxygen uptake	Control	+ Epi- nephrine (5 μg/ml)
			µl/mmole TG/hr	µmole. Ti	s/mmole G/hr
16 17 26 27	F.P., 67, male B.W., 66, female D.C., 60, male H.R., 66, male	Spinal General General Spinal	41 33 29 40	0.42 1.6 0.9 0.30	2.74 4.8 2.2 2.1

	TABLE III	
The effect of anesthesia or	n the metabolism of isolated	human adipose cells*

* Fat cells (approximately 465 μ moles of triglycerides) were incubated in 2 ml of an albumin-phosphate buffer without glucose for 2 hours. Epinephrine, when present, was added to make a final concentration of 5 μ g per ml.

cubation period, the ¹⁴CO₂ from the medium was driven off with 0.5 ml of 0.5 N H₂SO₄ and trapped in a strip of filter paper soaked in 0.5 ml of Hyamine [p-(diisobutylcresoxyethoxyethyl) dimethylbenzyl ammonium hydroxide]. The paper was then transferred to 10 ml of Bray's solution (10) and counted in a liquid scintillation counter.

Calculations. All statistical comparisons were based on means of paired experiments. The significance of the difference was tested where necessary by a nonparametric sign test, since this does not assume that the distribution of the data was normal (11). Results are expressed as micromoles of substrate per millimole triglyceride of adipose cells.

Results

The respiratory activity of isolated human adipose cells is shown in Figure 1. It required adipose cells equivalent to 200 to 300 μ moles of triglycerides per flask before a reliable uptake of oxygen could be measured. Accordingly, these amounts of adipose cells were used in most of the following experiments.

Effects of epinephrine on lipolysis. Figure 2 is a dose-response curve which shows that epinephrine stimulates the production of fatty acids from isolated human fat cells. Adipose tissue cells as approximately 132 µmoles of triglycerides were incubated in 2 ml of an albumin-phosphate buffer with increasing concentrations of epinephrine for 1 hour without glucose. The values plotted as fatty acid production have zero time blanks and 1-hour control values subtracted from them. Under the experimental conditions used, it was difficult to observe an effect of epinephrine below a concentration of 0.6 μ g per ml, and the lowest point on Figure 1 is not significantly different from zero with the number of replications employed.

In a series of ten patients, epinephrine $(3 \ \mu g$ per ml) stimulated the production of fatty acids from isolated fat cells, and the oxygen consumption was also increased (Table IV). When insulin was added with epinephrine to the incubation medium, the increment in production of fatty acids and the stimulation of oxygen uptake were the same as with epinephrine alone (Table IV). In a further group of five patients (Table V) epinephrine (3 μg per ml) produced a concurrent increase in oxygen consumption (p = 0.03) and in production of fatty acids and glycerol. When a smaller quantity of adipose cells was incubated



FIG. 1. GRAPH OF OXYGEN CONSUMPTION OF ISOLATED HUMAN FAT CELLS (AS MICROMOLES OF TRIGLYCERIDE). Values are derived from individual plots of four experiments. This was done to allow for the variation in delivering 240, 300, and 390 μ moles of triglycerides to each flask. Incubations were carried out under conditions described in Table I, and the standard error of the mean is represented between the bars.

	Oxygen uptake			Fatty acid release		
Treatment	Control without hormone	With hormone	p versus control	Control without hormone	With hormon e	p versus control
	للر	/mmoleTG/hr		μm	oles/mmoleTG/hr	
Epinephrine	25 ± 4	36 ± 4	0.05	0.43 ± 0.09	3.17 ± 0.4	0.001
Epinephrine + insulin	26 ± 5	35 ± 6	0.05	0.53 ± 0.27	2.40 ± 0.45	0.009

TABLE IV Effects of epinephrine (3 µg/ml) and epinephrine with insulin (1 mU/ml) on oxygen uptake and fatty acid production by isolated human fat cells*

* Approximately 450 μ moles of triglyceride of adipose cells was incubated in 2 ml of albumin-phosphate buffer for 1 hour with the indicated concentrations of hormones in the absence of glucose. Results are means of 10 experiments \pm standard errors of the mean. The significance of the difference was calculated by a nonparametric sign test (11).

