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Hormonal Regulation of Lipolysis and Phosphorylase Activity in Human Fat Cells

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ABSTRACT Prostaglandin E_1 (PGE_1) at a concentration of 1 ng/ml antagonized theophylline, and norepinephrine induced release of glycerol and free fatty acids (FFA) in human fat cell preparations. Insulin at higher doses also inhibited theophylline-stimulated lipolysis. The N -2'- O -dibutyryl derivative of cyclic adenosine monophosphate (DCAMP) stimulated lipolysis. Prostaglandin E_1 did not significantly inhibit the lipid mobilizing effects of DCAMP. Changes in glycogen phosphorylase activity after treatment with theophylline, norepinephrine, DCAMP, and PGE_1 paralleled those of lipolysis. These results suggest that in man as in experimental animals lipolysis and phosphorylase activity are regulated through processes involving cyclic AMP and that PGE_1 appears to exert its antilipolytic effect in human fat cells, as in rat fat cells, by interfering at the level of adenylyl cyclase with the accumulation of cyclic AMP.

INTRODUCTION

Lipid mobilization is primarily controlled by the rate of hydrolysis of the triglyceride stores of the fat cells of adipose tissue (1). Catecholamines stimulate free fatty acid (FFA) mobilization by activating adenylyl cyclase, an enzyme involved in the production of 3',5'-cyclic adenosine monophosphate (AMP) (2). Methylxanthines are thought to potentiate lipolysis by inhibiting the cyclic nucleotide phosphodiesterase, the enzyme responsible for the destruction of 3',5'-cyclic AMP (2, 3). The accumulation of the cyclic AMP presumably activates an inactive lipase which hydrolyzes triglycerides (4).

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Prostaglandin E_1 (PGE_1) has been found to antagonize the *in vitro* stimulation by catecholamines and theophylline of lipolysis in rat adipose tissue (5-7). In man, the infusion of PGE_1 causes a rise in plasma free fatty acids (8). It was originally reported that PGE_1 *in vitro* reduced basal lipolysis, but not that due to catecholamines in human adipose tissue (9). Recently Carlson and Hallberg (10) were able to show partial inhibition of catecholamine-induced lipolysis by PGE_1 in subcutaneous and omental human adipose tissue slices.

The present studies were designed to test the possibility that cyclic AMP is involved in human fat cell lipolysis and glycogenolysis. These studies suggest that the actions of prostaglandin E_1 , methylxanthines, catecholamines, and dibutyryl cyclic AMP (DCAMP) on these processes in human fat cells are quite similar to the effects which have been observed with fat cells from rats.

METHODS

Human adipose tissue was obtained during the course of abdominal surgery. Severely obese patients and those with diabetes and jaundice were excluded. Preparative treatment included an overnight fast and medication with Nembutal, Demerol, and scopolamine. Patients were given either spinal or general anesthesia or both. Adipose tissue samples were usually obtained from the abdominal wall just before closure of the wound. Within 5 min after removal the tissue was placed in 0.9% saline (37°C) and cut into small pieces. Connective tissue was removed and the tissue was incubated in saline for several minutes at 37°C. The pieces of adipose tissue were then removed and placed in plastic bottles for collagenase digestion. Rat white fat cells were isolated from the parametrial and periovarian adipose tissue of immature female rats (Sprague-Dawley, CD, Charles River Breeding Laboratories). The rats were fed *ad lib.* on laboratory chow and sacrificed by decapitation.

The cells were isolated by a modification of the procedure of Rodbell (11) in 4% albumin solution (bovine fraction V albumin powder obtained from the Pentex, Inc., Kankakee, Ill. (P-55) (Fig. 4 only) or the Armour Pharmaceutical Co., Chicago, Ill.) (A-27) made up fresh daily

