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Biochemical Aspects of Drug and Hormone Action on Adipose Tissue*

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I. Introduction

DURING the past 15 years there has been an increased interest in the effects of drugs and hormones on adipose tissue. The first review article on the physiology of adipose tissue appeared in 1948 (377) only 8 years after Wells had written a series of articles entitled "Adipose Tissue, a Neglected Subject" (373). There was little further progress until it was reported in 1956 that lipid is mobilized from adipose tissue in the form of fatty acids produced by hydrolysis of stored triglycerides (98, 154). The fatty acids are released into the plasma where they bind to albumin. The albumin-bound fatty acids are removed from the plasma by muscle, liver, and other tissues where they are either oxidized or reesterified. In the liver they can also be converted into ketone bodies.

The major factor regulating the utilization of fatty acids by tissues is the plasma level which is controlled by the net rate of fatty acid release from fat cells. This has stimulated interest in the effects of drugs and hormones on the release of fatty acids by fat cells. The possibility that abnormalities in the uptake or release of lipids by fat cells might play a role in obesity and atherosclerosis has also contributed to an increasing interest in effects of drugs on fat cell metabolism.

By 1965 an entire volume of the *Handbook of Physiology* could be devoted to adipose tissue with over 4000 references (303). In 1970 a book containing over 35 articles on regulation of adipose tissue metabolism was published (191). Ellis (102) and Himms-Hagen (167) reviewed the metabolic effects of catecholamines in 1967 with emphasis on *in vivo* effects, including lipid mobilization. A symposium on adrenergic receptors mediating metabolic responses was published in 1970 and included articles on lipid mobilization (111, 168). Kupiecki has reviewed the pharmacological control of fatty acid mobilization with emphasis on *in vivo* effects (223). The present article emphasizes the biochemical mechanisms by which drugs affect the metabolism of white fat cells.

Investigations of drug and hormone effects on adipose tissue were facilitated when Winegard and Renold (384) reported that pieces of epididymal adipose tissue taken from untraumatized rats, if not chilled during isolation, responded to insulin. Rodbell (312) later found that relatively pure preparations of free adipose tissue cells could be obtained by digestion of white adipose tissue with crude preparations of bacterial collagenase. More recently Rodbell (315) reported that hypotonic lysis of fat cells resulted in delipidated cell "ghosts" which serve as a convenient preparation for

investigations on the regulation of adenylate cyclase activity. Most of the reports of drug and hormone effects reviewed here have been on either white adipose tissue incubated *in vitro*, free fat cells, or fat cell ghosts.

Drugs have proven valuable as tools for the elucidation of the biochemical mechanisms involved in regulation of adipose tissue metabolism. Claude Bernard was one of the first to point out the usefulness of drugs in physiological analysis, and they have been equally important for biochemical investigations. However, one must remember that there are probably no truly specific pharmacological agents which inhibit only one enzymatic step. This is particularly true when the concentrations used *in vitro* are far greater than are required for *in vivo* effects. If enough of a given drug is added to fat cells, one can often obtain an effect. Effects of drugs or hormones at millimolar concentrations can usually be observed, but most agents are seldom present at such high concentrations in plasma. Effects of drugs obtained at relatively high concentrations on fat cells might be seen at much lower concentrations in the target cells or represent side effects from overdosage *in vivo*.

Generally, the enzymatic processes in fat cells which are affected by drugs are the same as in other tissues or cells. Furthermore, hormones and drugs have effects on lipolysis and glucose metabolism of free cells which are similar to those on pieces of adipose tissue, perfused adipose tissue, or intact adipose tissue. However, some agents, such as fluoride, have opposite effects on whole fat cells as opposed to broken cell preparations obtained from fat cells.

Free fat cells are a relatively homogeneous population of a highly differentiated mammalian cell with a limited number of enzymatic reactions. Fat cells are easily prepared by digestion of adipose tissue with crude bacterial collagenase (*Clostridium histolyticum*) followed by gentle centrifugation, during which fat cells float to the top while other cells types are sedimented. Free fat cells generally respond to all hormones which affect intact tissue and have a greater lipolytic response than pieces of intact adipose tissue incubated *in vitro*. The adipose tissue from several animals can be pooled and enough fat cells obtained so that one can test the effect of a large number of agents in paired experiments. We have found studies with isolated fat cells to be more convenient and reproducible than studies with slices of adipose tissue. The major advantage is that the effects of hormones and drugs can be attributed to a direct action on the fat cell since the other cells which occur in adipose tissue are not present.

Paired experimental replications are advisable since the variation in response to drugs and hormones of lots of fat cells prepared from the pooled white adipose tissue of several rats from one day to the next is generally much larger than the variation within a given lot of cells. The standard errors of the values for experiments done with four different batches of fat cells are always larger than the standard error for values from a single experiment based on quadruplicate tubes. It is unfortunate that data in some reports on fat cells are expressed as the mean $\pm$ standard error of a single selected experiment done in quadruplicate, for this gives a misleading impression of the variability in such studies.

Occasionally difficulties arise in studies with isolated fat cells. Our experience is that most of these are related to variability in the commercial preparations of albumin and collagenase (115). At the present time the most feasible procedure to avoid these problems is testing lots of bovine fraction V albumin powder and crude *C. histolyticum* collagenase prior to use (115).

One solution to the problems related to albumin would be to isolate and incubate cells in its absence. The addition of lipolytic agents to cells incubated in the absence of albumin results in a very high accumulation of intracellular free fatty acids (4) due to the absence of any substance to bind the fatty acids released during activation of lipolysis.

Incubation of fat cells under such conditions was shown by Rodbell to have deleterious effects (314). Even in studies in which no lipolytic agents are added, the incubation of fat cells in the absence of albumin reduces the response of the cells to hormones such as insulin and sometimes results in cell lysis (86, 198, 314).

II. Adrenergic Receptors of Fat Cells

The catecholamine content of rat adipose tissue is reduced by administration of reserpine analogues or monoamine oxidase inhibitors in the same manner as that of brain and heart (347, 349, 381). Adipose tissue contains enzymes involved with the synthesis and breakdown of catecholamines and behaves like a sympathetically innervated organ (347, 349, 381).

The concept of *alpha*- and *beta*-adrenergic receptors has been useful. There is increasing evidence that interaction of catecholamines with the *beta* receptor results in alterations in the plasma membrane and is associated with activation of adenylate cyclase (fig. 1). Sutherland suggested that adenylate cyclase might even be the *beta*-adrenergic receptor (355). More recent evidence suggests that the *beta* receptor for catecholamines is more likely to be a separate protein with the receptor-catecholamine complex activating adenylate cyclase and possibly other processes (309). There is no proof at the present time that the catecholamine-receptor complex plays an active role in ATP conversion to cyclic adenosine 3′,5′-monophosphate (cyclic AMP) as postulated in several models (16, 34, 41). It may be sufficient for the catecholamine to alter the conformation of the receptor protein as a result of binding to it and thus activate adenylate cyclase indirectly. Whether all of the *beta* effects of catecholamines are the result of activation of adenylate cyclase is not yet clear, particularly the inotropic effects of catecholamines (248).

Much less is known about *alpha* receptors, but the simplest hypothesis is that *alpha* effects are not due to activation of adenylate cyclase. It has been postulated that *alpha* effects are associated with inhibition of adenylate cyclase and reduced cyclic AMP accumulation (8, 311, 359). It is not clear whether all *alpha* effects of catecholamines are associated with a drop in cyclic AMP (309). One possibility is that *alpha* effects involve utilization of ATP, and, if both *alpha* and *beta* receptors are present, there is competition for substrate. Another is that stimulation of *alpha* receptors by catecholamines results in increased amounts of AMP or other compounds which might act as feedback inhibitors of adenylate cyclase.

Beta-adrenergic antagonists specifically block the effects of isoproterenol, partially

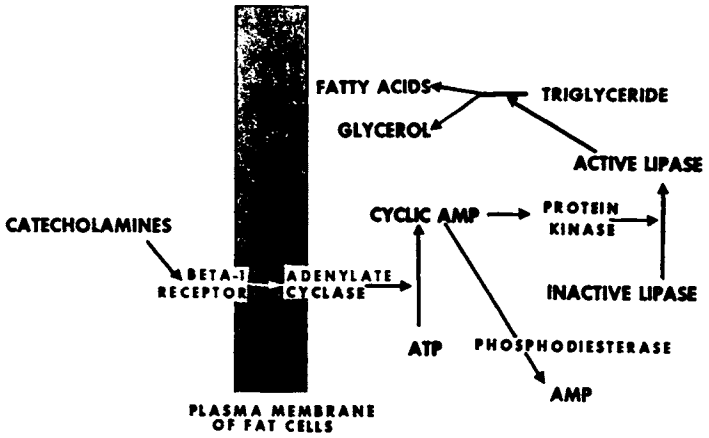


FIG. 1. A model for activation of lipolysis by catecholamine.

block those of epinephrine and have no effect on the response to phenylephrine (194). Conversely *alpha* blockers specifically inhibit the response to phenylephrine, partially block the response to epinephrine, and have no effect on the response to isoproterenol (194). In biochemical terms, *beta* blockers inhibit the activation of adenylate cyclase by catecholamines, whereas *alpha* blockers have little effect or actually increase adenylate cyclase activity.

The effects of catecholamines on fatty acid mobilization are primarily mediated through *beta*-adrenergic receptors (102, 111, 167, 168). The only known mechanism by which catecholamines stimulate fatty acid mobilization is by accelerating the rate at which the triglycerides in fat cells are hydrolyzed, and this is associated with activation of adenylate cyclase. There may be inhibitory *alpha* receptors in human fat cells, but under ordinary conditions the presence of inhibitory *alpha* receptors cannot be demonstrated in rat adipose tissue (236). However Himms-Hagen was able to see potentiation by 3 μM phentolamine [Regitine, 2-[N-(*m*-hydroxyphenyl)-*p*-toluidinomethyl]imidazoline] of the lipolytic response to 0.3 μM epinephrine in fat cells prepared from rats which had been starved for 5 days and refed for 1 day (168). In human fat cells the activation of lipolysis by 3 μM epinephrine was increased in the presence of 10 μM phentolamine to values seen with an equivalent amount of isoproterenol (310). Phentolamine did not affect the lipolytic action of isoproterenol. There was a much greater potentiation of cyclic AMP accumulation due to epinephrine by phentolamine than there was of lipolysis, but no values were given for the effects of phentolamine alone (310).

Wenkeová, Kuhn, and Wenke (376) confirmed that phentolamine elevated the response of human fat cells to norepinephrine but phenoxybenzamine [Dibenzyline, N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)-benzylamine] had a significantly smaller effect. They suggested that the effect of phentolamine might be due to something other than blockade of *alpha* receptors.

Perry and Hales (292) found an initial, rapid and transient increase in $^{42}K^+$ loss from fat cells in the presence of catecholamines which was blocked by phentolamine. A slower but more prolonged increase in $^{42}K^+$ efflux was dependent on the activation of lipolysis by catecholamines and blocked by propranolol (292). Loss of K^+ may account for all or some of the increase in glucose uptake due to catecholamines which has occasionally been observed in pieces of adipose tissue (37, 39).

Another effect of epinephrine which was reported not to be blocked by *beta*-adrenergic antagonists was the increase in uptake of ^{32}P into total fat cell phospholipids (340). However, in the presence of propranolol there was a marked decrease in ^{32}P incorporation into phosphatidylcholine accompanied by a large increase in counts in phosphatidic acid and phosphatidylinositol (340). The increase in incorporation of ^{32}P into phospholipids was unaffected by phentolamine but was blocked by high concentrations of phenoxybenzamine (340). Unfortunately it is not clear just what incorporation of label means, for no measurements of effects of epinephrine on the specific activity of the precursor pools for phospholipid synthesis were made (340).

Bray (37, 39) postulated that catecholamines could affect glucose oxidation independently of their effects on lipolysis. The stimulation of glucose oxidation by 50 to 140 μM epinephrine was only partially inhibited by large concentrations of propranolol or phenoxybenzamine (37). However, Blecher *et al.* (32) found that 1 μM Kö 592 [1-(3-methyl-phenoxy)-2-hydroxy-3-isopropylamine-propane] or propranolol [Inderal, 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol] blocked both the activation of lipolysis and of glucose oxidation by 1 μM epinephrine. This suggested that all of the effects of catecholamines on glucose metabolism were mediated through *beta* receptors. We have similarly never found

any circumstances in which the increased glucose oxidation due to epinephrine could be dissociated from the lipolytic action of epinephrine (371).

It is unlikely that the stimulation by catecholamines of glucose metabolism is due to the oxidation of epinephrine to adrenochrome or other products. Adrenochrome (371), like insulin and other anti-lipolytic agents (75), stimulates D-[1-^{14}C]glucose, oxidation much more than D-[6-^{14}C]glucose oxidation.

In contrast, catecholamines, adrenocorticotropic hormone (ACTH), and other lipolytic agents decrease D-[1-^{14}C]glucose oxidation, increase D-[6-^{14}C]glucose oxidation, and increase glucose utilization for fatty acid reesterification in fat cells and adipose tissue (56, 169, 241). A stimulation of D-[1-^{14}C]glucose oxidation but not of D-[6-^{14}C]-glucose oxidation indicates that the agent has preferentially increased glucose oxidation *via* the hexose monophosphate shunt pathway (190). In contrast agents which increase glucose oxidation through the tricarboxylic acid cycle will have an equal or greater stimulatory effect on D-[6-^{14}C]glucose oxidation (190).

In fat cells added fatty acids decreased glucose metabolism through the shunt pathway (169). One difference between lipolytic agents which increase cyclic AMP accumulation and added fatty acids is that the latter stimulate lactate formation (124). The effects of added fatty acids are similar to those of an uncoupler of oxidative phosphorylation such as 2,4-dinitrophenol which decreased D-[1-^{14}C]glucose oxidation and increased lactate accumulation (320). Apparently added fatty acids increase aerobic glycolysis as reflected in lactate formation, and elevating cyclic AMP by the addition of lipolytic agents or dibutyryl cyclic AMP decreases glycolysis (124). Increasing free fatty acids by any means also increases fatty acid re-esterification using α-glycerophosphate derived from glucose metabolism. The net effect of lipolytic agents on glucose metabolism may depend on the balance between cyclic AMP and free fatty acids. Under conditions in which the increase in cyclic AMP is small, as in the presence of relatively low concentrations of lipolytic agents, the increase in free fatty acids may act to increase lactate formation. In contrast, high concentrations of cyclic AMP produced by the addition of lipolytic agents in the presence of methyl xanthines may increase cyclic AMP to such concentrations that they antagonize the stimulation of glycolysis by fatty acids. Agents which increase lipolysis (catecholamines, methyl xanthines, and dibutyryl cyclic AMP) and added fatty acids all inhibit glucose oxidation by the shunt pathway (124, 169). This is in contrast with the effects of insulin and many other agents (see section XI) which preferentially increase glucose oxidation *via* the hexose monophosphate shunt pathway.

The effects of catecholamines on glucose metabolism are thus opposite from those of insulin and result in decreased oxidation of D-[1-^{14}C]glucose, whereas under some circumstances the oxidation of D-[6-^{14}C]-glucose is increased. No stimulation of glucose oxidation in fat cell ghosts is seen in the presence of concentrations of catecholamines which increase adenylate cyclase (316). This indicates that the stimulation of D-[6-^{14}C]glucose oxidation due to catecholamines may be secondary to increased free fatty acids and is in agreement with the finding that added fatty acids mimic the action of catecholamines on D-[6-^{14}C]glucose oxidation (56, 169).

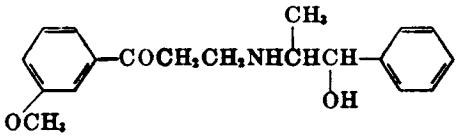
III. Catecholamines as Beta 1 Stimulators of Lipolysis

The structural requirements for *beta* stimulatory effects of catecholamines appear to be a secondary amine nitrogen linked to a phenyl ring by an alkyl bridge (41, 194). Previously it was thought that *beta*-adrenergic stimulation of lipolysis required a catechol nucleus, an alcoholic hydroxyl group, and a bulky substituent on the amino-nitrogen of the ethylamino side chain (324). However, the discovery that trimetoquinol

[1-(3′,4′,5′-trimethoxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline] was only slightly less active than isoproterenol on lipolysis has caused a reexamination of previous ideas (338). The structural relationship of trimetoquinol and isoproterenol is shown in figure 2. Trimetoquinol can be considered as a cyclized N-substituted catecholamine in which the lack of an alcoholic hydroxyl group is compensated for by a bulky ara-alkyl group on the amine nitrogen.

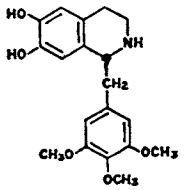
The data also indicate the stereospecificity of the effects of both catecholamines and tetrahydroisoquinolines on lipolysis (fig. 2). The deoxy derivatives of isoproterenol and norepinephrine were equipotent to the *d* isomers of these catecholamines (338).

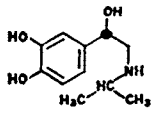
The lipolytic activity of trimetoquinol, like that of isoproterenol, was reversibly inhibited by propranolol. Oxyfedrine [L-3-[(β-hydroxy-α-methylphenethyl)amino]-3′-methoxypropiophenone] is another compound which is not a catechol but does contain an amine nitrogen linked to a phenyl ring through an alkyl bridge with a bulky group attached to the amine group.



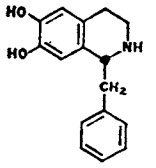
OXYFEDRINE

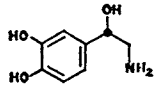
Oxyfedrine was slightly less effective than norepinephrine as a stimulator of lipolysis in rat adipose tissue, and its effects were





	Trimetoquinol	Isoproterenol
pD_2 *l* isomer	7.0	6.7
d isomer	4.1	4.2



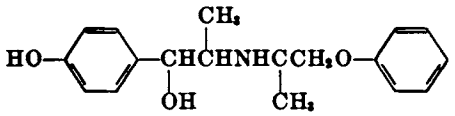


	1-Benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline	Norepinephrine
pD_2 *l* isomer	5.7	5.8
d isomer	4.2	3.8

FIG. 2. Structure-activity relationships for lipolytic action of catecholamines and tetrahydroisoquinolines.

The circle denotes the asymmetric carbon atom. The pD_2 values represent the negative logarithm of the drug concentration required to produce a response equal to 50% of the maximal response on rat adipose tissue lipolysis *in vitro*. The maximal response produced by all of the *l* isomers was equivalent. The data are from the work of Shonk, Miller, and Feller (338).

abolished by propranolol (379). Isoxsuprine [Vasodilan, *p*-hydroxy-α-[1-((1-methyl-2-phenoxyethyl)-amino)ethyl]benzyl alcohol) is another noncatechol compound which stimulated lipolysis, but its potency was about one-tenth that of norepinephrine (137).