with glucose-1-¹⁴C or glucose-6-¹⁴C, the addition of epinephrine (10 μ g per ml) still stimulated an approximately two- and threefold production of glycerol and fatty acids, respectively (Table VI); however, there was no concurrent stimulation in production of ¹⁴CO₂ from glucose-¹⁴C labeled in either the 1 or 6 position, although the formation of ¹⁴CO₂ from glucose-1-¹⁴C was 20 times greater than from glucose-6-¹⁴C (p = 0.017). The larger values for glycerol and fatty acid production in this Table are due to the subtraction of a medium



FIG. 2. DOSE-RESPONSE CURVE OF EPINEPHRINE ON PRODUCTION OF FATTY ACID BY ISOLATED HUMAN FAT CELLS. Points represent means of five experiments, with the standard error of the mean represented between the bars. Experimental conditions were the same as in Table I.

blank instead of tissue blank from the experimental values.

Effects of growth hormone and cortisol. With an incubation system similar to that used in the studies on epinephrine, it was found that growth hormone (5 μ g per ml) in the presence of cortisol $(1 \ \mu g \text{ per ml})$ led to a small production of fatty acids by human fat cells after an incubation period of 4 hours (Table II). This effect was not increased by raising the concentration of growth hormone to 10 μ g per ml. When either hormone was used alone or when bovine growth hormone was used with cortisol, no significant rise in concentration of fatty acids was observed (Table VII). Since there is evidence which suggests that the pituitary gland contains hormones other than growth hormone that might be responsible for the mobilization of fatty acids from adipose tissue (4, 12, 13), the effects of a crude pituitary powder were examined to see if it contained more active lipolytic agents than growth hormone. The pow-

TABLE V Effects of epinephrine (3 µg/ml) on oxygen uptake and fatty acid and glycerol production by isolated human fat cells*

Treatment	Oxygen uptake	Fatty acid release	Glycerol release
	µl/mmole/ hr	µmoles/ mmole/hr	µmoles/ mmole/hr
None	30 ± 5	0.17 ± 0.04	0.47 ± 0.08
+ Epinephrine (3 μg/ml)	43 ± 6	3.3 ± 1.2	3.4 ± 0.9
p versus control	0.03	0.03	0.03

* Fat cells (approximately 624 μ moles of triglyceride) were incubated in 2 ml of albumin-phosphate buffer for 1 hour in the absence of glucose. Results are means of five experiments \pm standard error of the mean, and the significance of the difference (p) was calculated by a nonparametric sign test (11).

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label in medium	Hormone	Fatty acids	Glycerol	to CO ₂	control
		µmoles/m	moleTG/hr	mμ moles/mmole TG/hr	
Glucose-1-14C	None Epinephrine	7.4 ± 2.1 25.9 ± 7.1	7.8 ± 1.1 16.2 ± 2.8	$\begin{array}{ccc} 0.99 & \pm \ 0.3 \\ 1.23 & \pm \ 0.2 \end{array}$	0.31
Glucose-6-14C	None Epinephrine	7.5 ± 2.7 24.3 ± 6.0	7.5 ± 1.8 15.6 ± 2.1	$\begin{array}{c} 0.054 \pm 0.01 \\ 0.064 \pm 0.01 \end{array}$	0.31

TABLE VI Effect of epinephrine on lipolysis and glucose oxidation in human adipose cells*

* Fat cells (approximately 20 μ moles of triglycerides) were incubated in 1 ml of an albumin-bicarbonate buffer in the presence of glucose (5.6 mM), glucose-¹⁴C (final specific activity, 0.02 μ c/ μ mole), and epinephrine (10 μ g/ml) for 1 hour. Results are means of five experiments \pm standard errors of the mean. Tissue blanks were not subtracted from values in the Table.