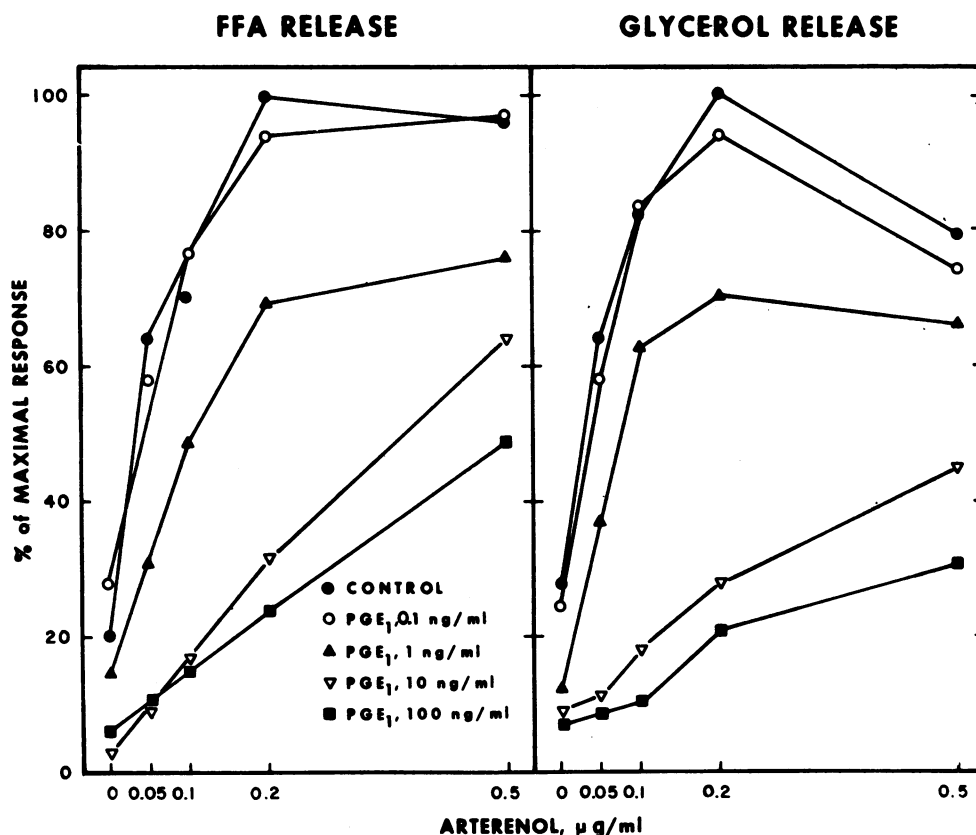


FIGURE 1 Inhibition by prostaglandin E_1 of the lipolytic action of norepinephrine on isolated human fat cells. Free fat cells (35.6 μ moles triglyceride/flask) were incubated for 4 hr in 1.5 ml of 4% albumin (A-27) in buffer without added glucose. Prostaglandin E_1 and norepinephrine bitartrate (artertenol) were added at the start of the incubation. Each point is the mean of eight paired experiments. Prostaglandin E_1 at concentrations of 1 ng/ml or greater significantly inhibited the increase in lipolysis due to all doses of artertenol ($P < 0.05$ by paired comparisons).

in phosphate buffer. The phosphate buffer contained: NaCl, 129 mmoles/liter; KCl, 5.15 mmoles/liter; $CaCl_2$, 1.39 mmoles/liter; $MgSO_4$, 1.4 mmoles/liter; and Na_2HPO_4 , 10 mmoles/liter. The pH was adjusted to 7.4 with NaOH. Pieces of adipose tissue were placed in 1-ounce plastic bottles containing 3 ml of 4% albumin in buffer and 1.5 mg of crude bacterial collagenase (*Clostridium histolyticum*, Worthington Biochemical Corp., Freehold, N. J.), incubated for 1 hr at 37°C in a Dubnoff metabolic shaker and then filtered through nylon chiffon to remove any remaining stromal material and undigested adipose tissue. The separated cells were washed three times in 1% albumin in buffer and resuspended in 4% albumin in buffer for incubation studies.