ISOXSUPRINE

A number of catecholamine derivatives have been tested on hamster (324) and rat adipose tissue and cells (107) as activators of lipolysis. Isoproterenol was about 10 times more potent than norepinephrine, which was more potent than epinephrine. All derivatives with a potency at least equal to epinephrine contained a *beta* alcoholic hydroxy group and a *p*-hydroxy group on the phenyl ring (137).

Compounds containing large ara-alkyl or alkyl groups on the amine nitrogen were more active as compared to norepinephrine (137). Norepinephrine analogs containing a methyl group on the *alpha* carbon were just as active as norepinephrine (137, 324). Removal of the *beta*-hydroxyl group (dopamine) or the presence of a *beta* keto instead of hydroxy group caused a 160-fold or greater decrease in the activity of catecholamines (137). Removal or methylation of the meta-ring hydroxyl group markedly reduced activity as did removal of the para-ring hydroxyl group (137).

The reduction in activity of norepinephrine derivatives without a meta-hydroxy group on the phenyl group (oxedrines) could be overcome by adding bulky groups to the amine group (235). Compounds which are preferential *alpha* stimulators such as phenylephrine and phenylpropanolamine were relatively inactive as stimulators of lipolysis (137).

Lands *et al.* (228) suggested in 1967 that *beta* receptors could be divided into two groups which were arbitrarily called β-1 and β-2. The β-1 adreno-receptors present in heart, fat cells, and other tissues can be differentiated from β-2 receptors in lung and other tissues as follows.

1) Norepinephrine is equal or even more potent than epinephrine as a stimulator of β-1 receptors, whereas epinephrine is often 5- to 60-fold more potent than norepinephrine as a stimulator of β-2 receptors (46, 107, 137).

2) Isoproterenol is only about 5-fold more potent than norepinephrine as a stimulator of β-1 receptors, whereas it is up to 100-fold more effective than norepinephrine as a stimulator of β-2 receptors (46).

3) Catecholamines with a tertiary butyl group linked to the nitrogen are usually equipotent to isoproterenol as stimulators of vasodepression and bronchodilation but are far less active than isoproterenol as activators of lipolysis and cardiac activity (228).

4) Although propranolol is an inhibitor of catecholamine action on both types of receptors, other *beta*-adrenergic antagonists such as practolol [4-(2-hydroxy-3-isopropylaminopropoxy)-acetanilide] are preferential antagonists of those effects of catecholamines which fall in the β-1 category. Butoxamine (N-tert-butylmethoxamine) in contrast is considered a β-2 antagonist (46).

The differences between β-1 and β-2 effects are also observed with regard to the effects of *beta* stimulators and blockers on adenylate cyclase activity of homogenates from rat heart and lung (46). The data from experiments with fat cells suggest that the adrenergic receptors are of the β-1 category for the following reasons.

1) Norepinephrine is more potent than epinephrine as an activator of rat fat cell lipolysis (107).

2) Isoproterenol is also about 5-fold more potent than norepinephrine as an activator of lipolysis (107).

3) As the size of the group attached to the amine group of norepinephrine is increased, the ability to relax the guinea pig tracheal chain was markedly increased but

there was only a small increase in activation of lipolysis in adipose tissue (374). An example of this is salbutamol [AH-3365; α_1-[(t-butylamino)methyl]-4-hydroxy-*m*-xylene-α^1,α^3-diol] which was equipotent to isoproterenol on bronchial muscle but had only 5% and 1% of the activity of isoproterenol on cardiac muscle (91, 160) and fat cell lipolysis, respectively (111).

4) Butoxamine is relatively ineffective as an inhibitor of fat cell lipolysis, and what inhibitory action it possessed was nonspecific (117). Practolol, in contrast, is active as an inhibitor of fat cell lipolysis (254).

A. Inhibition by Beta-adrenergic Antagonists

It is now well established that *beta*-adrenergic antagonists preferentially block the action of catecholamines on lipolysis. There are also nonspecific effects at high concentrations of some *beta* blockers due to local anesthetic activity. Both optical isomers of *beta*-adrenergic antagonists have equipotent local anesthetic activity, whereas only the *l* isomer is a specific *beta* blocker (194). At a concentration of 1 mM the *l* and *d* isomers of N-isopropyl-para-nitrophenylethanolamine (INPEA) were equipotent as inhibitors of glucose metabolism in isolated fat cells (1). However, 0.1 mM *l*-INPEA was an inhibitor of epinephrine-induced lipolysis, whereas the same concentration of *d*-INPEA was without effect (1). The structures of INPEA and several other *beta* blockers are shown in figure 3.

Mayer, Moran and Fain (250) originally demonstrated that β-hydroxy-N-isopropyl-3,4-dichlorophenethylamine (DCI; dichloroisoproterenol) markedly inhibited the rise in plasma free fatty acids seen after the administration of catecholamines to dogs. There was some stimulatory effect of DCI alone in intact dogs (250) and similar findings were reported *in vitro* (182, 239, 333, 375) where DCI was an agonist-antagonist of catecholamine-induced lipolysis. While DCI was the first experimental *beta* blocker, its high intrinsic or agonist activity limited its usefulness (194).

Pronethalol [Nethalide; Alderlin, α-[(isopropylamino)methyl]-2-naphthalene-methanol] was the first drug specifically designed as a *beta* blocker. However, like DCI it competitively antagonized catecholamine-activated lipolysis and had intrinsic lipolytic activity which hampered its usefulness as a *beta* blocker (25, 333).

The most potent and useful *beta* blockers are catecholamine derivatives containing an alkyl bridge of 3 carbon units between the amine group and the phenyl ring. Propranolol [1-(isopropylamino)-3-(1-naphthyloxy)-2-propranol] is the prototype to which more recent derivatives are compared (194). Kö 592 is identical to propranolol except for a methylphenyl group instead of a naphthyl group. Alprenolol [Aptin; H 56/28; 1-(*o*-allylphenoxy)-3-isopropylamino-2-propanol] has an allylphenyl group instead of a naphthyl group. Oxyprenolol [1-(*o*-allyloxyphenoxy)-3-isopropylamino-2-propanol] is similar to alprenolol except for an allyloxyphenyl group instead of an allylphenyl group. Practolol [4-(2-hydroxy-3-isopropylaminopropoxy)acetanilide] has an acetanilide instead of a plain phenyl group.

Propranolol was found to be a competitive antagonist of the activation by catecholamines on lipolysis in fat cells but had no effect on the lipolytic action of ACTH at concentrations up to 20 μM (107, 117). Similar effects were obtained with Kö 592 (348). However, concentrations of propranolol above 20 μM noncompetitively inhibited the lipolytic action of ACTH, theophylline, and dibutyryl cyclic AMP (9, 332, 350). Propranolol (70 μM) also blockde the increase in lipolysis seen after the addition of cyclic AMP to extracts of rat fat incubated with a coconut oil emulsion (331).

Schulz and Stock have shown recently that high concentrations of either optical isomer of propranolol will block the lipolytic action of theophylline and ACTH to the same extent, whereas only the *l* isomer of propranolol is a competitive antagonist of

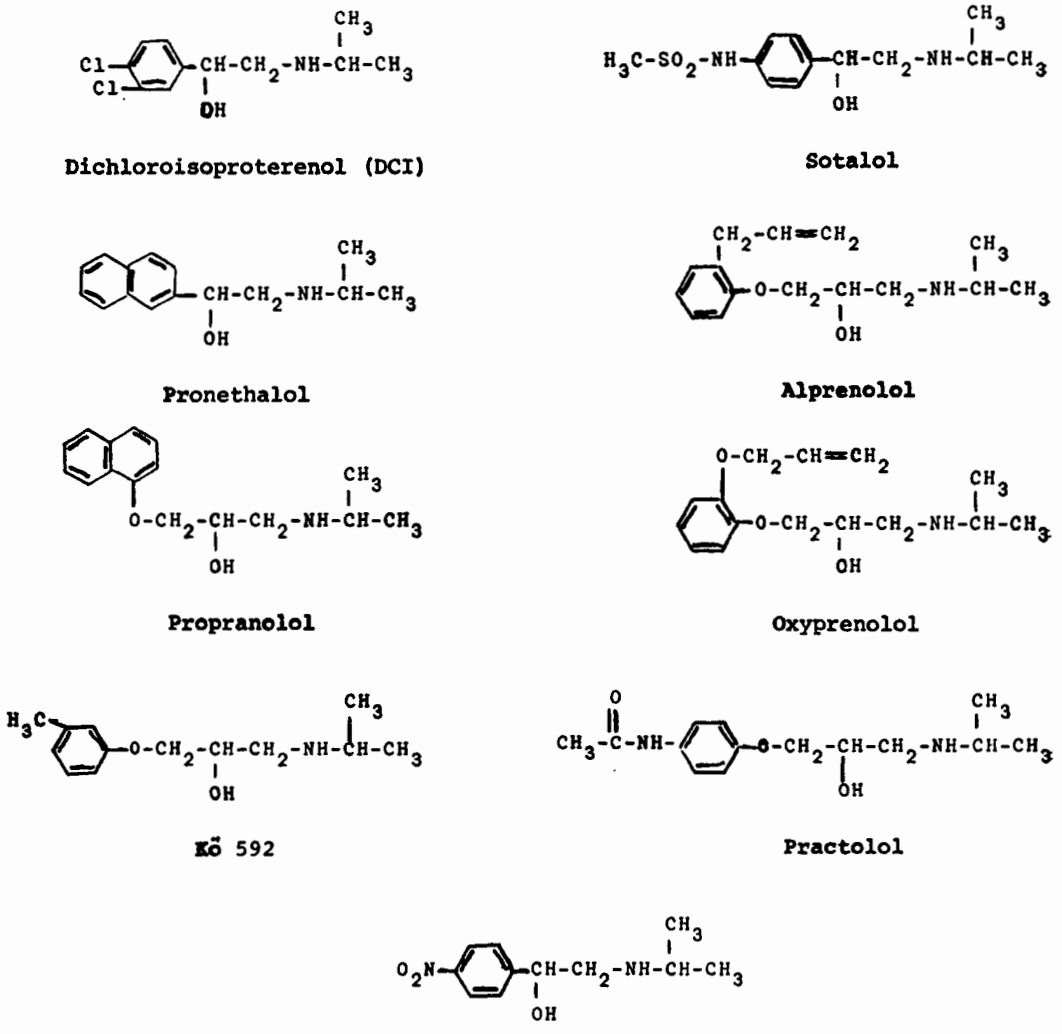


FIG. 3. Structures of *beta*-adrenergic antagonists.

catecholamine action (332). The equal potency of the optical isomers as nonspecific antagonists is in agreement with their effects as local anesthetics (194). In studies aimed at examining the specific *beta* blocker effects of propranolol, these findings suggest that the *l* isomer should be used with the *d* isomer as the control. Another way to get around the nonspecific effects of propranolol and Kö 592 is to use practolol which has no local anesthetic activity but is a specific blocker of β-1 effects of catecholamines (7, 254).

Other *beta* blockers such as INPEA, alprenolol, and sotalol [MJ-1999, 4-(2-isopropylamino-1-hydroxyethyl)-methane sulfonanilide] were considerably less potent than propranolol as antagonists of catecholamine action on adipose tissue (28, 107, 225, 232).

B. Inhibition by Alpha-adrenergic Antagonists

Considerable confusion was initially caused by the finding that phentolamine and phenoxybenzamine inhibited lipolysis due to ACTH, dibutyryl cyclic AMP, and norepi-

nephrine in adipose tissue (294, 330). However, it was found that low concentrations of phenoxybenzamine preferentially blocked the activation of lipolysis by growth hormone and glucocorticoid under conditions in which no effect on ACTH or catecholamine-induced lipolysis was seen (107). This suggested other actions of phenoxybenzamine besides activity as an *alpha*-adrenergic blocking agent. Since the addition of phentolamine (182, 331) or phenoxybenzamine (325) to homogenates reduced lipase activity, it is likely that these drugs inhibit the activation of lipase by cyclic AMP. This agrees with the non-competitive nature of phentolamine inhibition of lipolysis (9). These findings indicate that there are many non-specific effects of *alpha* blockers on adipose tissue. The finding that *alpha* blockers inhibit or potentiate effects of epinephrine does not necessarily mean specific action on *alpha*-adrenergic receptors.

IV. Catecholamines as Activators of Adenylate Cyclase

Agents which rapidly activate lipolysis all appear to increase the accumulation of cyclic AMP in fat cells and this is postulated to activate a protein kinase which converts inactive lipase to active lipase (fig. 1). This hypothesis was originally derived by assuming that the activation of lipolysis by catecholamines occurred by processes analagous to those involved in the activation of glycogen phosphorylase in muscle and liver. Vaughan (361) first provided support for this hypothesis when she found that epinephrine increases the phosphorylase activity of adipose tissue *in vitro* at the same time that it increased lipolysis.

If cyclic AMP is responsible for the activation of lipolysis by catecholamines, then caffeine or other methyl xanthines, which augment the accumulation of cyclic AMP by catecholamines in many other tissues, should potentiate the effect of catecholamines on lipolysis. Vaughan and Steinberg (367) reported a potentiation of the lipolytic action of catecholamines by methyl xanthines, and Butcher *et al.* (53) found similar results with respect to accumulation of cyclic AMP.

There is protein kinase activity in adipose tissue which is activated by cyclic AMP (77), and activation of rat adipose tissue lipase can be seen after the addition of protein kinase from muscle in the presence of ATP and cyclic AMP (78, 179, 180). There is also adenylate cyclase activity present in membranes obtained from adipose tissue which is activated by catecholamines, as first shown in 1963 in a report from Sutherland's laboratory (195).

If the mechanism of the lipolytic action of catecholamines involves cyclic AMP and protein kinase then one would expect to see parallel increases in adenylate cyclase, cyclic AMP, protein kinase, triglyceride lipase, and glycerol release. Mayer *et al.* (249) have reported that after incubation for 5 min with varying concentrations of epinephrine from 0.01 to 1 μM there were parallel increases in all of these parameters. Interestingly there were similar increases in glycogen phosphorylase but not of phosphorylase kinase. Correlations do not prove causation and, subsequently, some cases will be discussed in which parallelism in effects of agents on cyclic AMP and lipolysis is not seen.

Activation of adenylate cyclase is readily examined in ghosts prepared by hypotonic lysis of fat cells (22, 315). The adenylate cyclase activity of ghosts is responsive to those agents which increase cyclic AMP accumulation in intact fat cells, but larger concentrations of hormones are generally required to activate adenylate cyclase in ghosts than lipolysis in intact fat cells (11, 12, 22, 23, 79, 317). It has been difficult to further purify ghosts and obtain plasma membrane fractions which maintain hormonally sensitive adenylate cyclase activity (188). However, it has been found that a 5- to 10-fold increase in catecholamine-stimulated adenylase cyclase activity can be obtained by sucrose density gradient cen-

trifugation of disrupted fat cell ghosts in the presence of ATP (76).

In adipose tissue there are many agents which activate adenylate cyclase, but it appears that while they interact with different receptors they all affect the same enzyme (22, 23). Maximal concentrations of catecholamines and ACTH gave no greater stimulation of cyclic AMP accumulation than was seen with either hormone alone (53, 209, 219). Similar results have also been seen with respect to activation of adenylate cyclase in fat cell ghosts (11, 22).

The activation of fat cell ghost adenylate cyclase by various agents can be separated in that the stimulation by catecholamines is preferentially blocked by *beta*-adrenergic antagonists and that of ACTH by calcium chelators or ACTH analogues (11, 12, 22, 23). Similar results have also been seen in particles prepared by freezing and thawing fat cells in hypotonic medium (366).

Birnbaumer and Rodbell (23) first pointed out, that, even though a large number of hormones and other substances activate fat cell adenylate cyclase, there is considerable specificity in the action of these agents. There appear to be a number of discrete hormone-specific sites (receptors) to which hormones bind, and this binding results in subsequent activation of a single adenylate cyclase (23). This means that catecholamines do not bind to the same sites as ACTH and the reverse is also true. Many agents such as ACTH, thyroid-stimulating hormone (TSH), luteinizing hormone, and other peptides will activate adenylate cyclase in adipose tissue but only at much higher concentrations than are required to activate the same enzyme in their target tissues. This suggests that there are a few receptor sites for these hormones in fat cells but not nearly as many as in target tissues.

There are many hormones which activate adenylate cyclase and some question has been raised about how specificity of hormone action is achieved. One mechanism is that in target tissues for hormones there are many more receptor sites than in non-target tissues, which means that low concentrations of hormones (equivalent to physiological concentrations) specifically activate adenylate cyclase in target tissues. The other major mechanism is specificity in the enzymatic reactions and substrates present in a given cell which can be activated by cyclic AMP. In fat cells both triglyceride lipase and glycogen phosphorylase are activated by cyclic AMP, but under ordinary conditions there is almost no glycogen present in fat cells.

In rat and human fat cells catecholamines appear to be the only agents activating adenylate cyclase which are physiologically significant as activators of lipolysis. Growth hormone is also important in the regulation of lipolysis and adenylate cyclase, but its action is delayed in onset and appears to involve RNA and protein synthesis (section IX).

V. Beta-adrenergic Blockers as Antagonists of Catecholamine Activation of Adenylate Cyclase

Murad *et al.* (270) were the first to report that *beta*-adrenergic antagonists such as DCI blocked the stimulatory effect of catecholamines on adenylate cyclase activity of dog heart particles and also had a slight stimulatory effect in the absence of catecholamines. Vaughan and Murad (366) found that 42 μM DCI or pronethalol doubled the adenylate cyclase activity of fat cell particles, whereas the same concentration of epinephrine gave a 4-fold increase. Adenylate cyclase activity in the presence of epinephrine and DCI together was about 50% of the value seen with epinephrine alone, whereas with epinephrine and pronethalol the value was 20% of that with epinephrine alone.

Birnbaumer and Rodbell (23) found no effect of 34 μM propranolol or phentolamine on basal adenylate cyclase activity in fat cell ghosts. The activation of cyclase by glucagon or ACTH was also unaffected by either drug. Whereas the stimulation of adenylate cyclase due to 1 μM epinephrine

was unaffected by phentolamine, it was completely abolished by propranolol (23). Similar results have been reported for Kö 592 (11). Propranolol, 400 μM, also blocked the activation of adenylate cyclase in purified fat cell plasma membranes by 40 μM epinephrine (188).