TABLE VII Effects of growth hormones and cortisol on fatty acid production by isolated human fat cells*

	No. of	Fatty acid	р	
Additions to medium	ments	Control	+ hormone(s)	control
		µmoles/mmol	e TG/4 hr	
Human growth hormone (5 µg/ml)	8	-0.25 ± 1.8	$-0.10 \pm .8$	0.22
Cortisol (1 µg/ml)	8	-0.25 ± 1.8	-0.16 ± 1.4	0.22
Bovine growth hormone (5 μ g/ml) + cortisol (1 μ g/ml)	4	$+0.72 \pm 1.2$	$+0.59 \pm .5$	0.25

* Isolated fat cells (199 μ moles of triglyceride) were incubated for 4 hours in 2 ml of albumin-bicarbonate buffer containing glucose (5.6 mM). The results are expressed as means \pm standard errors of the mean, and the significance of the difference (p) was calculated by a nonparametric sign test (11).

der was an acetone-dried preparation of whole human pituitary glands and would be expected to contain about 5% by weight of human growth hormone and 0.1 to 0.3% by weight of corticotropin. We first tested the extract on isolated fat cells of the rat to determine whether it contained any substances that might interfere with lipolysis. As shown in Table VIII, the powder markedly increased the production of glycerol and fatty acids from adipose cells of the rat, but when the same pituitary extract and cortisol (1 μg per ml) were added to the incubation medium with human adipose cells, no effect on lipolysis was seen (Table VIII). This suggests that corticotropin and other pituitary peptides, such as thyrotropin, do not stimulate lipolysis in human adipose cells. Support for this came from four experiments which showed that when Oxycelpurified corticotropin was added at a concentration of 5 μ g per ml to a preparation of human adipose cells, the fatty acid level only altered from 0.295 ± 0.1 to $0.30 \pm 0.35 \ \mu$ moles per ml of cell suspension per 2 hours. This preparation of hormone had been previously shown to be active on rat adipose cells.

Discussion

Previous studies have reported that epinephrine has only a small effect on lipolysis in isolated human adipose tissue (14–16). Hamosh, Hamosh, Bar Maor, and Cohen (14) concluded that subcutaneous adipose tissue of man either responds sluggishly to epinephrine per se or that their experimental conditions were unsuitable for revealing it. The present studies with subcutaneous adipose cells isolated from man demonstrate a marked effect of epinephrine on lipolysis, as judged by the formation of fatty acids and glycerol, although relatively large amounts of hormone were required to produce it. At the same time, the oxygen consumption of these cells was

Experi- mental series	Addition to medium	Species	No. of experi- ments	Fatty acid production	p versus control	Glycerol production
	ан ан ал ан			µmoles/mmole TG/4 hr		µmoles/mmole TG/4 hr
Α	None	Rat	3	-0.02 ± 0.8		$+0.6 \pm 1.1$
	Human pituitary powder (0.5 mg/ml) + cortisol (1 μ g/ml)	Rat	3	16.3 ± 2.3		3.9 ± 0.4
	Human pituitary powder (1.0 mg/ml) + cortisol (1 μ g/ml)	Rat	3	12.9 ± 1.7		3.4 ± 0.4
в	None	Man	8	$+0.01 \pm 1.0$		
	Human pituitary powder (0.2 mg/ml) + cortisol (1 μ g/ml)	Man	7	-0.64 ± 0.5	0.27	
	Human pituitary powder (0.4 mg/ml) + cortisol (1 µg/ml)	Man	7	-0.82 ± 1.1	0.16	
С	None	Man	6	0.1 ± 0.01		
	Porcine pituitary powder (.8 mg/ml)	Man	6	0.1 ± 0.01	0.23	

TABLE VIII

* Free fat cells (227 μ moles of triglyceride) were incubated for 4 hours in 2 ml of an albumin-bicarbonate buffer containing glucose (5.6 mM). Three male rats of the Charles River breed (mean weight, 150 g) were killed by decapitation, and their epididymal adipose tissue was treated in a similar manner to the human tissue. Results are means \pm standard errors of the mean, and the significance of the difference (p) was calculated by a nonparametric sign test (11).

stimulated by epinephrine; this probably represents activation of the esterification pathway by the fatty acids liberated during lipolysis (17). The effect of epinephrine on lipolysis was observed in both Krebs-Ringer phosphate and bicarbonate buffers in the presence and absence of glucose (Tables V and VI). When ¹⁴C-labeled glucose was added to the medium, 20 times more glucose-1-¹⁴C was converted to ¹⁴CO₂ than glucose-6-¹⁴C (p = 0.017), although utilization of either was not augmented by epinephrine when, at the same time, lipolysis was accelerated.