In all studies the fat cells were incubated in 17×100 mm plastic culture tubes. Initial control values were obtained on cells incubated for 5 min. The values for glycerol and fatty acid release in each experiment were the average of duplicate tubes, except for studies in which phosphorylase activity was measured which were done in quadruplicate, using pooled fat cells obtained from a single patient or three rats. All experiments were replicated from five to eight times and each replication was done on a separate

day. Glycerol was determined on either a 50 or 100 μ l aliquot of the medium by a micromodification of the enzymatic procedure of Vaughan (12) in which diphosphopyridine nucleotide (DPNH) formation was measured with a filter fluorometer (American Instrument Co. Inc., Silver Springs, Md.) Free fatty acids were determined on the remainder of the incubation medium by a modification of the procedure of Dole and Meinertz (13) in which hexane was substituted for heptane. Phosphorylase activity was determined by a modification of the procedure of Diamond and Brody (14). After the incubation period, the fat cells from the four tubes for each point were separated by centrifugation from the albumin-containing medium, resuspended in 2 ml of cold (0°–5°C) 50 mM Tris buffer (pH 6.8) containing 1 mM ethylenediaminetetraacetate (EDTA), 20 mM NaF, and 0.3% albumin, and homogenized immediately with a Potter-Elvehjem homogenizer (Teflon pestle). After centrifugation at 26,000 g for 10 min at 0°C, 0.2 ml aliquots of the infranatant, the soluble layer between the pellet and the fat cake, were incubated in 50 mM Tris buffer pH 6.8, 0.4% glycogen, 10 mM glucose-1-phosphate, 1 mM EDTA, 20 mM NaF, and 0.3% albumin in a total volume of 1 ml in the presence and absence of 1 mM AMP. The addition of 5'-AMP

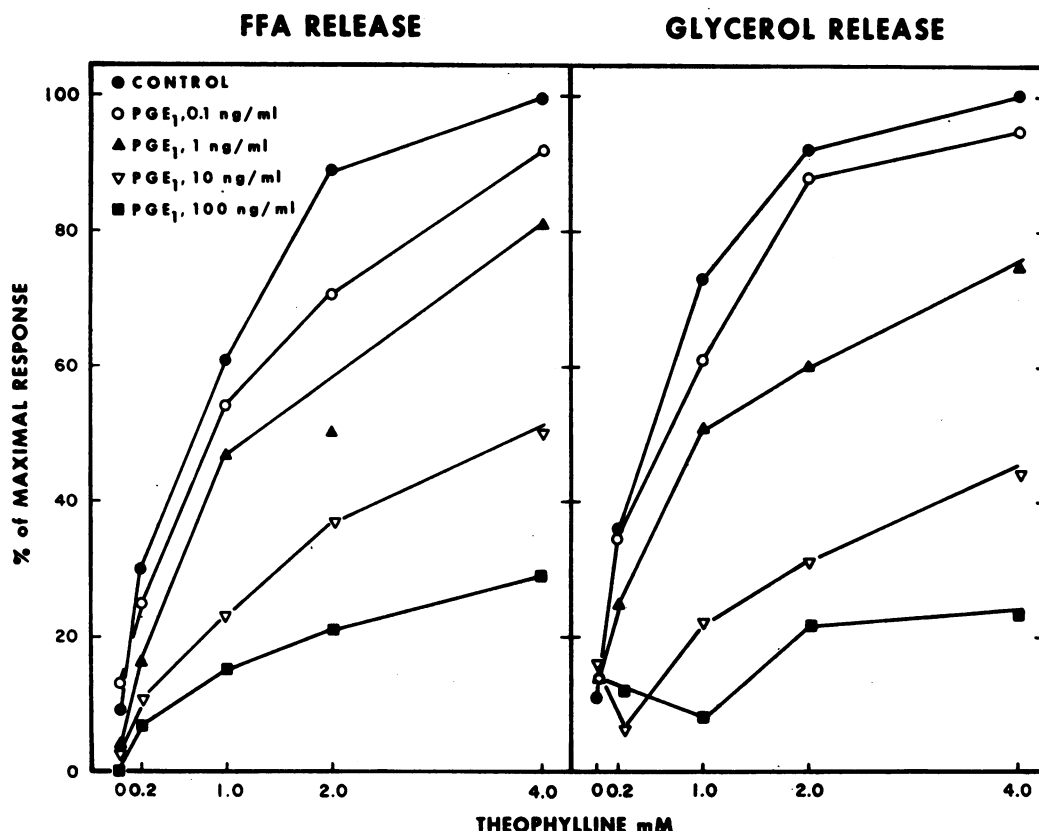


FIGURE 2 Inhibition by prostaglandin E_1 of the lipolytic action of theophylline on isolated fat cells. Free fat cells ($49.3 \mu\text{moles triglyceride/flask}$) were incubated for 4 hr in 1.5 ml of 4% albumin (A-27) in buffer without added glucose. Prostaglandin E_1 and theophylline were added at the start of the incubation. Each point is the mean of six paired experiments. Prostaglandin E_1 at concentrations of 1 ng/ml or greater significantly inhibited the increase in lipolysis due to all doses of theophylline ($P < 0.05$ by paired comparisons).