Agents which affect the availability of ATP to serve as substrate for adenylate cyclase would have little effect on adenylate cyclase in the assays currently used where very high concentrations of ATP are present. This is due to the finding that conversion of ATP to ADP and AMP is often far greater than that to cyclic AMP in plasma membrane fractions. However, Rodbell and associates have circumvented this problem by using an ATP analogue in adenylate cyclase assays which is relatively resistant to membrane ATPases but can be converted to the cyclic AMP derivative (318).

The only antilipolytic drugs whose effects on adenylate cyclase activity of preparations obtained from fat cells can account for their inhibitory effects on lipolysis are *beta*-adrenergic blockers. There are various effects of other drugs on adenylate cyclase which are discussed in section VIII.

VI. Lipolytic Agents as Stimulators of Cyclic AMP Accumulation

The determination of cyclic AMP was formerly a tedious process, but the protein-binding assay of Gilman (150) has facilitated these determinations. Another useful assay, particularly if combined with measurements of total cyclic AMP, was introduced by Kuo (210, 211), who found that cells incubated with labeled adenine incorporate label into ATP. The cells are then washed free of adenine, and the accumulation of label in cyclic AMP is measured. The original assay is more reliable if cyclic AMP is separated from ATP and other labeled compounds by barium-zinc precipitation and chromatography on Dowex 50 columns (121). To date, we have found no agent which preferentially stimulated the accumulation of total as contrasted to labeled cyclic AMP (58, 112, 123, 124, 126). The incorporation of labeled ATP into cyclic AMP has been described as a procedure for measuring adenylate cyclase activity in intact cells (177, 211). However, even in the presence of methyl xanthines, what is actually being measured is the net difference between the formation of cyclic AMP and its destruction. There is no evidence that high concentrations of methyl xanthines inhibit completely the degradation of cyclic AMP in fat cells.

The incorporation of labeled adenine into cyclic AMP should not be considered as an assay for adenylate cyclase. In more recent papers, Kuo described the assay as a procedure to determine cyclic AMP levels in fat cells by prior labeling with radioactive adenine (209, 210). Caution should be exercised in the interpretation of changes in radioactivity in cyclic AMP as representing alterations in cyclic AMP. In this type of assay an effect of a drug or hormone on the specific activity of the precursor pool of ATP used for cyclic AMP synthesis would appear as a change in cyclic AMP accumulation.

Butcher *et al.* (49, 53) originally reported that in pieces of white adipose tissue the basal value for cyclic AMP was about 0.2 nmole per g of fat, and maximal activation of lipolysis was obtained by increasing the cyclic AMP content to only 0.36 nmole per g. Manganiello, Murad, and Vaughan (224) added an amount of epinephrine (1.1 μM) sufficient to give maximal stimulation of lipolysis and found that cyclic AMP was increased from about 0.8 to 1.6 nmoles per g at 5 min after hormone addition, but by 10 min cyclic AMP content had returned to values only slightly higher than in the controls. Jarett *et al.* (189) found that 0.6 μM epinephrine increased cyclic AMP accumulation 4-fold in isolated fat cells within 2 min after its addition, and 30 min later the values were still 3-fold greater than in controls. There is considerable variation in the extent to which the initial increase at 5 min in cyclic AMP accumulation due to catecholamine is maintained over the next 50 to

60 min. The point is that very small increases in cyclic AMP are associated with increases in lipolysis and measurements of cyclic AMP accumulation 10 to 60 min after the addition of lipolytic agents may show no detectable elevation in cyclic AMP content.

In isolated fat cells the cyclic AMP content at 45 (21) or 60 min (267) after the addition of 1 to 3 mM theophylline was no higher than in controls. The addition of catecholamines gave no further enhancement of lipolysis, but cyclic AMP content was increased 3-fold (210, 267). Butcher, Baird, and Sutherland (52) similarly found no increase in cyclic AMP content after 10 min incubation with 1 mM caffeine. However, Jarett *et al.* (189) reported that 2 min after the addition of 2 mM theophylline cyclic AMP content had increased 3-fold and still was 2-fold greater than in controls 30 min later.

If one assumes an intracellular water content of 5% in adipose tissue or cells, then the cyclic AMP content per g of adipose tissue would represent a concentration of 20 μM. Corbin and Krebs (77) found that 0.1 μM cyclic AMP would give maximal activation of adipose tissue protein kinase. Presumably, even under basal conditions in which lipolysis is not elevated, the cyclic AMP content is more than 20- to 200-fold greater than is required for activation of protein kinase. Possibly much of the cyclic AMP is either inactive by virtue of compartmentation or binding to tissue proteins, unless there are factors present in the intact cells which antagonize the activation of protein kinase by cyclic AMP. Thus caution should be exercised in the interpretation of cyclic AMP determinations. One example of this is the reported claim by Lang and Schwyzer (229) that ACTH activated lipolysis by a mechanism independent of stimulation of cyclic AMP. However, these workers found a basal concentration of cyclic AMP of 60 nmoles per g using a luciferin-luciferase assay which is about 10-fold higher than that found by other workers and could well be due to a lack of specificity in their assay. Cyclic AMP content was increased by about 30 nmoles per g at 5 min after the addition of 1.5 μg per ml of ACTH, and this increase was blocked by a very high concentration of dactinomycin (50 μM). However, dactinomycin does not inhibit lipolysis due to ACTH (117, 119, 229). Inhibition by dactinomycin of the increase in cyclic AMP accumulation due to ACTH from 30 nmoles per g to as little as 0.3 nmole per g may be insufficient to reduce cyclic AMP content below levels at which it is rate limiting for lipolysis (229). The problem is simply that the increase in cyclic AMP accumulation due to lipolytic agents which is required to maximally activate lipolysis may be so small that it is difficult to detect because of the high basal value for total cyclic AMP. Higher concentrations of lipolytic agents alone or lower concentrations in the presence of methyl xanthines cause hugh increases in cyclic AMP accumulation with no physiological significance. The cyclic AMP content under such circumstances can approach 25% of the value for ATP (112).

Some of the problems encountered in correlating lipolysis and cyclic AMP accumulation are shown in figure 4. In the experiment shown in figure 4 the addition of 0.2 mM theophylline alone gave a maximal stimulation of lipolysis but cyclic AMP was increased to 0.5 nmole per g from a basal value of 0.2 nmole per g. In the presence of norepinephrine and theophylline lipolysis was no greater but the cyclic AMP content increased to 9.7 nmoles per g. This large increase in cyclic AMP does not appear to have any physiological function.

The data in figure 4 also illustrate the variations seen with two different types of antilipolytic agents. Both adenosine and valinomycin inhibited lipolysis due to theophylline alone. But in the presence of norepinephrine and theophylline the effect of adenosine was no longer seen, whereas valinomycin inhibited lipolysis. In contrast, valinomycin had no effect on cyclic AMP content due to theophylline alone and only a small effect when both lipolytic agents

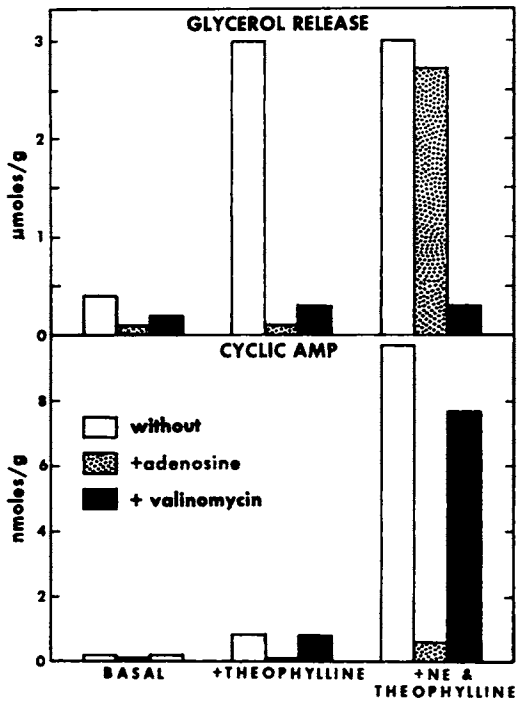


FIG. 4. Comparison of effects of adenosine *versus* valinomycin on lipolysis and cyclic AMP accumulation in white fat cells.

Fat cells (50 mg/tube) were incubated in 0.5 ml of phosphate buffer containing 4% albumin for 10 min in the absence and presence of either adenosine (100 μM) or valinomycin (2 μM). Theophylline (200 μM) was then added either without or with norepinephrine (1.5 μM), and the incubation was continued for 10 min more. Glycerol release was measured over the same period. The data are from unpublished studies by J. N. Fain (1972).

were added. Adenosine, on the other hand, markedly inhibited cyclic AMP accumulation in the presence of both norepinephrine and theophylline. However, if increasing the cyclic AMP content to only 0.5 nmole per g gives maximal activation of lipolysis, then the cyclic AMP content in the presence of adenosine, theophylline, and norepinephrine may still be too high for any inhibition of lipolysis to be seen (fig. 3).

Insulin was originally reported to inhibit cyclic AMP accumulation due to lipolytic agents in the presence of methyl xanthines (54). However, there are conditions in which lipolysis is reduced by insulin but cyclic AMP accumulation is unaffected (124, 189).

In order to correlate effects of drugs or hormones on cyclic AMP accumulation with those on lipolysis, measurements should be made at early time periods and under conditions in which cyclic AMP accumulation is rate limiting for lipolysis. It can be misleading to measure effects of agents on the large increase in cyclic AMP accumulation due to lipolytic agents in the presence of methyl xanthines. Preferably the effects of drugs should be examined on the small physiological increase in cyclic AMP due to the lowest concentration of a lipolytic agent which gives maximal activation of lipolysis.

VII. Effects of Exogenous Cyclic Nucleotide Addition to Fat Cells

If cyclic AMP mediates the lipolytic action of catecholamines and other agents, then exogenous addition of the nucleotide might stimulate lipolysis. Dibutyryl cyclic AMP (1 mM) but not cyclic AMP (2.5 mM) stimulated lipolysis in isolated fat cells (53). The general experience is that added cyclic AMP has little lipolytic action in regular Krebs-Ringer phosphate or bicarbonate buffer. Weiss, Davies, and Brodie (372) did find that the lipolytic action of 10 mM theophylline was inhibited by 1 mM adenosine, whereas the addition of 0.2 mM cyclic AMP restored lipolysis almost to the level seen with theophylline alone.

Mosinger and Vaughan (264) found a marked lipolytic effect of cyclic AMP (0.2 to 1 mM) in phosphate buffer from which calcium, magnesium, and potassium were omitted (saline-phosphate). In phosphate buffer containing Ca^{++} and Mg^{++}, cyclic AMP was anti-lipolytic (264). This may be due to the conversion of cyclic AMP to metabolites which inhibit endogenous adenylate cyclase as discussed in the section on nucleosides.

The addition of guanosine 3′,5′-cyclophosphate (cyclic GMP) at concentrations in the range of 0.4 to 1 mM to fat cells

incubated in saline-phosphate buffer (no other cations) increased lipolysis (271). In regular phosphate buffer cyclic GMP, like cyclic AMP, was an inhibitor of theophylliee-induced lipolysis. In either buffer cyclic GMP addition increased the total cyclic AMP content, but this increase in cyclic AMP accumulation in the Krebs-Ringer phosphate buffer was not associated with an increase in lipolysis. The increase in cyclic AMP accumulation was attributed to inhibition of the low K_m phosphodiesterase since cyclic GMP (5 μM) was a competitive inhibitor of cyclic AMP hydrolysis (271). The lack of correlation between cyclic AMP accumulation and lipolysis with cyclic GMP is unexplained and might be due to multiple effects of cyclic GMP. Braun, Hechter, and Bar (36) and Blecher, Ro'Ane, and Flynn (33) compared the effect of the cyclic 3′,5′-monophosphates of uracil, cytosine, thymine, inosine, guanosine, and adenine on lipolysis in fat cells incubated in saline-phosphate buffer. Cyclic CMP, IMP, GMP, UMP, and 2′-deoxy cyclic AMP all had some lipolytic activity (33, 36). It was postulated that the cyclic nucleotides were not affecting the hydrolysis of cyclic AMP by phosphodiesterase.

The finding that dibutyryl cyclic AMP is more effective than cyclic AMP when added to fat cells was originally attributed to greater uptake into cells or protection from destruction during entrance to the sites of deacylation and action, or both (163). The N^6,$O^{2'}$-dibutyryl cyclic AMP is inactive as a stimulator of protein kinase activity of adipose tissue (77). This suggests that the dibutyryl cyclic AMP must either be metabolized to a compound which can activate protein kinase or that it acts *via* an indirect mechanism. Both possibilities have been suggested.

In bone cells dibutyryl cyclic AMP apparently inhibits phosphodiesterase, which results in increased cyclic AMP accumulation (161). However, in fat cell homogenates we have found dibutyryl cyclic AMP to be a very poor inhibitor of the degradation of labeled cyclic AMP by the low K_m soluble phosphodiesterase (369).

The other possibility is that the $O^{2'}$-ester group is removed by an esterase and the resulting N^6-monobutyryl cyclic AMP is able to activate protein kinase or is further metabolized (31). It is difficult to detect dibutyryl cyclic AMP conversion to cyclic AMP by fat cells (31), since the concentrations of dibutyryl cyclic AMP required are so large that less than 1% conversion to cyclic AMP would be sufficient to maximally activate lipolysis.

The lipolytic activity of a number of 8-substituted derivatives of cyclic AMP has been examined using fat cells incubated in regular phosphate buffer (142). Cyclic AMP derivatives containing a hydroxy or thiol group on the 8-position gave half-maximal stimulation of lipolysis at a concentration of 0.2 mM, whereas 8.5 mM cyclic AMP was required for an equivalent increase (142).

Replacement of the 6-amino group of cyclic AMP by methylthio, ethylthio, or benzylthio has been reported to increase the potency of cyclic AMP (143). Substitution of the ribose group by arabinose or acylation at the $O^{2'}$ position was reported to decrease activity (143). The 8-substituted derivatives of cyclic AMP were either as potent as cyclic AMP or somewhat more active in stimulating brain protein kinase (269). They are relatively resistant to hydrolysis by cyclic AMP phosphodiesterase preparations from brain and kidney (269). The 8-methylthio cyclic AMP also inhibited cyclic AMP hydrolysis (269). The 8-methylthio cyclic AMP was the most effective of the derivatives tested as an activator of adrenal steroidogenesis and fat cell lipolysis (142). Probably the relative resistance to hydrolysis or greater ease of penetration of 8-substituted cyclic AMP derivatives, or both, account for their effectiveness in whole cells.

There are many problems in experiments in which cyclic nucleotides are added to fat cells since very large concentrations (>1 mM) are required before lipolysis is stimulated. At low concentrations cyclic AMP

or dibutyryl cyclic AMP actually inhibits lipolysis (264) and stimulates glucose oxidation (30). Apparently, with the appropriate concentration of added cyclic nucleotide you can obtain any desired effect. Probably the relative usefulness of studies in which fat cells are incubated with millimolar concentrations of cyclic AMP derivatives has been exaggerated in the past.

Stimulation of adipose tissue protein kinase by different cyclic nucleotides was examined by Corbin and Krebs (77). At a concentration of 2 μM only cyclic IMP was active, and it was 80% as effective as 2 μM cyclic AMP. Dibutyryl cyclic AMP, cyclic GMP, and cyclic uridine monophosphate at 2 μM had some effect which could have been due to contamination with cyclic AMP since 0.02 μM cyclic AMP stimulated the protein kinase and maximal activation was seen with 0.1 μM cyclic AMP. Nucleotides with little activity at 2 μM were cyclic thymidine monophosphate, 2′-deoxy cyclic AMP, cyclic cytidine monophosphate, adenosine 3′- or 5′-monophosphates, and inosine 5′-monophosphate (77).

Further studies on the regulation of protein kinase activity by drugs and hormones are needed. Some agents may affect the availability of ATP at the site where protein kinase phosphorylates triglyceride lipase. Thus drugs may interfere both with the amount of cyclic AMP and ATP present at the sites for phosphorylation as well as block the ability of cyclic AMP to activate protein kinase.

VIII. Drugs Affecting Lipolysis and Cyclic AMP Accumulation or Action

A. Methyl Xanthines, Papaverine, and Other Phosphodiesterase Inhibitors

Methyl xanthines were first shown to inhibit phosphodiesterase by Butcher and Sutherland (55). Vaughan and Steinberg (367) reported that caffeine increased lipolysis in adipose tissue and Butcher *et al.* (53) found that it markedly potentiated the stimulation of cyclic AMP accumulation by catecholamines. There are two cyclic AMP phosphodiesterase activities in fat cells (196, 271, 356). These activities have been separated into a high molecular weight fraction which hydrolyzes both cyclic AMP and GMP with an apparent Michaelis constant for cyclic AMP of around 20 to 400 μM. There is also a lower molecular weight fraction with a Michaelis constant for cyclic AMP of approximately 2 to 5 μM and less ability to hydrolyze cyclic GMP (196, 356). The lower molecular weight phosphodiesterase is inhibited by cyclic GMP and appears to be subject to negative cooperative control (356).

Many of the early studies on effects of agents on phosphodiesterase activity are of limited value since they were done in the presence of high cyclic AMP concentrations. Most recent studies on phosphodiesterase have been done at concentrations of cyclic AMP in the physiological range (0.1 to 5 μM). Many agents which are competitive inhibitors of the low K_m diesterase will have little effect on phosphodiesterase activity if measured at high substrate concentrations, for under those conditions activity due to the high K_m enzyme is primarily being measured.

One interesting problem is that cyclic AMP both activates protein kinase and is degraded by phosphodiesterase. There should be some similarity in the nature of the cyclic AMP recognition sites on the two enzymes. Some inhibitors of phosphodiesterase may be even more potent inhibitors of protein kinase and actually inhibit lipolysis. There have been few studies on the effects of drugs on protein kinase in adipose tissue so far, but obvious candidates are drugs which inhibit both lipolysis and phosphodiesterase activity.

Beavo *et al.* (15) tested over 64 drugs as activators of lipolysis and inhibitors of phosphodiesterase activity in fat cells. Most of the compounds were xanthine derivatives with substitutions at the 1, 3, 7, or 8 positions. The most active compounds were those which contained small nonpolar groups on both positions 1 and 3 (15). There was a

close correlation between the potency of a xanthine as a lipolytic agent and as an inhibitor of cyclic AMP phosphodiesterase.

Papaverine is a good example of a compound which is a potent inhibitor of phosphodiesterase but actually blocks lipolysis (14, 334). Papaverine was 10 to 50 times more potent as an inhibitor of phosphodiesterase than theophylline in the absence of albumin but was equipotent to theophylline in the presence of 3% albumin (14). The effect of theophylline was unaffected by the presence of albumin (14). Papaverine did not mimic the potentiation by theophylline of cyclic AMP accumulation due to lipolytic agents in fat cells (14, 113).