Ξ

It has been observed in the rat that insulin, in concentrations as low as 10 μ U per ml, can reduce the release of fatty acids from adipose tissue that has been stimulated with epinephrine, even when glucose is not present in the incubation medium (18). This antilipolytic effect of insulin was examined with human adipose cells in Table IV. Although the mean release of fatty acids was less when insulin was added with epinephrine to the incubation medium, the difference was not statistically significant (p = 0.16).

The effect of epinephrine on the formation of fatty acids by isolated human adipose cells was sixfold greater than the combined effect of growth hormone and cortisol. The response to growth hormone could not be increased by raising its concentration in the medium to 10 μ g per ml. In any case, the amounts of hormones that were used

exceeded the maximal amounts reported to occur in the plasma of man. Raben and Hollenberg (19) used 20 times the dosage of human growth hormone as in the present experiments (in the absence of a glucocorticoid) and obtained no greater response from human adipose tissue.

Human growth hormone at a concentration of 5 μ g per ml and cortisol at a concentration of 1 μg per ml used separately did not stimulate formation of fatty acids; when used together they caused a small production of fatty acids. This observation supports the experiments of Fain, Kovacev, and Scow (20), which suggested that a synergistic action exists between growth hormone and a glucocorticoid in the stimulation of lipolysis in isolated adipose cells of the rat. It also extends the observations of Burns and Hales (21), who found that human growth hormone (100 μ g per incubation flask) and prednisolone (0.8 μg per flask) stimulated the release of glycerol from human adipose cells of perirenal tissue, whereas prednisolone alone had no such effect.

The effect of human growth hormone and cortisol on fatty acid production is small but probably real. Two of the 17 experiments in Table II did not show a response to these hormones, whereas the others displayed a small and variable release of fatty acids. This variation is not solely attributable to the different types and amounts of anesthetic agents administered to each patient; for instance, patient W. C. had spinal anesthesia, yet his tissue released very small amounts of fatty acids when stimulated with growth hormone and cortisol. Since all patients had undergone an overnight fast before operation, it would be expected that their adipose tissue would release fatty acids into the incubation medium. However, the control incubations of 4 of 11 patients were taking up fatty acids, although this did not appear to interfere with the response to growth hormone and cortisol. When bovine growth hormone was substituted for human growth hormone, there was no increment in the production of fatty acids by human adipose cells.

There is evidence which suggests that the pituitary gland contains hormones other than growth hormone that might be involved in the mobilization of fat (4, 12, 13). However, a crude human pituitary powder, which was active on isolated fat cells of the rat, did not stimulate the production of fatty acids by isolated human adipose cells. This makes it unlikely that the human pituitary gland contains more active lipolytic agents than growth hormone, although it does not exclude the possibility that there are lipolytic agents present in human pituitary glands which are not in an active form in the unfractionated gland, or that the pituitary preparation contains substances which interfere with the action of such lipolytic agents. On the other hand, it suggests that corticotropin, which is a potent lipolytic agent in the rat, does not stimulate lipolysis in human adipose tissue in vitro. This was found when it was used at a concentration of 5 μ g per ml and confirms the reports of others (19).

It is difficult to estimate the relative importance of epinephrine and growth hormone with cortisol in the mobilization of fatty acids from adipose tissue from the present studies. In man, intramuscular injections of epinephrine (1) or human growth hormone (3) will produce approximately similar rises of plasma fatty acids (from about 0.5 to about 2 μ Eq per ml). It was surprising that growth hormone and cortisol were so much less effective than epinephrine in stimulating the production of fatty acids from subcutaneous adipose cells of the anterior abdominal wall of man in vitro. This could be partly due to a difference in the mechanism of action of growth hormone and The effect of epinephrine on rat epinephrine.

adipose tissue occurs more quickly than that of growth hormone and a glucocorticoid, the effect is greater in magnitude, and it cannot be suppressed by such agents as puromycin and actinomycin D, which block the effect of growth hormone and dexamethasone (20). This points to a difference in the mechanisms of action of epinephrine and growth hormone, and the conditions of incubation in vitro might not be entirely suitable for the expression of the latter. However, it is possible that adipose tissue in various locations responds differently to the same lipolytic agent, so that it is difficult to draw conclusions on the relative efficacy of these agents in elevation of the plasma fatty acids in man when adipose tissue from only one location has been examined in vitro.

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