increased the activity of the phosphorylase. The increment was, however, the same in the presence of lipolytic and antilipolytic agents. Vaughan (15), working with rat adipose tissue, reported that the effect of 5'-AMP does not seem to be related to prior treatment of the tissue. At present there are no quantitative data available concerning the dependence of various forms of adipose tissue phosphorylase on 5'-AMP. The rate of production of inorganic phosphate was constant for 30 min in both the presence and absence of AMP. No phosphorylase was detected if glycogen was omitted from the incubation medium. The dependence on glycogen in the assay system indicates that organic phosphate formation is due to phosphorylase activity rather than non-specific phosphatase activity.

Prostaglandin E_1 was a gift of Dr. John E. Pike (The Upjohn Co., Kalamazoo, Mich.); L-norepinephrine bitartrate (arterenol), theophylline, dibutyl cyclic AMP (DCAMP), and AMP were obtained commercially. Stock solutions of these agents were stored at -15°C . In the presentation of the data, the arithmetic mean is used. However, the significance of the differences between the various experimental pairs was determined by the use of the Student's t test (16) on the logarithms of the paired differences. The distribution of the data was not normal, as is

often the case in biological systems, and for data of this kind the logarithms are distributed more normally (16). The original ranges and means were nearly proportional, but after logarithmic transformations the ranges were almost equal and uncorrelated with the means.

RESULTS

Low concentrations of norepinephrine bitartrate (arterenol) markedly stimulated lipolysis by human fat cells (Fig. 1). Maximal stimulation of lipolysis was seen with $0.2 \mu\text{g/ml}$ of arterenol and a higher concentration actually gave somewhat lower lipolysis as based on glycerol release.

As little as 1 ng/ml of prostaglandin E_1 was able to inhibit lipolysis due to all concentrations of arterenol (Fig. 1). Maximal inhibition of the lipolytic action of arterenol was obtained at PGE_1 concentration of 100 ng/ml.

The effect of PGE_1 on the theophylline-induced lipolysis was similar to that seen with norepinephrine (Fig.

2). The average increase in glycerol release due to the highest concentration of theophylline in the studies shown in Fig. 2 was 12.5 μ moles/mmmole triglyceride as contrasted to 10.8 μ moles/mmmole triglyceride in the presence of norepinephrine (Fig. 1). The results in both figures are shown as the per cent of maximal response, since the per cent differences varied much less between experiments than the actual differences due to PGE_1 . Lipolysis was significantly inhibited, at all doses of theophylline and arterenol, in the presence of 1 ng/ml or more of PGE_1 . Insulin had an antilipolytic action on theophylline-induced lipolysis (Fig. 3). However, the effects of insulin were more variable than those of PGE_1 , and only the effect of 100 μU of insulin/ml was statistically significant.

The addition of the dibutyl derivative of cyclic AMP, N^2 -2'-O'-dibutyl cyclic AMP (DCAMP) increased lipolysis in the human cells (Fig. 4). In experiments with isolated rat fat cells significant increases in DCAMP-stimulated lipolysis were achieved with lower concentrations in human cells than in rat fat cells. The maximal response per millimole of triglyceride was much greater, however, in the rat fat cells. The effects of PGE_1 on the DCAMP-stimulated lipolysis are given in Table I. While the effects of high doses of theophylline were markedly inhibited by 100 ng/ml of PGE_1 (64% inhibition) PGE_1 had little or no effect on DCAMP-stimulated lipolysis.