Papaverine at concentrations in the range of 0.5 to 5 μM inhibited oxygen uptake and ATP formation by rat liver mitochondria incubated in the presence of glutamate but not if succinate was the substrate (327). These data indicated that papaverine is a potent inhibitor of NAD-linked respiration. Papaverine, cyanide, and dinitrophenol all have similar effects on acetylcholine-induced contractions of guinea pig ileum (327). The inhibition of oxidative metabolism by papaverine in fat cells could reduce the energy-dependent activation of triglyceride lipase and account for its inhibitory effects on lipolysis (14, 334).

Schwabe, Berndt, and Ebert (334) found that dipyridamole [2,2′,2″,2‴-(4,8-dipiperidinopyrimido-[5,4-d]pyrimidine-2,6-diyldinitrilo) tetraethanol], which like papaverine is a vasodilator, and imipramine [5-(3-dimethylaminopropyl)-10,11-dihydro-5H-dibenz[b,f]azepine], which is an antidepressant, blocked lipolysis at concentrations from 20 to 200 μM. Their effects were almost identical to those of papaverine. Dipyridamole was a much more potent inhibitor of phosphodiesterase than was theophylline. The K_i for inhibition of adipose tissue phosphodiesterase was 1.8 μM for papaverine, 4.5 μM for dipyridamole, 91 μM for theophylline, and 222 μM for imipramine at cyclic AMP concentrations of 0.5 to 2 μM. The K_i for inhibition of phosphodiesterase was increased 4- to 7-fold if assayed at cyclic AMP concentrations in the range of 100 to 400 μM, but all drugs were competitive inhibitors in both substrate ranges (334). It was suggested that papaverine, dipyridamole, and imipramine might block the activation of protein kinase (334).

Methyl xanthines are in effect substituted imidazopyrimidine compounds. Imidazopyrazines such as 5-chloro-6-(ethylamino)-1,3-dihydro-2H-imidazo [4,5-b]pyrazin-2-one and 5-chloro-6-[[2-(dimethylamino)ethyl]amino]-1,3-dihydro-2H-imidazo [4,5-b] pyrazin-2-one were equipotent with theophylline as activators of lipolysis in fat cells and also increased the accumulation of cyclic AMP (242).

A compound known as SQ 20009 [1-ethyl-4-isopropylidenehydrazine)-1H-pyrazole-(3,4-b)-pyridine-5-carboxylic acid, ethyl ester] was as lipolytic as theophylline and was also a very potent inhibitor of phosphodiesterase (69, 141). The amount of the drug required to inhibit cyclic AMP phosphodiesterase preparations from adipose tissue was 21 μM if the concentration of cyclic AMP used as substrate was 0.16 μM (69).

Apparently some drugs which are much more potent inhibitors of cyclic AMP phosphodiesterase than methyl xanthines are unable to mimic the effects of methyl xanthines on either lipolysis or cyclic AMP accumulation. However, it should be realized that it has not yet been conclusively established that the activation of lipolysis by methyl xanthines is the result of their inhibitory effects on cyclic AMP phosphodiesterase.

B. Dihydroergotamine

Dale in 1906 found that low concentrations of ergot preferentially blocked those responses to catecholamines which we now classify as *alpha*-adrenergic effects (88). While ergot derivatives are *alpha*-adrenergic antagonists, at high concentration they have a variety of other effects. It has been reported that dihydroergotamine blocked the

glycogenolytic action of cyclic AMP in rat liver (277, 278), but recently this has been disputed (154a). Gothelf and Ellis (154a) found that in intact rats concentrations of dihydroergotamine which inhibited glycogenolysis due to catecholamines had no effect on that due to glucagon or cyclic AMP. Ergotamine was one of the first drugs reported to inhibit catecholamine-activatable adenylate cyclase in dog heart and liver membrane preparations (270).

Dihydroergotamine (0.1 mM) inhibited the activation of lipolysis by catecholamines in rat fat cells (111) or adipose tissue (42). But dihydroergotamine (0.1 mM) potentiated the lipolytic action of dibutyryl cyclic AMP and theophylline on fat cells (111) or adipose tissue (42). Ward and Fain (368) found that dihydroergotamine (0.02 to 0.1 mM) was a potent inhibitor of cyclic AMP phosphodiesterase activity in white fat cells at cyclic AMP concentrations in the range of 1 to 5 μM and also preferentially blocked the activation of adenylate cyclase by catecholamines without affecting basal or fluoride-sensitive activity (368).

In dog fat cells dihydroergotamine was a potent activator of lipolysis at concentrations in the range of 0.1 to 1 μM and its lipolytic action was unaffected by propranolol (72). It was reported that dihydroergotamine did not affect cyclic AMP phosphodiesterase activity in fat cells, but the concentration of cyclic AMP used in the assays was 300 μM (72). If dihydroergotamine preferentially inhibits the low K_m enzyme, it may not be possible to see inhibition when the cyclic AMP concentration in the assay is 300 μM. However, inhibition of phosphodiesterase probably accounts for the lipolytic action of dihydroergotamine in fat cells from dog adipose tissue. Dihydroergotamine apparently blocks the lipolytic action of catecholamines by interfering with stimulation of adenylate cyclase while it potentiates lipolysis due to other agents as the result of inhibition of cyclic AMP phosphodiesterase.

C. Imidazoles

Butcher and Sutherland (55) originally reported that high concentrations of imidazole activated heart phosphodiesterase. However, Allen and Clark (2) found that 75 mM imidazole actually inhibited the phosphodiesterase activity of the high speed supernatant fraction of fat cells by 11% in the presence of 1 μM cyclic AMP. Imidazole at concentrations in the range of 5 to 50 mM blocked the lipolytic action of norepinephrine, ACTH, and theophylline but not that of dibutyryl cyclic AMP (60, 89, 151, 272, 275). 1-Methylimidazole or 2-methylimidazole at concentrations in the range of 10 to 50 mM were also antilipolytic (272, 275). Allen and Clark (2) reported that 75 mM imidazole gave a 25% inhibition of the activation of adenylate cyclase in fat cell homogenates by epinephrine but had no effect on basal or fluoride-sensitive cyclase. It appears that imidazole reduces lipolysis by inhibition of adenylate cyclase rather than activation of phosphodiesterase.

An imidazolidinone compound 4-(3, 4-dimethoxybenzyl)-2-imidazolidinone (Ro 7-2956) has been found to stimulate lipolysis (89). Ro 7-2956 inhibited the low K_m phosphodiesterase activity present in homogenates of fat cells prepared by hypotonic lysis (89). The addition of 10 mM cyclic AMP to cells incubated with 0.1 mM theophylline resulted in a 50% inhibition of lipolysis, whereas cyclic AMP increased lipolysis 2- to 3-fold if added to cells in the presence of 0.1 mM Ro 7-2956 (89). This could be due to differences between the drugs with respect to uptake into fat cells of cyclic AMP, its rate of degradation, or its conversion to antilipolytic factors. Nicotinic acid (1 μM) was also much more effective as an inhibitor of theophylline-induced lipolysis than of that due to Ro 7-2956 (89). In other respects, such as potentiation of the lipolytic action of norepinephrine, the effects of Ro 7-2956 appeared to be similar to those of theophylline (89). The lipolytic activity of theophylline and Ro 7-2956 was unaffected by con-

centrations of propranolol (1 to 10 μM) which abolished the effect of norepinephrine (89).

D. Cyclic Carboxylic Acids

Nicotinic acid (1 mM) was originally found to stimulate the oxidation of [1-^{14}C]-glucose but not that of [6-^{14}C]glucose by adipose tissue (233). Carlson (61) subsequently reported that 0.01 to 0.1 mM nicotinic acid inhibited the lipolytic action of catecholamines. Nicotinic acid also inhibited the action of lipolytic agents such as ACTH or growth hormone plus glucocorticoid as well as that of epinephrine during a 4-hr incubation (117).

Nicotinic acid is a competitive antagonist of the lipolytic action of catecholamines, ACTH, and theophylline with little effect on lipolysis due to dibutyryl cyclic AMP (10, 26, 61, 293, 294). One report did claim a slight inhibition by nicotinic acid of dibutyryl cyclic AMP-induced lipolysis (273).

Butcher, Baird, and Sutherland (52) reported that after 10 min incubation of fat cells or pads, 33 μM nicotinic acid had no effect on cyclic AMP accumulation in the presence of 1 mM caffeine. However, there was almost complete inhibition of the large increase in cyclic AMP seen when 5.5 μM epinephrine was added in the presence of caffeine (52). Andersson *et al.* (3) found 0.9 nmole per g of cyclic AMP after incubation of white adipose tissue for 1 hr, which was 36% higher after incubation with 0.15 μM norepinephrine and 22% higher after incubation with 1 mM theophylline for 1 hr. Nicotinic acid (2 mM in the presence of norepinephrine or 50 μM in the presence of theophylline) completely blocked the effects of the lipolytic agents on both cyclic AMP accumulation and lipolysis without affecting basal values for either parameter (3).

The most likely mechanism by which nicotinic acid inhibits lipolysis and cyclic AMP accumulation is that it is a nonspecific inhibitor of adenylate cyclase. Allen and Clark (2) did find an inhibition by 0.1 mM nicotinic acid of basal adenylate cyclase activity in fat cell homogenates as well as of the increase due to epinephrine, ACTH, and sodium fluoride.

In a preliminary report it was claimed that nicotinic acid was an activator of adipose tissue phosphodiesterase (204), but as yet no full report has been published confirming this suggestion. Several workers could find no effect of nicotinic acid on the low K_m cyclic AMP phosphodiesterase (2, 165, 224, 293).

A large number of nicotinic acid derivatives has been tested for ability to inhibit theophylline-induced lipolysis in fat cells. None were more active than nicotinic acid but there was some activity of 2-OH, 6-Cl and 5-F, Cl, Br, OH, CH_3, or NH_2 substituted nicotinic acid (90). Other carboxylic acids such as 5-methylpyrazole-3-carboxylic acid (145, 146, 149, 223, 224) and 3-methylisoxazole-5-carboxylic acid (288, 289) inhibit lipolysis and appear to act by the same mechanisms as nicotinic acid.

In vivo the administration of β-pyridylcarbinol (3-pyridine-methanol, nicotinic alcohol) results in the slow accumulation of nicotinic acid (64). The administration of β-pyridylcarbinol or nicotinic acid to intact rats lowered the cyclic AMP content of adipose tissue from 0.4 to 0.2 nmole per g at 15 or 30 min after administration, while at 1, 3, or 5 hr the cyclic AMP content was increased to 0.6 nmole per g (47). The plasma free fatty acids stayed down for 6 hr but then rebounded to higher values than in the controls (47). Although nicotinic acid lowers the plasma free fatty acids *in vivo* during the 1st hr after its administration to man (64, 157, 295) or dogs (286), by 90 to 120 min the fatty acids values have rebounded to values higher than in controls. Pereira (286) suggested that the rebound phenomena was the result of stimulation of the pituitary-adrenal axis since plasma corticosterone values were doubled 1 hr after the administration of nicotinic acid.

5-(3-Pyridyl)tetrazole is an analogue of nicotinic acid which does not have a carboxyl group but has been reported to be active *in*

vitro as an antilipolytic agent (290). However, the drug was incubated with a large amount of adipose tissue for 3 hr and it remains to be demonstrated that it was not metabolized to nicotinic acid (290). This is particularly true since the concentration of the pyridyltetrazole required to produce 50% inhibition of the lipolytic action of norepinephrine was 300 times greater than that of nicotinic acid, while *in vivo* the pyridyltetrazole was several fold more potent (290). There was no rebound seen in plasma free fatty acids after administration of the pyridyltetrazole as is encountered with the other carboxylic acids (290). Further studies will be needed to determine the mechanism of the antilipolytic action of pyridyltetrazole and the relationship of its antilipolytic action to its ability to lower plasma triglycerides and cholesterol in man.

E. Prostaglandins and Aspirin-like Drugs

Steinberg *et al.* (342) reported in 1963 that prostaglandin E_1 inhibited the lipolytic action of catecholamines on incubated rat adipose tissue. Subsequently it was found that prostaglandins blocked the effects of other fast-acting lipolytic agents such as ACTH, glucagon, and theophylline (107, 109, 176, 268, 343, 345) as well as slow-acting lipolytic agents such as growth hormone and glucocorticoids (107, 109). Prostaglandin E_1, like insulin and nicotinic acid, did not inhibit the lipolytic action of dibutyryl cyclic AMP in rat (176, 341) or human (266) fat cells. Prostaglandin E_1 was also without effect on the activation of phosphorylase by dibutyryl cyclic AMP in human fat cells (266).

The ability of prostaglandin E_1 to inhibit the activation of phosphorylase and lipolysis in fat cells (266, 343) suggested that it might inhibit the accumulation of cyclic AMP. Measurements of cyclic AMP accumulation in fat pads and fat cells indicated that prostaglandin E_1 was a potent inhibitor of the large accumulation of cyclic AMP seen in the presence of methyl xanthines plus catecholamines, ACTH, glucagon, or TSH (48, 50, 176). Another feature of prostaglandin E_1 is that it is a potent inhibitor of cyclic AMP accumulation due to catecholamines in the presence of methyl xanthines in intact fat cells but is without effect on cyclic AMP accumulation in fat cell homogenates, whereas propranolol is equally effective in either system (50). In homogenates of fat cells prostaglandin E_1 actually doubled cyclic AMP accumulation in the presence of 6.7 mM caffeine, while it either reduced, slightly increased, or had no effect on cyclic AMP accumulation due to 1 mM caffeine in intact cells (48, 50, 51).

In intact adipose tissue prostaglandin E_1 alone increases cyclic AMP accumulation in contrast to the general findings in isolated fat cells, and this has been attributed to stimulation of adenylate cyclase in the non-fat cells of adipose tissue (48). Indeed fat cells appear to be the exception now to a general rule that in most tissues prostaglandin E_1 stimulates adenylate cyclase (48).

Prostaglandin E_1 like nicotinic acid increased glucose uptake, oxidation, and conversion to fatty acids in adipose tissue or cells (35, 108, 364). However, the maximal stimulation of glucose metabolism by prostaglandin E_1 was usually about 10% of what was seen with insulin (35, 364). The general finding is that prostaglandin E_1 and nicotinic acid are more potent as anti-lipolytic agents than they are as stimulators of glucose metabolism.

There are unexplained complexities in the effects of prostaglandins on adipose tissue and cells. Fain (107) found that prostaglandin E_1 inhibited basal lipolysis by fat cells from starved rats and Kupiecki (222) found similar results using incubated adipose tissue. However, several groups found an inhibition by prostaglandin E_1 of lipolysis by adipose tissue from fed but not from starved rats (19, 62, 63, 253, 346). The effects of nicotinic acid and prostaglandins *in vitro* are similar except that nicotinic acid was able to inhibit basal lipolysis in adipose tissue from starved rats under conditions where prostaglandin E_1 was ineffective (19, 346).

Paradoxically, if starved rats were injected with 300 mg per kg of nicotinic acid 3 hr before adipose tissue was removed, then prostaglandin E_1 stimulated lipolysis *in vitro* (253). The mechanism by which prostaglandins are activators of lipolysis and cyclic AMP accumulation in intact adipose tissue under some conditions warrants further investigation and is unexplained at the present time.

The mechanism of action of prostaglandins may involve other factors besides regulation of cyclic AMP, such as calcium uptake and permeability by cells (337). Fassina and Contessa (131) implicated calcium movements in prostaglandin action on adipose tissue. However, the absence of calcium from buffers used for incubation of fat cells (109) or adipose tissue (131) did not alter the inhibition by prostaglandin E_1 of the lipolytic action of theophylline or catecholamines.

The possibility that prostaglandins might act as negative feedback modulators of adenylate cyclase arose when it was realized that they are released from incubated pieces of adipose tissue as a result of electrical stimulation or addition of catecholamines (336). However, acetylcholine and histamine at concentrations (5 μg/ml) which had no effect on lipolysis were as potent as catecholamines in stimulating prostaglandin release (336). Fredholm, Rosell, and Strandberg, in a preliminary report, found that there was some release of prostaglandins during and after nerve stimulation of canine subcutaneous adipose tissue, but the minute amounts released were generally lower than those required to inhibit lipolysis (140). They also noted rapid inactivation of infused prostaglandins by adipose tissue.

Illiano and Cuatrecasas (184) have concluded that endogenous prostaglandins are regulators of lipolysis in adipose tissue. 7-Oxa-13 prostynoic acid (184) or SC-19220 [1-acetyl-2-(8-chloro-10,11-dihydrodibenz-(b,f) (1,4) oxazepine-10-carbonyl hydrazine] (184, 302) overcame the inhibitory effects of prostaglandin E_1 on catecholamine-induced lipolysis. The SC-19220 compound was reported by one group to potentiate the lipolytic action of epinephrine in the absence of added prostaglandin E_1 (184), whereas another group found no effect (302). However, neither of the so-called prostaglandin antagonists interfered with the binding of prostaglandin E_1 to a specific binding protein present in fat cells (205).

Vane (360) found that 2 to 20 μg per ml of acetylsalicylic acid (aspirin), 10 to 200 μg per ml of salicyclic acid, or 0.1 to 1 μg per ml of indomethacin [1-(*p*-chlorobenzoyl)-5-methyl-indole-3-acetic acid] blocked the formation of prostaglandins in guinea pig lung. This has created a flurry of interest in explaining all effects of these drugs as being secondary to inhibition of prostaglandin formation. Illiano and Cuatrecasas (184) found that preincubation at room temperature for 20 min with indomethacin (10 μg/ml) resulted in an enhancement of lipolysis and cyclic AMP accumulation in the presence of catecholamines. However, attempts in our laboratory to reproduce these findings have been unsuccessful (113).

Salicylates should stimulate lipolysis in adipose tissue if they act by blocking prostaglandin formation, but they actually reduced basal lipolysis by incubated adipose tissue (64a) and fat cells (353). Sodium salicylate also inhibited the activation of lipolysis in fat cells by catecholamines, ACTH, glucagon, theophylline, growth hormone plus glucocorticoid, as well as that due to dibutyryl cyclic AMP (353). The latter observation clearly differentiates the action of salicylate from that of other carboxylic acids such as nicotinic acid which have little effect on lipolysis due to dibutyryl cyclic AMP.