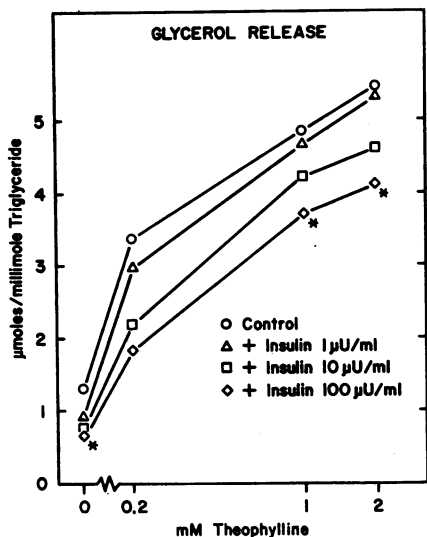


FIGURE 3 The effect of insulin on the lipolytic action of theophylline in isolated human fat cells. Free fat cells (45.0 μ moles triglyceride/flask) were incubated for 4 hr in 1.5 ml of 4% albumin (A-27). Theophylline and insulin were added at the start of the incubation. Each point is the mean of six paired experiments. Significant effects of insulin are shown by asterisks ($P < 0.05$ by paired comparisons).

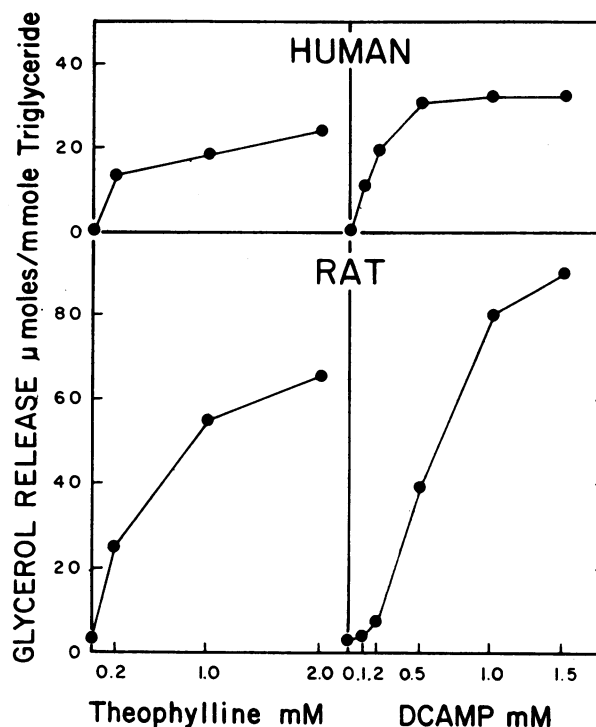


FIGURE 4 Sensitivity of human fat cells to lipolytic action of dibutyl cyclic AMP as compared to rat fat cells. Free rat fat cells (15.7 μ moles triglyceride/flask) and human fat cells (15.8 μ moles triglyceride/flask) were incubated for 4 hr in 1.5 ml of 4% albumin (P-55) without added glucose. Dibutyl cyclic AMP (DCAMP) and theophylline were added at the start of the incubation period. Each point is the mean of five paired experiments. The rat and human cells were incubated at the same time under identical conditions.

To measure the effects of PGE_1 on a parameter of cyclic AMP action other than lipolysis, we measured glycogen phosphorylase activity in extracts of the isolated fat cells. Theophylline and norepinephrine increased both phosphorylase activity and lipolysis in the human fat cells (Table II). PGE_1 inhibited the increase in phosphorylase activity due to norepinephrine and theophylline, both in the presence and absence of AMP (Table II). The total lipolytic response due to these agents were lower than that in our other studies because of the large amount of cells (87.1 μ moles triglyceride/flask) needed to obtain detectable phosphorylase activity. PGE_1 inhibited both phosphorylase activity and glycerol release in the theophylline and arterenol-stimulated cells.

The effects of PGE_1 on DCAMP-stimulated lipolysis and phosphorylase activity were also examined (Table III). Both phosphorylase activity and lipolysis were stimulated by the addition of DCAMP. Prostaglandin E_1 did not affect the stimulation of lipolysis and

TABLE I
Failure of Prostaglandin E₁ to Inhibit the Lipolytic Action of Dibutyryl Cyclic AMP on Human Fat Cells

Additions	Free fatty acid release		Glycerol release	
	Increment due to lipolytic agent	Inhibition by PGE ₁	Increment due to lipolytic agent	Inhibition by PGE ₁
	$\mu\text{moles/mmole triglyceride}$	%	$\mu\text{moles/mmole triglyceride}$	%
DCAMP	24.5	5 \pm 3	12.5	2 \pm 3
Theophylline 4 mmole/liter	17.0	61 \pm 13	8.0	64 \pm 1