If prostaglandins are physiologically important as negative modulators of lipolysis, then one should be able to prevent their formation by feeding animals diets deficient in essential fatty acids. One problem is that rats fed such diets weigh much less than controls (18, 24). It is therefore difficult to compare data, unless expressed on a per cell basis, for the essential fatty acid-deficient animals invariably are leaner and have much

less triglyceride per cell. However, all of the reported data are expressed per g of fat or per unit of triglyceride (18, 24). On this basis there was an enhanced response to lipolytic agents including dibutyryl cyclic AMP in tissue or cells from essential fatty acid-deficient animals. However, if the data were expressed on a per cell basis, the reported differences between control and deficient rats might disappear.

F. Inhibitors and Uncouplers of Oxidative Metabolism

Mosinger (257, 258) originally reported little effect of cyanide, azide, and malonate but some inhibition by 2,4-dinitrophenol on the activation of lipolysis by catecholamines. But the requirement for oxygen by adipose tissue is so low that unless strict anaerobic conditions are maintained lipolysis is not abolished. Subsequent studies have indicated marked inhibition of lipolysis by anaerobiosis and drugs affecting oxidative metabolism. Drugs affecting oxidative metabolism generally inhibit the lipolytic action of dibutyryl cyclic AMP and probably affect the activation of lipolysis by cyclic AMP.

1. Amytal, piericidin A, and rotenone. Incubation of adipose tissue with 3.5 mM amytal for 80 min abolished the subsequent lipolytic response to norepinephrine (27). In contrast to nicotinic acid which blocked the lipolytic action of catecholamines but had no direct effect on the lipase activity of homogenates (26), the addition of amytal had a direct inhibitory effect on lipase (27). Amytal, piericidin A, and rotenone are primarily inhibitors of the respiratory chain prior to the entry of succinate (147). Papaverine also blocks respiration in liver mitochondria prior to succinate entry (327), and its inhibitory effect of lipolysis (discussed earlier) may be mediated through the same mechanisms.

Piericidin A (12 μM) was a noncompetitive inhibitor of lipolysis (261). Rotenone (1 to 50 μM) inhibited the lipolytic action of catecholamines and of dibutyryl cyclic AMP in a noncompetitive fashion after a lag period of about 30 min (129, 130, 134, 136). There was only a very small effect of rotenone on cyclic AMP accumulation due to norepinephrine and theophylline (133).

Probably inhibition of mitochondrial electron transfer by amytal, piercidin, rotenone, and papaverine reduces the availability of energy required for activation of triglyceride lipase. The lack of effect on cyclic AMP accumulation, the inhibition of dibutyryl cyclic AMP action, and direct inhibition of lipase support this hypothesis.

2. Antimycin A, oligomycin, and dinitrophenol. Antimycin A is another inhibitor of electron transport in mitochondria like amytal, rotenone, and piericidin A except that it blocks the respiratory chain between cytochromes *b* and c_1 (192). Antimycin (10 μM) inhibited lipolysis due to both catecholamines and dibutyryl cyclic AMP (245) and appeared to act in a similar fashion to the other inhibitors of electron transport. Antimycin A (112) at 6 μM and rotenone (134) at 10 μM blocked respiration by over 50% in white fat cells.

Uncouplers of oxidative phosphorylation such as 2,4-dinitrophenol (1 mM) also blocked the activation of lipolysis by catecholamines (129) and dibutyryl cyclic AMP (130) in a noncompetitive fashion. Dinitrophenol reduced the maximal accumulation of cyclic AMP which was seen in the presence of norepinephrine and theophylline from 23 to 1.3 nmoles per g (133). The inhibition of cyclic AMP accumulation may be secondary to reduction in availability of ATP for adenylate cyclase. It is of interest that the effect of 1 mM dinitrophenol on cyclic AMP accumulation was much greater than that of 10 μM oligomycin or rotenone which gave equivalent inhibitions of lipolysis.

Oligomycin is an antibiotic which blocks energy-transfer reactions involved in the formation of ATP from the conserved energy of electron transport. Oligomycin (1 to 10 μM) was a potent noncompetitive inhibitor of lipolysis due to lipolytic agents and dibutyryl cyclic AMP (122, 130, 136). The antilipolytic action of oligomycin was ac-

companied by a reduction in fat cell cyclic AMP content in the presence of norepinephrine and theophylline from 23 to 5 nmoles per g (133). A reduction of this magnitude was probably not sufficient to reduce cyclic AMP below levels which are rate limiting for lipolysis. The effect of oligomycin is probably not on cyclic AMP accumulation except as an indirect consequence of reduction in ATP availability for adenylate cyclase.

Oligomycin inhibited glucose oxidation in white fat cells (122, 164) and increased lactate accumulation (122). Oligomycin (1 μM) lowered total ATP in white fat cells under conditions in which it had only a small inhibitory effect on respiration (21, 125). There was little inhibition by oligomycin of basal respiration except in the absence of glucose (125, 134). Oligomycin did inhibit respiration of fat cells in the presence of insulin and glucose but not the increase in respiration due to epinephrine (164).

None of the inhibitors of electron transport such as piericidin A (207, 216), avenaciolide (207, 218), rotenone (113), antimycin A (112), or oligomycin (122, 164) have been reported to increase glucose metabolism. They are usually inhibitors of glucose metabolism in fat cells. Uncouplers such as dinitrophenol have not been found to stimulate glucose metabolism in fat cells (320). In marked contrast are the naphthoquinones, which are also antilipolytic but stimulate glucose metabolism (112, 208, 217).

3. Naphthoquinones. Naphthoquinones are a group of antilipolytic agents which actually increase cyclic AMP accumulation in fat cells (112, 208). Menadione (2-methyl-1,4-naphthoquinone) or the products obtained from auto-oxidation of vitamin K_5 (2-methyl-4-amino-1-naphthol) stimulated cyclic AMP accumulation (112). These effects were seen within 5 min after addition of the naphthoquinones, while lipolysis was not inhibited at this early time period (112). The naphthoquinones did not reduce the glucose-6-phosphate or ATP content of fat cells but did stimulate respiration, lactate formation, glucose oxidation, and fatty acid synthesis (112). No stimulatory effects of naphthoquinones on adenylate cyclase activity of fat cell ghosts could be demonstrated (112).

The effects of naphthoquinones on fat cell metabolism were similar to those of insulin with respect to inhibition of lipolysis and stimulation of glucose metabolism (112, 208, 217). However, menadione did not stimulate protein synthesis in fat cells (124), and naphthoquinones increased cyclic AMP accumulation whereas insulin had no effect (112).

Naphthoquinones have been shown to be uncouplers of oxidative phosphorylation in rat liver mitochondria (70). However, classical uncouplers such as dinitrophenol (133) or carbonyl cyanide *m*-chlorophenylhydrazone (113) decreased the accumulation of cyclic AMP due to catecholamines in the presence of methyl xanthines. The sensitivity of menadione action to inhibition by antimycin A and cyanide (112) suggests that it may act as an artificial shuttle accepting electrons from NADH and then donating electrons into the electron transport chain at the level of cytochrome *b*.

As yet, we have no clear understanding of how naphthoquinones are able to mimic some of the actions of insulin and actually increase cyclic AMP accumulation. Natural oxidation-reduction carriers such as quinones or flavin derivatives may have a more important role in the regulation of fat cell metabolism than has been realized to date. One role could be as shuttles for the transfer of reducing equivalents between the cytoplasm, mitochondria, and other compartments.

4. Ionophorous antibiotics. Ionophorous antibiotics such as gramicidin, nonactin, and valinomycin have been shown to be inhibitors of the lipolytic action of catecholamines and of dibutyryl cyclic AMP (110, 121, 132, 211). Valinomycin was originally postulated to block adenylate cyclase activity in fat cells (211). However, what was actually measured was the accumulation of radio-

activity in cyclic AMP in cells where the ATP pool was labeled by prior incubation with radioactive adenine. There is no procedure currently available which measures just the adenylate cyclase activity of intact cells. Fain and Loken (121) found that valinomycin actually increased basal adenylate cyclase activity of fat cell ghosts and cyclic AMP accumulation by fat cells. The studies in figure 4 show that valinomycin did not block the small increase in cyclic AMP accumulation due to theophylline under conditions in which it blocked the lipolytic action of theophylline. If norepinephrine was added to fat cells in the presence of theophylline there was a marked increase in cyclic AMP accumulation which was only slightly reduced by valinomycin. Valinomycin is actually representative of a group of agents which markedly inhibit lipolysis in the presence of both norepinephrine and theophylline with little effect on cyclic AMP accumulation. Our studies on the mechanism of action of valinomycin suggest that its small inhibitory effect on cyclic AMP accumulation does not account for its antilipolytic action in fat cells (121).

The effects of valinomycin on fat cell lipolysis are more likely to be on the activation of lipolysis by cyclic AMP as it blocks the lipolytic action of dibutyryl cyclic AMP (121). Fain (110) originally suggested that the ability of valinomycin to increase passive K^+ efflux from cells and stimulate an energy-dependent influx of K^+ might utilize large amounts of energy. This would reduce the energy available for the activation of lipolysis. It is known that the presence of K^+ is required in order to observe an antilipolytic action of valinomycin, and, in the absence of K^+, valinomycin actually enhanced the activation of lipolysis by theophylline (110). If valinomycin acts as an uncoupler of oxidative phosphorylation by dissipating energy in cyclic K^+ flux which might ordinarily be used for activation of lipolysis and glucose metabolism, then total ATP content might be reduced. However, in brown fat cells valinomycin did not lower ATP content (121); even with a classical uncoupler of oxidative phosphorylation such as *m*-chlorocarbonyl cyanide phenylhydrazone, only a transient drop in total ATP is seen (126). At the present time valinomycin appears to be a K^+-dependent inhibitor of lipolysis (110, 121, 211) and glucose metabolism (110) in fat cells whose mechanism of action remains to be elucidated.

G. Nucleosides as Inhibitors of Cyclic AMP Accumulation and Lipolysis

Dole (99, 100) and Vaughan (361, 362) originally found that the addition of cyclic AMP (0.5 mM or higher) to incubated adipose tissue inhibited fatty acid release and increased glucose metabolism. The addition of RNA, ATP, AMP, cyclic AMP, or adenosine (0.2 to 1 mg/ml) reduced the release of fatty acids due to epinephrine (99). However, adenine, 2,6-diaminopurine, and 6-mercaptopurine (2 to 4 mM) enhanced fatty acid release in the presence of epinephrine (99, 301). Hypoxanthine, xanthine, purine, or adenosine (5 to 50 μM) were potent inhibitors of epinephrine but not corticotrophin-induced fatty acid release (301). Uric acid was without effect, and the adipose tissue converted hypoxanthine to uric acid. The addition of allopurinol (10 μg/ml), which is an inhibitor of xanthine oxidase, blocked the inhibitory effects of hypoxanthine and adenosine on epinephrine-induced glycerol release in adipose tissue (301). Allopurinol also blocked the marked increase in epinephrine degradation seen in the presence of hypoxanthine and adenosine. The mechanism of inhibition by the purines appeared to be the result of increased catecholamine degradation by hydrogen peroxide formed during the oxidation of the purine bases by xanthine oxidase (301).

The effects of high concentrations of nucleosides on adipose tissue are complex, for Davies (94) found equal inhibition of ACTH and norepinephrine action by 400 μM adenosine, inosine, or guanosine. Davies also found an inhibition by 1 mM inosine and adenosine of the lipolytic action of dibutyryl

cyclic AMP. Kappeler (193) found a marked inhibition of the increase in glycerol release due to glucagon by adenosine, AMP, ADP, ATP, cyclic AMP, coenzyme A, NAD, NADP, NADH, and NADPH but not by adenine or ribose in incubated fat pads. The amount of adenosine required to give half-maximal inhibition of lipolysis was around 50 μM (193). Pereira and Holland (287) found that 1 μM NADP, AMP, or adenosine but not adenine inhibited the norepinephrine-induced fatty acid release in incubated adipose tissue.

In isolated fat cells low concentrations of adenosine (5 to 75 μM) completely blocked lipolysis due to theophylline, had less effect on that due to norepinephrine in fat cells, and enhanced lipolysis due to dibutyryl cyclic AMP (101, 123). No effect was noted of allopurinol on the anti-lipolytic action of adenosine on fat cells (113) which indicates that these effects are not due to activation of xanthine oxidase.

The levoisomer of N^6-phenylisopropyl adenosine has been reported to be a potent inhibitor of the *in vitro* lipolytic action of norepinephrine and ACTH on isolated fat pads at concentrations in the range of 0.03 to 0.1 μM. The drug was much less active as an inhibitor of theophylline-induced lipolysis and had inconsistent effects on the basal lipolysis of fat pads from starved rats (380). In isolated fat cells *dl*-phenylisopropyl adenosine at a concentration of 5 μM was a potent inhibitor of lipolysis due to either theophylline or norepinephrine but did not inhibit the lipolytic action of dibutyryl cyclic AMP except at a concentration of 50 μM (123). A major difference between the effects of adenosine and phenylisopropyl adenosine is that the latter was without effect on the adenylate cyclase activity of fat cell ghosts (123).

The adenylate cyclase activity of fat cell ghosts incubated in the presence of saturating concentrations of norepinephrine (0.2 mM) was inhibited by adenosine, 2′-deoxyadenosine, 3′-deoxyadenosine, arabinosyladenosine, and 2-fluoroadenosine at concentrations between 20 and 75 μM (123). The most potent adenosine nucleoside tested was 2′,5′-dideoxyadenosine, which was active at concentrations in the range of 2 to 10 μM (123). There was no inhibition of the cyclase by adenine, inosine, 2′-deoxyinosine, guanosine, 7-deazaadenosine, 8-amino-2′-deoxyadenosine, 2-chloroadenosine, or 6-methoxyadenosine (123). It was considered unlikely that the inhibitory effect of the adenosine nucleosides was the result of conversion to the nucleotides since 2′-deoxyadenosine monophosphate was less active than 2′-deoxyadenosine. The most potent inhibitor of adenylate cyclase was 2′,5′-dideoxyadenosine which cannot be converted to a 5′-nucleotide (123). Fain, Pointer, and Ward (123) suggested that adenosine or related nucleosides might play a physiological role as regulators of adenylate cyclase activity.

Although guanosine or 2′-deoxyguanosine were ineffective as inhibitors of adenylate cyclase, guanosine tri- or tetraphosphates (30 to 40 μM) were potent inhibitors of fat cell ghost adenylate cyclase (123). Cryer, Jarett, and Kipnis (79) had originally reported that 400 μM GTP, GDP, GMP, CTP, and UTP but not guanosine or cyclic guanosine 3′,5′-monophosphate were inhibitors of adenylate cyclase activity in fat cell ghosts. Guanosine triphosphate (GTP) was effective as an inhibitor at concentrations in the range of 1 to 10 μM (79). GTP (4 μM) did not inhibit the adenylate cyclase activity of adrenal particulate preparation while it enhanced by 40% the activity of kidney particulate preparations. Rodbell *et al.* (318) found that GTP at concentrations in the range of 0.01 to 0.1 μM markedly potentiated the activation by glucagon of adenylate cyclase in rat liver membrane preparations.

Adenosine and 2′,5′-dideoxyadenosine inhibited the large accumulation of cyclic AMP seen in intact fat cells 5 min after the addition of catecholamines in the presence of methyl xanthines (123; fig. 4). However, there was no antilipolytic effect of the nu-

cleosides under these conditions. Even the small increase in cyclic AMP accumulation seen after incubation of fat cells with theophylline for 5 min was almost completely blocked by 2′,15′-dideoxyadenosine, but this was not accompanied by any reduction in the lipolytic action of theophylline even if lipolysis was measured after 60 min incubation (123). The results with 2′,5′-dideoxyadenosine indicate that almost complete inhibition of the increase in cyclic AMP is not necessarily accompanied by a reduction in lipolysis. One explanation of these results is that the cyclic AMP pool involved in the physiological regulation of lipolysis is small and unequilibrated with the total cyclic AMP of fat cells. This suggests that measuring effects of drugs or hormones on cyclic AMP accumulation may indicate little about effects on the small compartment of cyclic AMP which regulates lipolysis. It is also possible that the interaction of catecholamines with the *beta*-adrenergic receptor results in the enhanced release of bound cyclic AMP or increased sensitivity of the protein kinase to cyclic AMP, or both, and secondarily an increase in adenylate cyclase.

Possibly adenosine or some other metabolite of cyclic AMP could explain the findings of Ho and Sutherland (171) and of Manganiello, Murad, and Vaughan (244). They found that something accumulated after stimulation of cyclic AMP accumulation by catecholamines in the presence of methyl xanthines which prevented further activation of adenylate cyclase. It may be that many agents which either inhibit or stimulate cyclic AMP accumulation due to catecholamines in the presence of methyl xanthines influence the formation of a negative feedback inhibitor of adenylate cyclase.

H. Sulfonylureas and Biguanides

Tolbutamide (0.1 to 1 mg/ml) is a potent inhibitor of lipolysis due to catecholamines, ACTH, theophylline, glucagon, growth hormone and dexamethasone, and dibutyryl cyclic AMP in white adipose tissue or cells (43–45, 352). The accumulation of cyclic AMP in white fat cells was enhanced by tolbutamide under conditions in which it inhibited lipolysis (43). A similar nonspecific inhibition of lipolysis by tolbutamide has been seen in brown fat cells (126).

The data in figure 5 illustrate the rapid inhibitory effect of tolbutamide on catecholamine-induced lipolysis in the presence of theophylline in brown fat cells and the inability of tolbutamide to affect the large increase in cyclic AMP accumulation. The data also show the relatively small effect of tolbutamide on total ATP (fig. 5). The effect of tolbutamide on cyclic AMP, ATP, and lipolysis was similar to that of a classical uncoupling agent such as *m*-chlorocarbonyl

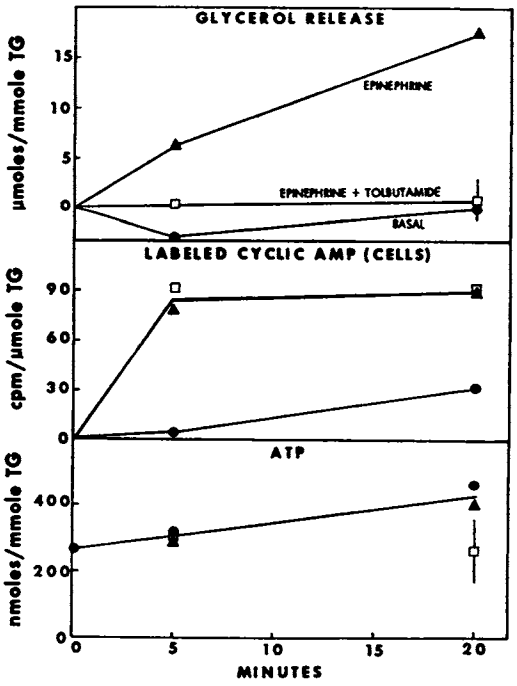


FIG. 5. Failure of tolbutamide to affect cyclic AMP or ATP accumulation in brown fat cells.

Brown fat cells (11 μmoles of triglyceride per tube) were incubated in 1.5 ml of buffer containing 2% albumin, 0.8 mM theophylline, and 2.8 mM glucose. The values are the means of nine paired experiments, and the error bar for the cells incubated for 20 min with tolbutamide represents the standard error of the paired differences between epinephrine (1.3 μM) without and with tolbutamide. The data are taken from table 1 of reference 126.

cyanide phenylhydrazone after 20 min incubation (126). The uncoupler differed from tolbutamide in that there was a lag period before it had an antilipolytic action, and, after 5 min incubation, there was a transient drop in ATP and cyclic AMP accumulation (126). Both tolbutamide and *m*-chlorocarbonyl cyanide phenylhydrazone increased respiration in brown fat cells and markedly enhanced glucose oxidation in the presence of insulin (68). There was little effect of tolbutamide on glucose metabolism of white fat cells (300, 304). These data are compatible with the hypothesis that tolbutamide acts by interferring with energy metabolism in fat cells and acts by a mechanism similar but not identical to that of a classical uncoupler of oxidative phosphorylation.

Biguanides such as phenformin (DBI, 1-β-phenethylbiguanide), buformin (1-butylbiguanide), and metformin (1,1-dimethylbiguanide) inhibited lipolysis (45, 351) and oxidative metabolism in adipose tissue (186, 291, 339, 370). Phenformin (0.05 to 0.2 mM) nonspecifically blocked lipolysis due to lipolytic agents and dibutyryl cyclic AMP (45, 351). The biguanides probably work by the same mechanisms discussed previously for inhibitors of electron transport linked to oxidative phosphorylation. The biguanides increased glucose conversion to lactate and decreased that to carbon dioxide, lipid, and glycogen in intact fat pads (370). Either in the absence or presence of insulin, phenformin (0.05 to 0.5 mM) inhibited [1-^{14}C]-glucose and [6-^{14}C]glucose oxidation, decreased respiration, and markedly increased the lactate to pyruvate ratio (291, 339). Phenformin also inhibited the oxidation of pyruvate, acetate, and glutamate by adipose tissue (186). Buformin was equivalent to phenformin, but metformin was somewhat less active (186). In adipose tissue there were relatively small effects of biguanides on glucose uptake and their primary effect was to divert glucose taken up by cells from oxidative to glycolytic pathways (186, 291).

The effects of biguanides on adipose tissue are in agreement with the hypothesis of Steiner and Williams (344) that they are inhibitors of aerobic processes. Whether the effects of biguanides observed in adipose tissue are seen under conditions in which they are effective in lowering blood glucose in man remains to be established, but is unlikely in view of the high concentrations required to affect fat cell metabolism.

I. Inhibitors of Glycolysis: Fluoride, Iodoacetate

Fluoride (10 mM) is the most potent known activator of adenylate cyclase in broken cell systems (22, 79, 188, 366), while in intact fat cells it almost completely blocks the stimulation of cyclic AMP accumulation by catecholamines plus methyl xanthines (112, 135, 210). Fain and Saperstein (127) found a stimulation by fluoride of cyclic AMP accumulation in fat cells but the cells had been incubated for 3 hr prior to the addition of fluoride. The cells were probably damaged just sufficiently to see fluoride stimulation of cyclase as in fat cell ghosts. In subsequent experiments no stimulation by fluoride of cyclic AMP accumulation could be obtained using intact fat cells (112). Fluoride also inhibited lipolysis in white fat cells due to all known lipolytic agents (112, 135, 257, 258). The nonspecific inhibitory effect of fluoride on lipolysis in intact fat cells may be the result of a depletion of ATP in fat cells. We have found equivalent decreases after 10 min incubation with oligomycin (1 μM) or fluoride (10 mM) of the ATP content in fat cells by 40% in the absence and by 70% in the presence of theophylline plus norepinephrine (113).

Fluoride (20 mM) was originally reported to be without effect on lactate formation by incubated adipose tissue (257). However, we have found that fluoride (10 mM) inhibited lactate formation by fat cells in the presence of glucose and insulin by over 60% (113).

Iodoacetate (1 mM) was found to stimulate basal lipolysis and cyclic AMP accumulation in adipose tissue. Iodoacetate (1 mM) inhibited the lipolytic action of norepinephrine or dibutyryl cyclic AMP and reduced

by 66% the large increase in cyclic AMP accumulation seen with norepinephrine and theophylline (135). The antilipolytic action of iodoacetate and fluoride is probably the result of interference with energy availability for activation of both adenylate cyclase and lipase by cyclic AMP.

J. Cardiac Glycosides and ATPase Activity in Fat Cells

High concentrations of ouabain (G-strophanthin), digitoxin, or strophanthin-K stimulate glucose oxidation and inhibit catecholamine-activated lipolysis in fat cells. The effects of ouabain (0.01 to 1 mM) on both parameters were dependent on the presence of K^+ in the buffer and abolished in the absence of K^+ (110, 169, 170, 262, 263). The antilipolytic action of ouabain is not dependent on the presence of either Ca^{++} or Mg^{++} in the buffer and is actually enhanced by their absence (263). Ho *et al.* (170) suggested that ouabain acted to inhibit adenylate cyclase. The available evidence suggests that any effect ouabain has on adenylate cyclase is indirect and is due to reduction in intracellular K^+. It is known that ouabain inhibits the uptake of K^+ (75) and amino acids (74) by fat cell ghosts, but no direct effect of ouabain on ghost adenylate cyclase has yet been reported.

Ouabain is relatively specific as an inhibitor of lipolysis due to low concentrations of agents which act by increasing adenylate cyclase. Under conditions in which ouabain blocks the lipolytic action of catecholamines it can actually potentiate the lipolytic action of theophylline (110) or dibutyryl cyclic AMP (227). High concentrations of potent glycosides, such as digitoxin, nonspecifically inhibit lipolysis due to all known lipolytic agents (391, 392). This probably reflects little more than a toxic side effect of glycosides.

The mechanism by which ouabain stimulates glucose metabolism and inhibits catecholamine-activated lipolysis may well be secondary to inhibition of (Na^+, K^+)-linked ATPase since these effects are mimicked by omission of K^+ from the incubation medium. However it is not established that the effects of cardiac glycosides on fat cells are all secondary to inhibition of K^+ uptake *via* a (Na^+, K^+)-dependent ATPase. For that matter it is not clear whether the effect of glycosides on ATPase activity has anything to do with the inotropic action of glycosides (234).

Ouabain only inhibited the ATPase activity of fat cell plasma membranes by 6% (187). Similarly omission of Na^+ and K^+ from the ATPase reaction medium produced no significant change in ATP hydrolysis (187, 256). In the presence of sodium azide it was possible to determine that at most 6 to 10% of the total plasma membrane ATPase activity was due to a specific (Na^+, K^+)-ATPase (187).

Ouabain has little effect on basal respiration in white (125) or brown (118) fat cells, and its ability to block the increase in respiration due to catecholamines appears to be secondary to inhibition of lipolysis. The stimulation of respiration due to methyl xanthines, dibutyryl cyclic AMP, and added fatty acids was unaffected by ouabain in brown fat cells (118). Ouabain was active in the presence of the above-mentioned agents since glucose oxidation was enhanced and lactate accumulation was diminished (118). These data do not support the hypothesis that the K^+-dependent stimulation of respiration in brown fat cells by added exogenous free fatty acids and endogenous fatty acids produced by activation of lipolysis by catecholamines involves stimulation of a ouabain-sensitive process (118).

K. Antimalarials

Quinine (6 mM) was found to be a potent inhibitor of catecholamine-induced fatty acid release (257). Chloroquine [7-chloro-4-(4-diethylamino-1-methylbutyl-amino) quinoline] at a concentration of 0.06 mM inhibited the stimulation of fatty acid release by lipolytic agents and the uptake of glucose by fat pads (17). Quinine, quinacrine [6-chloro-9-[(4-(diethylamino)-1-methylbutyl)-

amino]-2-methoxyacridine], chloroquine, primaquine [8-(4-amino-1-methylbutylamine)-6-methoxy-quinoline], and hydroxychloroquine inhibited lipolysis by both incubated adipose tissue slices and homogenates in the range of 0.2 to 1 mM (246). Quinidine at concentrations less than 1 mM inhibited lipolysis due to catecholamines and theophylline but potentiated that due to dibutyryl cyclic AMP, whereas higher concentrations non-specifically inhibited lipolysis due to all agents (299). Primaquine (1 mM) and quinacrine (0.1 or 1 mM) also inhibited the activation of fat cell ghost adenylate cyclase activity due to norepinephrine by over 50% (299).

The antimalarials appear to have many effects on adipose tissue or fat cells in the millimolar concentration range and they should probably not be considered as specific inhibitors of triglyceride lipase as suggested by Markus and Ball (246). Schimmel and Goodman (328) have measured the efflux of free fatty acids from adipose tissue and concluded that this was an energy-dependent process, but large amounts of antimalarials were used in all of the studies to block triglyceride lipase. In view of the many effects of antimalarials it is doubtful if anything can be concluded about whether fatty acid efflux from cells is passive or an active process requiring energy until studies are performed in the absence of antimalarials.

L. Chlorophenoxyisobutyrate

Chlorophenoxyisobutyrate [CPIB, Atromid-S, clofibrate, 2-(*p*-chlorophenoxy)-2-methylpropionic acid ethyl ester] lowers plasma triglycerides of man and experimental animals (13, 29, 357). Only at high concentrations of chlorophenoxyisobutyrate (0.5 to 2 mM) could an inhibition of epinephrine-induced fatty acid release be seen. Tolman, Tepperman, and Tepperman (357) found that in rats fed ethyl chlorophenoxyisobutyrate for 2 weeks, the major effect on adipose tissue was an increase in lipoprotein lipase activity.

Greene *et al.* (155) reported that in rats fed chlorophenoxyisobutyrate for 2 weeks the adenylate cyclase activity of fat, liver, and intestine homogenates was one-third to one-half less than in pair-fed controls. No direct inhibitory effect of chlorophenoxyisobutyrate on adenylate cyclase was seen. Whether the increase in lipoprotein lipase is related to the decrease in adenylate cyclase is not known.

M. Sulfhydryl Inhibitors

Exposure of fat cells to N-ethylmaleimide for only 10 sec results in an increase in basal lipolysis, decreased response to ACTH, TSH, or epinephrine and an increased response to theophylline and dibutyryl cyclic AMP (148, 247). We have similarly observed a marked increase in basal lipolysis after incubation of fat cells with N-ethylmaleimide (113). However, Calvert and Lech (59) found no increase in basal lipolysis due to N-ethylmaleimide, iodoacetamide, *p*-chloromercuribenzoate, and iodoacetic acid under conditions in which these agents inhibited the lipolytic action of catecholamines, theophylline, and dibutyryl cyclic AMP.

Phenylmercuric chloride is also a noncompetitive inhibitor of the lipolytic action of ACTH and catecholamines (148). In contrast charged mercurials, such as Hg^{++} and *p*-chloromercuribenzoate, had little effect on the action of TSH, ACTH, or epinephrine during the 1st hr after their addition, but they tripled the lipolytic response to theophylline and dibutyryl cyclic AMP (148). Charged mercurials may penetrate cells poorly in contrast to the uncharged phenylmercuric chloride. In view of the ubiquitous nature of sulfhydryl proteins, it is not clear what biochemical mechanisms are responsible for the observed effects. In all studies thiols have proven to be nonspecific inhibitors of lipolysis due to catecholamines and other activators of adenylate cyclase, whereas under some conditions they markedly increase basal lipolysis and the response to dibutyryl cyclic AMP. Little useful information with regard to possible thiol involvement in drug or hormone action

of fat cells has come from studies to date using sulfhydryl inhibitors.

N. Diethylchelidonate

Diethylchelidonate, the diethyl ester of 4-oxo-4H-pyran-2,6-dicarboxylic acid, was tested as a possible structural analog of triglycerides (393). It did not inhibit the triglyceride lipase of adipose tissue homogenates or pancreatic lipase (393). However, 0.1 to 0.3 mM diethylchelidonate blocked the activation of lipolysis by dibutyryl cyclic AMP, ACTH, theophylline, and norepinephrine without affecting the stimulation of phosphorylase (393). This compound may interfere with the activation of triglyceride hydrolysis by cyclic AMP.

O. Cocaine

Cocaine (2β-carbomethoxy-3β-benzoxytropane) administration to dogs potentiated the effects of intravenous norepinephrine on heart rate, mean systemic arterial blood pressure, blood glucose, and the concentration of plasma free fatty acids (159, 274). Cocaine blocks the indirect lipolytic action seen after administration *in vivo* of tyramine or metaraminol (α-1-aminoethyl)-*m*-hydroxybenzyl alcohol). However, cocaine did not activate lipolysis or potentiate the lipolytic action of norepinephrine on dog or rat fat cells *in vitro* (274). Cocaine (0.3 to 3 mM) actually inhibited lipolysis in the presence of norepinephrine (274).

P. Tyramine

There are many agents which are able to increase lipid mobilization *in vivo* by stimulating the release of norepinephrine. Many of these agents inhibit lipolysis rather than activate it after their addition *in vitro*. There was no direct lipolytic activity of tyramine on adipose tissue (349). However, tyramine increased lipid mobilization *in vivo* by causing the release of endogenous catecholamines since the tyramine effect was markedly reduced by prior treatment with cocaine or syrosingopine (methyl carbethoxysyringoyl reserpate) (349). The catecholamine content of adipose tissue was depleted by syrosingopine treatment (349).

The depletion of tissue catecholamines with α-methyl-3,4-dihydroxyphenylalanine (dopa) produced rather different results than treatment with α-methyl-*m*-tyrosine. The α-methyl-dopa was converted to a false transmitter α-methyl-norepinephrine which displaced norepinephrine. However, when tyramine was administered there was the usual increase in lipid mobilization *in vivo* (349, 378). *In vitro* studies indicated that the false transmitter α-methyl-norepinephrine which had displaced norepinephrine was just as potent as norepinephrine in activating lipolysis (378, 381).

Q. Antidepressants

Amitriptyline [10,10-dihydro-N,N-dimethyl-5H-dibenzo(a,d)cycloheptene-Δ^{5},γ-propylamine, Elavil]; imipramine [5-(3-dimethylamionopropyl)-10,11-dihydro-5H-dibenz(b,f)azepine, Tofranil]; and several other antidepressants inhibited the lipolytic action of epinephrine on adipose tissue at concentrations between 1 and 5 mM (261). Schneidermann and Opitz (329) found that protriptyline [N-methyl-5H-dibenzo(a,d)cycloheptene-5-propylamine] at a concentration of 0.2 mM increased basal fatty acid release by incubated adipose tissue but blocked the lipolytic action of norepinephrine at all concentrations tested (0.2 mM to 2 mM). Desipramine [10,11-dihydro-5-(3-(methylamino)propyl)-5-H-dibenz(b,f) azepine], protriptyline, and other antidepressants gave half-maximal inhibition of fatty acid release in the range of 0.2 to 0.7 mM (138, 329). Finger, Page, and Feller (138) found that desipramine was twice as potent as imipramine. Desipramine (0.1 mM) was a competitive antagonist of catecholamine-induced lipolysis, but at 0.5 mM it was a noncompetitive inhibitor (138).

Lovrien *et al.* (240) found that 0.2 mM amitriptyline inhibited lipolysis due to norepinephrine, theophylline, and dibutyryl cyclic AMP. The inhibition of the lipolytic action of norepinephrine and theophylline

was accompanied by an increase in the concentration of cyclic AMP (240). These results suggest that antidepressants are inhibiting lipolysis at some site other than on the accumulation of cyclic AMP. It is unlikely with the dosage of antidepressants used clinically that blood values are ever high enough to affect adipose tissue metabolism *in vivo*.

Amitriptyline administration to rats actually resulted in an elevated plasma free fatty acid level some 2 hr after acute administration (166, 281, 326). Since amitriptyline inhibited fatty acid release by adipose tissue *in vitro* the increase in plasma free fatty acid *in vivo* is probably an indirect effect.

All of the above antidepressants are of the dibenazepine group, but another group of antidepressants are the so-called monoamine oxidase inhibitors of the nonhydrazine type, such as pargyline (N-methyl-N-2-propynylbenzylamine) or tranylcypromine (2-phenylcyclopropylamine). Both of these agents have been reported to increase the concentrations of both glycerol and free fatty acids after their *in vivo* administration to rats (226). The effects of tranylcypromine and pargyline were markedly reduced in rats treated with propranolol or reserpine (226). It was claimed that neither drug had any direct lipolytic effect on adipose tissue (226).

R. Anorexigenic Drugs: Amphetamine, Fenfluramine

Anorexigenic compounds increase lipolysis *in vivo* but are without effect as activators of lipolysis *in vitro*. Amphetamine (*dl*-α-methylphenethylamine) increased the level of plasma free fatty acids after its administration to dogs (282) or human beings (296, 297), which appeared to be largely dependent on endogenous catecholamine release. Pinter and Pattee (296, 297) found that the increases in plasma free fatty acids seen after the administration of amphetamine or methamphetamine (*d*-N,α-dimethylphenethylamine, *d*-desoxyephedrine) were due to increased mobilization. Amphetamine, methamphetamine, fenfluramine (N-ethyl-α-methyl-*m*-(trifluoromethyl) phenethylamine), or tyramine at concentrations of up to 1 mM had no stimulatory effect on fat cell lipolysis (92, 93, 138). Fenfluramine (1 mM) was actually a nonspecific inhibitor of lipolysis which blocked the increase in lipolysis due to catecholamines, ACTH, caffeine, growth hormone + glucocorticoid, and dibutyryl cyclic AMP in fat cells (92, 93). In contrast, methamphetamine (1 mM) acted as a competitive and reversible inhibitor of catecholamine action and had little antilipolytic action against other agents such as caffeine or dibutyryl cyclic AMP (92, 282).

Fenfluramine administration to mice for periods of 1 to 4 weeks increased oxygen consumption, decreased the respiratory quotient and carcass fat content even though the controls were fed the same amount of food that the fenfluramine-treated mice consumed the previous day (285). Fenfluramine and other anorexigenic drugs also appear to depress transiently food consumption in man or experimental animals, which could contribute to a reduction of fat content. It should be realized that the adipose tissue triglyceride content is the reservoir for the calories consumed in excess of the energy expenditure. Thus any drug which depresses appetite and increases oxygen consumption, either by decreasing the efficiency of oxidative phosphorylation or by increasing the expenditure of energy indirectly, reduces the triglyceride content of adipose tissue.