Free fat cells (36 $\mu\text{moles triglyceride/tube}$) were incubated for 4 hr in 1.5 ml of 4% albumin with the additions added at the start of incubation period. The values are the means of 5 paired experiments \pm SE. Basal release of fatty acid and glycerol were 13 and 1.4 $\mu\text{moles/mmole triglyceride}$, respectively. PGE₁ concentration, when present, was 100 ng/ml. PGE₁ = prostaglandin E₁; AMP = adenosine monophosphate; DCAMP = dibutyryl cyclic AMP.

phosphorylase activity by DCAMP while that of theophylline was greatly reduced by PGE₁.

DISCUSSION

Previous investigators of human adipose tissue lipolysis have reported a poor response of this tissue to catecholamines (10, 17-19). Galton and Bray (20) have recently demonstrated that isolated fat cells respond better than tissue to large quantities of epinephrine. The isolated fat cells preparations used in this study responded to low concentrations of catecholamines, since 10^{-7} M norepinephrine significantly increased lipolysis.

Human adipose tissue lipolysis appears to be very sensitive to the antilipolytic agent, prostaglandin E₁. Carlson and Hallberg (10) have reported an antilipolytic effect of PGE₁ on omental and (less often) subcutaneous adipose tissue lipolysis stimulated by norepinephrine. A large dose of PGE₁ 100 ng/ml was utilized to inhibit the hormone-stimulated lipolysis and this inhibition was achieved in only two of the four subcutaneous specimens examined (10). The present studies indicate that free fat cell preparations are very sensitive to PGE₁ since 1 ng/ml inhibited norepinephrine-induced lipolysis (Fig. 1). The responsiveness of the human fat cell preparations to norepinephrine and PGE₁ are similar to the results of previous observations with rat fat cells (21).

Theophylline has not been utilized as a lipolytic agent with human fat cell preparations. In our hands, the maximal rate of lipolysis in human fat cells seen with theophylline was about the same as that due to catecholamines. Low doses of theophylline (0.2 mmole/liter)

TABLE II
Inhibition by Prostaglandin E₁ of the Activation of Lipolysis and Phosphorylase by Arterenol and Theophylline

	Basal	+ Arterenol	+ Theophylline
Phosphorylase activity in the presence of 1 mM AMP			
	$\mu\text{moles phosphate/mmole triglyceride}$		
No additions	4.12	6.92	6.72
Prostaglandin E ₁	3.52	5.32	4.92
Increment due to PGE ₁	-0.60	-1.60*	-1.80*
Phosphorylase activity in the absence of AMP			
	$\mu\text{moles phosphate/mmole triglyceride}$		
No additions	1.64	4.80	3.72
Prostaglandin E ₁	1.16	2.60	2.20
Increment due to PGE ₁	-0.48	-2.20*	-1.52*
Glycerol release			
	$\mu\text{moles glycerol/mmole triglyceride}$		
No additions	2.87	4.05	4.49
Prostaglandin E ₁	2.86	2.90	3.10
Increment due to PGE ₁	-0.01	-1.15*	-1.39*

Human fat cells (87.1 $\mu\text{moles triglyceride/flask}$) were incubated for 1/2 hr in 1.5 ml of 4% albumin in buffer without added glucose. Arterenol (norepinephrine bitartrate) 0.1 $\mu\text{g/ml}$, theophylline (1 mmole/liter), and prostaglandin (PGE₁) (100 ng/ml) were added at the start of the incubation period. The values are shown as the means of 8 paired experiments. The increases due to arterenol and theophylline in the absence of PGE₁ are significant ($P < 0.05$).

* A significant effect of PGE₁ is indicated, $P < 0.05$.

elicited a lipolytic response similar to 0.05 $\mu\text{g/ml}$ norepinephrine. As was the case with catecholamines, extremely low doses of PGE₁ (as low as 1 ng/ml) were potent inhibitors of theophylline-stimulated lipolysis.

Few reports on insulin as an antilipolytic agent on human cell lipolysis have appeared except for that of Galton and Bray (20), who found the human fat cell response to insulin to be quite erratic. Although insulin at 1000 $\mu\text{U/ml}$ decreased epinephrine-stimulated lipolysis, the difference was not significant (20). Burns and Langley (22) have more recently found epinephrine-stimulated lipolysis in human cells to be inhibited by low doses of insulin. We have also, like Galton and Bray (20), found the response of human cells to insulin quite erratic. However, the theophylline-stimulated lipolysis was significantly reduced with 100 $\mu\text{U/ml}$ insulin in our experiments.