Dannenburg and Kardian (93) suggested that fenfluramine might reduce adipose tissue triglycerides by depressing synthesis of fatty acids *via* a direct effect on adipose tissue. They found that 1 mM fenfluramine had a small inhibitory effect on glucose conversion to fatty acid without affecting glucose oxidation (93).

Wilson and Galton (383) found an inhibition by fenfluramine (>1 mM) of lipogenesis in homogenates of human adipose tissue. At the concentrations used, it is surprising

that fenfluramine did not have more of an effect on fat cells. No effect on lipogenesis in adipose tissue could be seen after the administration of fenfluramine to human beings. Since the blood levels of anorexigenic agents is unlikely to ever reach concentrations of 1 mM, the predominant effect, if any, of these drugs on lipid mobilization *in vivo* would be as indirect stimulators of lipid mobilization.

S. Serotonin

Serotonin (5-hydroxytryptamine) ordinarily has little lipolytic activity on white adipose tissue unless theophylline or a monoamine oxidase inhibitor is present (20). Serotonin is more rapidly deaminated by adipose tissue than is norepinephrine (20). The potentiation of serotonin action by theophylline might be explained if serotonin was a weak activator of adenylate cyclase.

In brown fat cells similar results were seen and serotonin was shown to increase cyclic AMP accumulation by a process which was preferentially inhibited by propranolol (118). These results suggest that serotonin (5 to 100 μM) has a direct lipolytic effect due to activation of *beta*-adrenergic receptors in fat cells. There is also an indirect lipolytic action of serotonin in intact adipose tissue due to release of bound catecholamines (390).

T. Thyroid Hormones

Thyroid hormone administration to rats enhances the response of adipose tissue to lipolytic agents but only after a lag period of at least 3 hr (38, 58). In contrast, the addition of large amounts of certain thyroxine derivatives to adipose tissue or fat cells *in vitro* has an immediate lipolytic effect. The *in vitro* effects are probably unrelated to the physiological potentiation of lipolysis by L-3,5,3′-triodothyronine (T-3) for the reasons discussed below.

Vaughan (363) first reported that direct addition of T-3 at concentrations greater than 3 μM enhanced the lipolytic response to epinephrine during the 1st hr after its addition. Several laboratories have subsequently reported direct effects of T-3 (5 to 1000 μM) on lipolysis in white fat cells (66, 67, 183, 243). L-Diodo-3,5-thyronine is a thyroxine derivative with little effect on lipolysis after *in vivo* administration (183), but it was as potent as T-3 in increasing basal and catecholamine-stimulated lipolysis *in vitro* (183, 243). Both compounds appeared to be equally effective as inhibitors of phosphodiesterase activity in adipose tissue. The amount of T-3 required for half-maximal inhibition of phosphodiesterase was around 400 μM (183, 243). The stimulation of basal lipolysis by 0.1 mM T-3 was reduced by the presence of insulin (183, 243) or prostaglandin E_1 (183, 243) but was unaffected by Kö 592 (0.1 mM) (243) or phenoxybenzamine (0.1 or 10 mM) (183). T-3 also potentiated the lipolytic action of theophylline or added cyclic AMP (183). These results indicate that there is a direct lipolytic effect of millimolar concentrations of T-3 which may be the result of inhibition of phosphodiesterase. The *in vitro* effects are probably unrelated to physiological effects of T-3 on lipolysis which are seen at nanomolar concentrations and only after the *in vivo* administration of L-T-3 or T-4 (L-3,3′,5,5′-tetraiodothyronine, L-thyroxine).

Hyperthyroidism is associated with elevated plasma free fatty acids (305) and an exaggerated lipolytic response in white adipose tissue or free cells to catecholamines (40, 58, 95, 96, 139, 152, 153, 203, 321). There is also an increased rate of respiration in adipose tissue (139) or fat cells (125) from hyperthyroid rats. Hypothyroidism results in a decrease in the lipolytic response to catecholamines (96, 139, 203, 321). A diminished lipolytic response to norepinephrine, isoproterenol and dibutyryl cyclic AMP has also been seen in adipose tissue from hypothyroid human beings (322). In hypothyroid animals the response of adipose tissue to very high concentrations of lipolytic agents is unimpaired (139, 153, 203, 322).

The main effect of T-3 is on the sensitivity

of the enzymes involved in lipolysis to activation not only by submaximal concentrations of catecholamines but also glucagon (153) TSH (153), and dibutyryl cyclic AMP (58, 322). Several possibilities could account for these findings. An inhibition of cyclic AMP phosphodiesterase by T-3 treatment is possible but has never been seen after *in vivo* treatment on rats with T-3 (58, 67, 203). Krishna, Hynie, and Brodie (203) saw an increased adenylate cyclase activity in adipose tissue homogenates after 5 days treatment with T-3. Caldwell and Fain (58) found no effect of T-3 administration 18 hr prior to sacrifice on the adenylate cyclase activity of fat cell ghosts. No increase in cyclic AMP accumulation due to norepinephrine alone was seen in fat cells from T-3-treated rats (58). There was an increase in the large accumulation of cyclic AMP due to catecholamines in the presence of theophylline which had a lag period of 4 and 6 hr (58). A similar lag period is required for T-3 effects on lipolysis (38).

The relationship between the effects of T-3 on cyclic AMP accumulation and lipolysis remains to be established. The data indicate that thyroid hormones influence lipolysis after a lag period which is longer than that for other lipolytic agents. If T-3 does affect the concentration of cyclic AMP or its ability to activate the triglyceride lipase, it is by processes which remain to be elucidated.

The effect of T-3 could be on mitochondrial metabolism. The increased respiration in fat cells from rats given T-3 was seen in the presence or absence of glucose, was abolished by oligomycin (1 μM), and was not associated with elevated ATP values (125). In fat cells from T-3-treated rats incubated with norepinephrine or theophylline, or both, the ATP content was considerably less than in controls.

The available evidence does not suggest that thyroid hormones affect the adrenergic receptor of fat cells. Rather the data are more compatible with the hypothesis that the ability of thyroid hormone to potentiate the effects of submaximal concentrations of catecholamines on lipolysis in fat cells and phosphorylase activation in the heart (175) is secondary to a primary effect on mitochondrial energy metabolism.

The calorigenic action of T-3 on fat cells appears to be due to either a decreased efficiency of oxidative phosphorylation or an increased utilization of energy derived from oxidative metabolism. No evidence was found (125) for an increased utilization of energy for fatty acid reesterification in fat cells from T-3-treated rats as suggested by Fisher and Ball (139).

Another hypothesis to explain the calorigenic action of T-3 is increased cation flux *via* a ouabain-sensitive process. Ismail-Beigi and Edelman (185) suggested this hypothesis to explain the calorigenic effect of prolonged T-3 treatment on respiration in liver slices. However, the increased respiration in fat cells seen after T-3 treatment was remarkably insensitive to ouabain even at a concentration of 1 mM (125).

Thyroid hormone action on lipolysis and calorigenesis in fat cells, as in other tissues, is probably the result of effects on mitochondrial metabolism. All of the observed effects of hyperthyroidism could be due to a decreased efficiency of mitochondrial oxidative phosphorylation. If this is the case, it remains to be established how this could potentiate the action of cyclic AMP.

U. Inhibitors of RNA and Protein Synthesis

There is no indication that inhibitors of RNA and protein synthesis affect the lipolytic action of ACTH (119, 229) or epinephrine (104, 117, 201, 210). High concentrations of puromycin (1.5 mM) have been shown to increase cyclic AMP accumulation and inhibit phosphodiesterase activity in rat diaphragm muscle (6). Rizack and Spencer (307) have claimed that the aminonucleoside of puromycin (2 mM), which does not block protein synthesis in adipose tissue, inhibited fat cell phosphodiesterase and increased lipolysis. Large amounts of dactinomycin (50 μM) inhibit the very high increase

in cyclic AMP seen after addition of ACTH to fat cells in the presence of methyl xanthines without affecting lipolysis (229). It is hardly surprising that large concentrations of inhibitors of RNA and protein synthesis influence fat cell metabolism, but the only known specific effects of such inhibitors are to block the lipolytic action of growth hormone and glucocorticoids (see section IX).

V. Parasympathomimetic Agents

The stimulation of lipolysis and glucose uptake by epinephrine was unaffected by 0.2 mM choline chloride carbamate (carbachol) or 0.2 mM acetylcholine plus 0.04 mM physostigmine (73). The combination of physostigmine plus acetylcholine also had no effect on basal lipolysis or glucose uptake (73).

IX. RNA and Protein Synthesis Involvement in the Lipolytic Action of Growth Hormone and Glucocorticoids

The addition of growth hormone to incubated pieces of white adipose tissue or free white fat cells increased lipolysis after a lag period of about 2 hr (119). There was no lipolytic action of glucocorticoid alone but it potentiated the action of growth hormone (57, 119). The amount of growth hormone required for activation of lipolysis under these conditions was in the range of 0.01 to 0.1 μg per ml with maximal activation obtained at a concentration of 0.1 μg per ml (119).

The lipolytic actions of growth hormone and glucocorticoids are unique in that they are blocked by inhibitors of RNA and protein synthesis, such as dactinomycin, puromycin, cycloheximide, ultraviolet light, and X-radiation (57, 105, 106, 116, 119, 127, 267). These inhibitors did not block the action of fast-acting lipolytic agents. Both the lipolytic effect of growth hormone alone and the ability of glucocorticoid to potentiate the action of growth hormone of fat cells from normal and hypophysectomized rats are sensitive to cycloheximide and dactinomycin (57).

The studies with inhibitors suggested that the stimulation of lipolysis by growth hormone and its potentiation by glucocorticoid both involve RNA synthesis. More direct evidence for the involvement of RNA synthesis was sought by measuring the incorporation of labeled uridine into fat cell RNA and of labeled ATP into RNA by isolated fat cell nuclei. A transient stimulation of uridine incorporation into total RNA of fat cells was seen in the presence of growth hormone and glucocorticoid (127). After 2½ hr of incubation, this was followed by a decrease in incorporation of labeled uridine into RNA (127). The incorporation of labeled ATP and RNA by a crude nuclear preparation from fat cells was enhanced if nuclei were isolated from fat cells after incubation for 1 hr with growth hormone and glucocorticoid (127). In nuclei isolated after 3 hr of incubation with hormones, the stimulation due to the hormones had disappeared (127).

The lipolytic action of growth hormone and glucocorticoid was blocked by inhibitors of protein synthesis such as puromycin (119) and cycloheximide (57, 105, 116, 267). Cycloheximide at a concentration of 0.1 μg per ml inhibited leucine incorporation into protein by 50%, in addition to inhibiting the lipolytic action of growth hormone by 50% (105). One microgram per ml of cycloheximide completely inhibited both protein synthesis and the activation of lipolysis by growth hormone and glucocorticoid (105). Cycloheximide inhibited lipolysis due to growth hormone alone as well as the potentiation of growth hormone action by glucocorticoid but not lipolysis due to norepinephrine or dibutyryl cyclic AMP (105).

The question arises as to how the protein(s) made under the influence of growth hormone and glucocorticoid affect lipolysis. If the protein(s) affect the accumulation of cyclic AMP, then one should see an increase in adipose tissue phosphorylase. Moskowitz and Fain (267) found that after incubation of fat cells with growth hormone and gluco-

corticoid for at least 3 hr there was an increased phosphorylase activity. Cycloheximide also specifically blocked the increase in phosphorylase activity in fat cell homogenates due to growth hormone and glucocorticoid (267). These studies suggested that growth hormone and glucocorticoids might be increasing the accumulation of cyclic AMP. After incubation of fat cells for more than 3 hr with growth hormone, it was possible to see an increase in the maximal accumulation of cyclic AMP after the addition of catecholamines in the presence of methyl xanthines (116, 127). There was no potentiation of the growth hormone effect on cyclic AMP accumulation by glucocorticoids (127). The effect of growth hormone on cyclic AMP accumulation was blocked by cycloheximide (127). These results suggested that a protein made under the influence of growth hormone might affect cyclic AMP accumulation but did not indicate whether it increased the formation or decreased the degradation of cyclic AMP. The effect of glucocorticoid appears to be more complex and, as in most other systems described to date, it apparently influences the action rather than the accumulation of cyclic AMP (114).

Efforts in our laboratory to find any effect of either growth hormone or glucocorticoids on phosphodiesterase activity of fat cells after incubation of the hormones for periods of up to 4 hr have been negative. However, it was possible to demonstrate an increase in the catecholamine-sensitive adenylate cyclase activity of fat cell ghosts prepared after incubation of fat cells for 3½ hr with growth hormone (table 1). There was no effect of prior incubation with growth hormone on either basal adenylate cyclase activity or the response to ACTH and fluoride (table 1).

The effect of growth hormone on lipolysis is summarized in figure 6. Growth hormone increases the synthesis of a protein(s) *via* DNA-dependent RNA synthesis, and this results in increased ability of catecholamines to activate adenylate cyclase. The protein(s) which affect adenylate cyclase are not lost during isolation of ghosts by hypotonic lysis of fat cells and may be associated with the plasma membrane. Figure 6 also reviews the currently accepted hypothesis for the lipolytic action of methyl xanthines and the activation of lipolysis by cyclic AMP.

TABLE 1

Increased norepinephrine-sensitive adenylate cyclase activity in fat cell ghosts after incubation for 3½ hr of fat cells with growth hormone

White fat cells were isolated from the parametrial adipose tissue of six rats by incubation in 14 ml of 4% albumin buffer containing 6 mg of trypsin and 8 mg of collagenase. After 45 min incubation the cells were isolated and then divided into four equal aliquots which were each incubated for 3½ hr in 13 ml of 4% albumin buffer. The concentration of growth hormone was 0.75 μg per ml. At the end of this incubation period ghosts were prepared from each group and then incubated for 20 min (approximately 45 μg of protein per tube) in duplicate in the absence or presence of 0.2 mM norepinephrine, 2 μg per ml of ACTH, or 10 mM sodium fluoride. The basal values are the means of 11 experiments and the changes due to growth hormone are the means ± standard error of the paired differences (J. N. Fain, unpublished experiments).

Additions to Ghosts during Assay for Adenylate Cyclase	Adenylate Cyclase Activity	
	Values for Ghosts from Control Cells	Difference Due to Incubation of Cells with Growth Hormone
	nmoles cyclic AMP formed/mg of protein × 20 min	
None	1.1	+0.8 ± 1.10
Norepinephrine	9.5	+3.8 ± 1.20
ACTH	3.9	−1.1 ± 1.00
Fluoride	29.2	+2.0 ± 2.8

X. Drugs and Lipase Activity in Cell-free Systems

A. Hormone-sensitive Triglyceride Lipase

The hydrolysis of the stored triglycerides in adipose tissue is catalyzed by a lipase which is activated by cyclic AMP and usually referred to as the hormone-sensitive lipase. It has been difficult to examine the factors regulating the activity of this enzyme. Unlike phosphorylase, most proce-

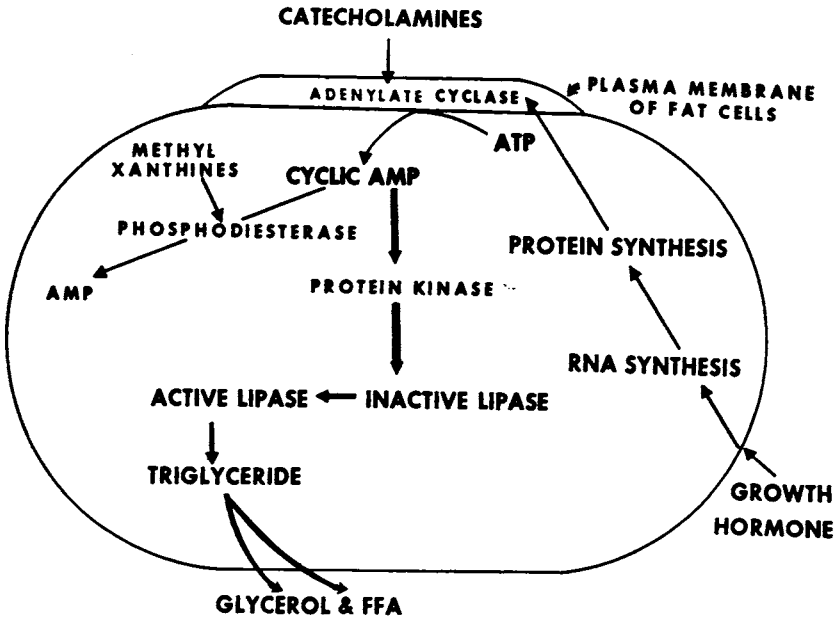


FIG. 6. A model for activation of lipolysis by growth hormone.

dures used for isolation of triglyceride lipase from fat cells result in a marked loss of activity. Under appropriate conditions, an increased lipase activity has been demonstrated in various cell-free systems prepared from adipose tissue which had been incubated with catecholamines or ACTH (173, 178, 335, 354, 365)

Rizack (306) found an activation of lipase in cell-free systems by added cyclic AMP, but until recently it was difficult to reproduce the findings (181, 358). Lipase activity using triolein as a substrate was enhanced by the combined addition of ATP, $MgCl_2$, and cyclic AMP to homogenates of rat fat which has been extracted with benzene (358) or the supernatant fraction obtained after centrifugation of adipose tissue homogenates at 78,000 $\times$ g (181).

If adipose tissue was homogenized in water and the crude homogenate was incubated in the presence of an inhibitor of protein kinase from muscle, an activation of the lipase activity could be seen by added cyclic AMP in the presence of added ATP and muscle protein kinase (78). Steinberg and associates have extensively investigated the triglyceride lipase activity prepared by homogenizing adipose tissue in isotonic sucrose containing 1 mM ethylene glycol bis(β-aminoethyl ether)-N, N'-tetraacetic acid after prior incubation in bicarbonate buffer for 4 hr to reduce the activity of lipoprotein lipase (179–181, 298). The homogenized adipose tissue was centrifuged for 1 hr at 78,000 $\times$ g, the pH adjusted to 5.2 and the precipitate was subjected to sucrose density gradient centrifugation (179–181). This resulted in a high molecular weight fraction with a $d < 1.12$ containing bound lipid which was over 90% phospholipid. The addition of cyclic AMP, ATP, and muscle protein kinase resulted in an increased triglyceride lipase activity which was accompanied by increased phosphorylation of the lipase by labeled ATP (179–181). There is also triglyceride lipase activity with a smaller molecular weight and little associated phospholipid which is activated by cyclic AMP in the presence of ATP and protein kinase (298).