Human cells have been shown to be sensitive to insulin as based on stimulation of glucose incorporation into CO₂ and fatty acids (23). The sensitivity of the

TABLE III
The Stimulation of Phosphorylase Activity and Lipolysis
by Dibutyryl Cyclic AMP in Human Fat Cells

Additions	Basal	Increment due to DCAMP	P value
Phosphorylase activity in the presence of 1 mM AMP <i>μmoles phosphate/mmole triglyceride</i>			
None	5.76	+4.40	0.01
PGE ₁	4.72	+4.64	0.05
Theophylline	9.32	+0.68	NS
Theophylline + PGE ₁	6.16	+2.96	0.01
Glycerol release <i>μmoles glycerol/mmole triglyceride</i>			
None	2.49	+0.87	0.01
PGE ₁	2.34	+0.56	0.01
Theophylline	3.29	+0.77	0.05
Theophylline + PGE ₁	2.46	+0.89	0.01

Free human fat cells (95.6 μ moles triglyceride/flask) were incubated for 1/2 hr in 1.5 ml of 4% albumin without added glucose. Dibutyryl cyclic AMP (0.25 mmole/liter), theophylline (1 mmole/liter), and prostaglandin E₁ (PGE₁) (100 ng/ml) were present from the start of the incubation. The basal values and the increments due to the addition of DCAMP are shown as the means of 5 paired experiments. The increment due to DCAMP was not affected by PGE₁ either in the presence or absence of theophylline ($P < 0.05$ by paired differences which is also used as the criteria for nonsignificant differences represented by NS).

cells appears to be reduced with age (23) and increased cell size (24). The variations in the antilipolytic effects of insulin in our studies may be due to high titers of insulin in the patients' plasma before excision of the tissue, since the patients in our studies received a glucose infusion during surgery.

Since both norepinephrine and theophylline are believed to increase the accumulation of cyclic AMP in the rat fat cell (2), we utilized the *N*⁶-2-*O*-dibutyryl derivative of cyclic AMP (DCAMP) to see if it would stimulate lipolysis in the human fat cells. Our data indicate that human adipocytes respond to DCAMP and are more sensitive to low doses of this compound than are the rat fat cells. However, high doses of DCAMP gave a greater stimulation of lipolysis in rat adipocytes than in human cells. The reason for the 5-fold greater sensitivity of human fat cells lipolysis to 0.1 mM DCAMP is not clear.

Prostaglandin E₁ did not inhibit the lipolytic response of the human cells to DCAMP. This lack of effect would be expected if PGE₁ influenced the lipolytic enzyme system by interfering with the accumulation of cyclic AMP. Thus, PGE₁ should have no effect on the acti-

vation of inactive lipase to active lipase or of glycogen phosphorylase by 3',5'-cyclic adenosine monophosphate. It has been suggested that PGE₁ might activate the phosphodiesterase involved in degradation of cyclic AMP (25). This appears unlikely since DCAMP is degraded by the phosphodiesterase (26) which attacks cyclic AMP, and if PGE₁ activates this enzyme it should have inhibited the action of DCAMP on human fat cells.

Prostaglandin E₁ lowered the increases of phosphorylase activity and lipolysis due to the addition of norepinephrine and theophylline. These results confirm data from animal studies which concluded that PGE₁ inhibited the activation of phosphorylase and lipase activity due to catecholamines (27).

From these studies it is evident that the human adipocyte preparation responds in a manner similar to rat fat cells when challenged with various lipolytic agents. Norepinephrine, theophylline, and dibutyryl cyclic nucleotide all stimulated both lipolysis and glycogenolysis. Insulin and prostaglandin E₁ reduce the stimulation of both processes by norepinephrine and theophylline but not by dibutyryl cyclic AMP. The data from the studies with PGE₁ support the hypothesis that cyclic AMP is involved in the activation of lipolysis and phosphorylase in human as in rat fat cells and that PGE₁ probably acts to inhibit adenyl cyclase.

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