Even in the most highly purified (100-fold) preparations of the high molecular weight, lipid-rich enzyme there is still five times

more mono- and diglyceride lipases activity than triglyceride lipase activity (162). The monoglyceride lipase activity was not enhanced by added cyclic AMP, Mg^{++}-ATP plus protein kinase, whereas the diglyceride lipase activity was increased by only 15% under conditions in which triglyceride lipase was increased by 70% (162). The high activity of the mono- and diglyceride lipases of adipose tissue and their relative insensitivity to cyclic AMP indicate that they are neither hormonally responsive nor rate limiting for triglyceride hydrolysis. Due to the high activity of the mono- and diglyceride lipases care must be taken in studies of the hormone-sensitive triglyceride lipase that substrates are free of mono- and diglycerides.

Various agents had different effects on the monoglyceride as contrasted to triglyceride lipase activity. Sodium chloride (1 M) inhibited monoglyceride lipase activity by more than 50% but did not affect triglyceride lipase activity. Isopropanol inhibited triglyceride lipase activity by 70% but had no effect on monoglyceride lipase activity (162). If the substrates were sonicated in the presence of 5 mM taurodeoxycholate, the triglyceride lipase was inhibited by 90% and the monoglyceride lipase activity was enhanced by 50% (162).

B. Activation of Lipase by EDTA and Catecholamines

A cell-free system has been described in which EDTA, serotonin, pyrophosphate, thyroxine, and catecholamines activated lipolysis and ACTH, glucagon, cyclic AMP, and theophylline were inhibitory (259, 260, 265, 279). In this system homogenized adipose tissue was incubated with EDTA or catecholamines (1.5 to 100 μM for each) for 1 hr in saline containing 25 mM Tris buffer without albumin. At the end of this time, phosphate buffer containing albumin was added, and the incubation was continued for 1 hr. The effect of EDTA was not abolished by addition of equimolar concentrations of calcium or magnesium (259). In this experimental design, originally proposed by Okuda *et al.* (279), EDTA and amines may affect lipid peroxidation. The effect might be specific for adipose tissue lipids since no activity was seen after substitution of adipose tissue lipids by artificial lipid emulsions such as coconut oil (Ediol) or Intralipid (260). In contrast, no specificity with respect to source of the triglyceride lipase has been observed in the following experimental design. The fat layer obtained after centrifugation of adipose tissue homogenates was sonicated, the sonicated suspension was centrifuged at 12,000 $\times$ g for 5 min, and the fatty layer was then incubated with 100 μM epinephrine for 1 hr (388). Lipolysis was measured by adding adipose tissue lipase (the supernatant fraction obtained after centrifugation of the sonicated fat layer), microbial lipase, or pancreatic lipase. Regardless of the source of the lipase, an effect of epinephrine to increase fatty acid release could be demonstrated (388).

Interestingly, 160 μM 3,4-dichloroisoproterenol inhibited by 70% the effect of 50 μM epinephrine if added 15 min prior to the 1-hr incubation of the sonicated adipose tissue lipids with epinephrine (280). In view of the high concentrations of amines that were used and the possibility that dichloroisoproterenol prevents the auto-oxidation of epinephrine, the results should probably be viewed as an example of a nonphysiological mechanism for increased fatty acid release in artificial systems. The mechanisms involved appear fairly complex but of no physiological significance.

C. Lipoprotein Lipase

There is another lipase present in adipose tissue usually referred to as lipoprotein or clearing factor lipase which is inhibited under conditions in which the hormone-sensitive lipase is active (174). The effects of hormones on the lipoprotein lipase are generally the opposite to their actions on triglyceride lipase. Lipoprotein lipase is concerned with the assimilation of circulat-

ing plasma triglycerides into adipose tissue in the fed state and is activated by insulin. In contrast, the hormone-sensitive lipase is involved in mobilization of stored triglycerides during starvation or stress and is inhibited by insulin.

Lipoprotein lipase is extracted from acetone-dried powders of adipose tissue by ammonium hydroxide and requires lipoprotein triglyceride as the substrate. Heparin activates the enzyme activity in extracts and accelerates its release from intact tissue (71). The lipoprotein lipase activity of incubated adipose tissue from fed rats falls rapidly in the absence of glucose and insulin (71, 172, 174, 284, 308, 387). The half-life of the lipoprotein lipase appears to be less than 2 hr.

In adipose tissue from starved rats incubated with glucose and insulin, one can see a 10-fold increase in lipoprotein lipase over a 9-hr incubation (386). The addition of lipolytic agents (276, 386) or dibutyryl cyclic AMP (284, 385) prevented the rise seen during incubation with insulin and glucose. The effect of added cyclic AMP or agents which increase cyclic AMP accumulation was similar to that of inhibitors of protein synthesis such as cycloheximide and puromycin (284, 385, 386). Patten (284) found that all of these agents blocked protein synthesis and that the correlation between inhibition of protein synthesis by dibutyryl cyclic AMP, cycloheximide, and other lipolytic agents and percentage of decrease in lipoprotein lipase was very good. These data suggest that cyclic AMP acts by inhibiting protein synthesis, but it is not clear whether the effect is direct or secondary to increased accumulation of fatty acids. The effect on cyclic AMP is more likely secondary to increased intracellular free fatty acid accumulation and the resulting decrease in fat cell ATP (284).

Dactinomycin increased the lipoprotein lipase activity of adipose tissue during incubation with glucose and insulin (386). However, the properties of this increase in lipase activity were different from those of the increases in enzyme activity seen in the absence of dactinomycin (386).

XI. Agents Which Stimulate Glucose Metabolism

The number of substances which can partially mimic the effects of insulin on the metabolism of fat cells is very large. Some agents stimulate glucose metabolism at low concentrations but inhibit it at higher concentrations. As yet, no substance has been discovered which is effective in the same concentration range as insulin and mimics all of its effects on fat cells. None of the agents discussed below have therapeutic value as substitutes for insulin in the treatment of diabetes. But investigations on the mechanisms by which agents partially mimic the ability of insulin to stimulate glucose transport into fat cells may provide insights into insulin action.

All of the agents discussed in this section preferentially stimulate the hexose monophosphate shunt pathway of glucose oxidation unlike lipolytic agents which increase glucose oxidation *via* the Embden-Meyerhof pathway. Whether any of the so-called insulin-like agents really mimic all of the effects of insulin such as increased glycogen, fatty acid and protein synthesis, and decreased lipolysis remains to be demonstrated. Naphthoquinones mimic the stimulation of lipogenesis seen with insulin (112, 217) but are unable to increase amino acid incorporation into protein (124). Ouabain increases glucose oxidation but decreases lactate accumulation (118). Dinitrophenol increases lactate formation in adipose tissue without affecting total glucose uptake (320).

In view of the uncertainty as to whether any of the agents which mimic the ability of insulin to increase D-[1-^{14}C]glucose oxidation and fatty acid synthesis are acting by the same mechanism as insulin or mimic all of the other effects of insulin, none of them will be referred to as "insulin-like." The use of this term in the past has created confusion and future use of the term should be restricted to those cases in which there

is definitive proof that the agent mimics all of the known effects of insulin.

It is difficult to measure glucose transport in adipose tissue and particularly in free cells because of the small intracellular water space. Jeanrenaud (190) has reviewed the evidence for glucose transport *via* facilitated diffusion. All of the stimulators of glucose metabolism mentioned in this section which have been tested appear to increase glucose transport rather than increase glucose uptake *via* simple diffusion. The usual tests employed have been inhibition by phloretin, deoxyglucose, and other inhibitors of glucose transport and the ability to increase 3-O-methylglucose counter-transport.

The simplest hypothesis is that agents which can slightly alter the conformation of the plasma membrane increase the facilitated diffusion of sugars. Many of these same agents at higher concentrations alter the plasma membrane so markedly that lysis of fat cells occurs and the insulin-like effect disappears.

A. Plasma Factors

At one time there was a flurry of interest in the ability of plasma factors other than insulin to stimulate glucose metabolism by fat pads or fat cells. Antoniades and Gunderson (5) postulated that one of these substances was really insulin bound to plasma factors which could be liberated by treatment with extracts of adipose tissue. However, little evidence is available to support this hypothesis (252). The major problem is that we do not have any clear evidence that there is any insulin present in the so-called "bound insulin" fraction of plasma (238, 252). A likely explanation is that proteolytic enzymes or other factors present in plasma are able to mimic the action of insulin on glucose oxidation by fat pads *in vitro*. There does not appear to be any physiological significance to plasma factors other than insulin in the regulation of glucose metabolism by muscle and adipose tissue.

B. Proteases, Phospholipases, and Neuraminidases

Trypsin, papain, ficin, chymotrypsin, subtilopeptidase A, and *Streptomyces griseus* protease all have the ability to stimulate glucose oxidation at concentrations of 2 to 10 μg per ml (213, 220, 221). Pepsin was without effect (220). Higher concentrations of trypsin (100 μg/ml or more) decreased basal glucose oxidation and abolished the response of fat cells and adipose tissue to insulin (120, 197–200).

Proteolytic enzymes affected only the V_{max} for glucose metabolism, whereas insulin lowered the apparent K_m values by one-half (207, 212). Thus the failure of proteases to affect the apparent K_m for glucose distinguishes their effects from those of insulin. The effect of proteases is apparently not to increase glucose uptake as a result of increasing the leakiness of cells because the increased glucose metabolism due to proteases was inhibited by phloretin (199) and 3-O-methylglucose (199, 213).

Kono and Barham (199, 200) found that in cells incubated for 2 hr after brief exposure to trypsin (1 mg/ml for 15 sec) the increase in glucose oxidation seen immediately after trypsin addition was lost. The "recovery" process was not blocked by 100 μM cycloheximide (199). Interestingly, cells that had recovered from brief trypsin treatment by a 2-hr incubation in the absence of trypsin had a 10-fold drop in sensitivity to insulin-stimulated glucose oxidation and of insulin binding (81, 199). Why brief exposure to trypsin should cause a transient stimulation of glucose metabolism which is followed by a marked drop in sensitivity of fat cells to insulin for both binding and stimulation of glucose oxidation is not known.

Kono reported that cells which had lost their sensitivity to insulin as a result of prolonged (15 min) incubation with trypsin also demonstrated recovery of the response to insulin. The recovery process was greatly reduced by exposure of fat cells to a large concentration (100 μM) of cycloheximide for 90

min prior to and for 120 min during the recovery period (199). Previously Kono (198) had found similar effects of 100 μM puromycin, but the aminonucleoside of puromycin which does not block protein synthesis in fat cells was also an effective inhibitor of recovery. This finding suggests that the inhibition by puromycin and cycloheximide of the recovery processes may be due to nonspecific effects unrelated to protein synthesis. The recovery process is not easy to demonstrate and under the conditions used by Cuatrecasas (80) and Rosenthal and Fain (323) there was no recovery from trypsin treatment. The difference is probably that conditions must be optimal to see any recovery. In the experiments of Kono and Barham (199, 200) there was a recovery of less than 5% of the insulin receptors. In cells which had recovered from trypsin treatment, 20 (200) to 100 (199) times more insulin was required to give an equivalent binding of insulin to the cells and stimulation of glucose oxidation as in controls.

In the presence of insulin the insulin receptors of fat cells were protected from inactivation by trypsin (83). The effect of trypsin is relatively specific for certain hormones (insulin and glucagon) and appears to be due to destruction of the ability of fat cells to bind insulin. Other agents such as phospholipase preparations are also insulin-like but unlike trypsin higher concentrations actually enhance insulin binding to fat cells (80).

Phospholipase C preparations (α toxin of *Clostridium perfringens*) were found by Rodbell to increase glucose oxidation at low concentrations, whereas higher concentrations lysed fat cells (313, 319). Cuatrecasas (80) found that concentrations of phospholipase C preparations which totally abolished insulin-stimulated glucose metabolism actually enhanced binding to fat cells. In brown fat cells there was no increase in glucose oxidation due to trypsin but various phospholipase C preparations were able to increase glucose oxidation (323). Rosenthal and Fain (323) suggested that impurities in the phospholipase C preparations might account for their effects.

Neuraminidase preparations also increase glucose oxidation by fat cells (82, 323). However, unlike trypsin, higher concentrations of neuraminidase appear to interfere nonspecifically with glucose metabolism (323) and insulin binding is unaffected (80). In studies using neuraminidase preparations from *C. perfringens* the effects have been attributed to contamination with an unknown agent with higher activity than any known enzyme (323) or to neuraminidase (82).

C. Thiols and Metal Ions

Cysteine and other thiols increased D-[1-^{14}C]glucose oxidation by fat cells at fairly high concentrations (230). More recently it has been found that the stimulation of glucose oxidation is dependent on the presence of certain metal ions present as contaminants in albumin (86). In albumin treated to remove these ions, one can see an effect of cysteine only in the presence of divalent copper (86).

Sulfhydryl inhibitors such as N-ethylmaleimide and iodoacetate have not been noted to increase glucose oxidation but rather inhibit basal and insulin-stimulated glucose metabolism in adipose tissue (97, 255, 283, 382) and fat cells (65). Sodium arsenite, on the other hand, stimulates glucose metabolism at low concentrations (5 to 50 μM), whereas at higher concentrations (0.5 to 1 mM) it inhibits basal and insulin-stimulated glucose metabolism (97, 215). A similar biphasic effect was seen with *p*-hydroxymercuribenzoate with regard to glucose metabolism (97).

Incubation of fat cells for 1 hr with Hg^{++} (0.1 mM) did not produce any increase in glucose oxidation, but the response to insulin was reduced (148). Zn^{++} (0.01 mM) has been reported to produce a small stimulatory effect on basal glucose metabolism of adipose tissue which was not increased if the concentration of Zn^{++} was raised to 10 mM (97). Cr^{+++}, Cd^{++}, Co^{++}, and Ni^{++} were without

effect at 0.01 mM, but 0.1 mM Ni^{++} or Co^{++} did increase glucose oxidation whereas neither 0.1 nor 1 mM Cr^{+++}, Fe^{++}, or Cd^{++} affected glucose oxidation (97). Ni^{++} but not Co^{++} at a concentration of 10 mM mimicked the stimulatory effect of insulin on glucose incorporation into glycogen (97). Both Ni^{++} and Co^{++} preferentially stimulated the oxidation of glucose, as did insulin (97).

D. Polyamines

Spermidine or spermine increased glucose oxidation by fat cells at concentrations in the range of 1 to 100 μM (237). No effect of putrescine was seen at similar concentrations (237). The stimulation of glucose oxidation due to spermidine was inhibited by 3-O-methylglucose and phloretin (237).

E. Saponins and Hemagglutinins

Concanavalin A is a divalent hemagglutinin which mimics the effects of insulin on glucose oxidation and lipolysis in fat cells (87). Aescins are plant saponins; they are able to stimulate glucose oxidation by fat cells at low concentrations, but higher concentrations are inhibitory (156). The effects of aescins were inhibited by 3-O-methylglucose and they increased the V_{max} rather than the K_m for [1-^{14}C]glucose oxidation (156).

F. Polyene Antibiotics

Polyene antibiotics such as filipin, nystatin, and pimaricin are thought to preferentially disrupt lipid membranes containing cholesterol. Filipin stimulated glucose metabolism at a concentration of 50 μM, whereas pimaricin and nystatin were effective at concentrations of 150 μM on fat cells (206). Increasing the concentration of the polyene antibiotics 4-fold resulted in an inhibition of glucose metabolism. Lipolysis is also inhibited by filipin, which had the unique property of increasiug the efflux of cyclic AMP from cells into the medium (210, 219). Another effect of filipin is to enhance insulin binding to a particulate preparation obtained by centrifugation of fat cell homogenates (80). The polyene antibiotics, like most of the agents mentioned in this section, appear to stimulate glucose oxidation secondarily to effects on membrane permeability.

XII. Drugs Acting as Inhibitors of Glucose Metabolism

The agents which inhibit glucose metabolism of adipose tissue can be divided into two categories. One group such as dexamethasone, 2-deoxyglucose, puromycin, puromycin aminonucleoside, and others preferentially blocks basal glucose metabolism. Other drugs have less effect on basal glucose metabolism than on the stimulation seen in the presence of insulin. It is most likely that the difference between the two conditions is the result of transport being rate limiting for glucose metabolism under basal conditions. In the presence of high concentrations of insulin, hexose transport is accelerated to such an extent that intracellular metabolism limits glucose oxidation. Thus a competitive inhibitor of hexose transport might have no detectable effect in the presence of insulin, whereas an inhibitor of metabolism might have little effect except in the presence of insulin. Avenaciolide and piericidin A are apparently inhibitors of glucose metabolism rather than transport, for they had little effect on basal hexose metabolism of fat cells but reduced the increase due to insulin (207, 216, 218).

The multiple processes for hexose entry into fat cells postulated by Fain (103, 104), Kuo (207), and Gutman *et al.* (158) appear to be a less likely explanation than the hypothesis mentioned above. All of their data can be explained by assuming a separate basal entry process for fructose and a single hexose carrier system which is responsible for basal glucose entry and for insulin-stimulated hexose entry.

The inhibition of glucose metabolism by dexamethasone and other glucocorticoids (103, 128, 231) appears to be primarily on transport into cells (85, 114, 389). Ordinarily glucocorticoids have little effect on insulin-

stimulated glucose metabolism, but with low concentrations of glucose and insulin it is possible to see inhibition of insulin action (85).

Inhibitors of protein synthesis such as puromycin (104, 158, 207) and cycloheximide (84) also inhibited basal glucose metabolism in fat cells. However, the aminonucleoside of puromycin mimicked the effects of puromycin on glucose metabolism but did not inhibit protein synthesis (104).

Dactinomycin had little effect on basal glucose metabolism of adipose tissue (84, 105, 158) but did block the inhibitory effect of glucocorticoids (84). Insulin action on glucose metabolism was unaffected by either inhibitors of RNA or protein synthesis except for inhibition by 10 μM dactinomycin of insulin action on fat cells (158). This concentration is almost 100-fold greater than that required to block RNA synthesis in fat cells (105, 119).

Avenaciolide preferentially inhibits insulin-stimulated glucose metabolism (218). This compound has been reported to be a specific inhibitor of glutamate transport in rat liver mitochondria (251). Examination of the structure of avenaciolide indicates that it can be considered as an analogue of glutamate (202). Avenaciolide is also an antilipolytic agent and inhibits palmitate oxidation and reesterification in fat cells (218). Avenaciolide had little effect on oxidation of a mixture of uniformly labeled amino acids or their incorporation into protein (218).

Phlorizin is an effective inhibitor of glucose uptake by adipose tissue (144) or cells (214). Phloretin (2′,4′,6′-trihydroxy-3-(*p*-hydroxyphenyl)propiophenol) is some 50 times as potent as phlorizin and arbutin (*p*-hydroxyphenyl-β-D-glucopyranoside) was also active (144). Phlorizin had no effect on glucose metabolism in fat cell homogenates (144).

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