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THE EFFECTS OF GLUCOCORTICOIDS ON THE DISTRIBUTION AND MOBILISATION OF ARACHIDONIC ACID IN FAT CELL GHOSTS

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1 The prostaglandin synthesizing enzymes were found to be present in fat cell ghosts isolated from rabbit adipose tissue.

2 Prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ were synthesized by ghosts after stimulation with adrenocorticotrophic hormone (ACTH).

3 Indomethacin was found to inhibit this synthesis but not the synthesis of lipoxygenase products.

4 When fat cell ghosts were stimulated by ACTH, fatty acid release was observed from both neutral lipids and phospholipids.

5 The arachidonic acid (AA) pool within the ghosts was identified: approximately 90% was present in the phospholipid fraction, 8.5% in the neutral lipids and 1.5% unbound.

6 The glucocorticoids were found to stimulate incorporation of [^{14}C]-AA into neutral lipids and inhibit its incorporation into phospholipids.

7 When fatty acid release was stimulated with ACTH, the glucocorticoids were found to inhibit the mobilisation of [^{14}C]-AA from the phospholipids and enhance its release from the neutral lipids.

8 The glucocorticoids inhibit prostaglandin formation in fat cell ghosts.

Introduction

Prostaglandin formation has been demonstrated in adipose tissue following nervous or hormonal stimulation (Shaw & Ramwell, 1968; Lewis & Matthews, 1970). The prostaglandin release associated with the vasodilatation accompanying lipolysis in rabbit was found to be prevented not only by prostaglandin synthetase inhibitors (Bowery & Lewis, 1973) but also by glucocorticoids (Lewis & Piper, 1975). These findings were confirmed *in vitro* in adrenocorticotrophic hormone (ACTH)-induced lipolysis in rabbit and catecholamine-induced lipolysis in human isolated fat cells (Chang, Lewis & Piper, 1977). Similar findings had also been made in lung tissue stimulated by anaphylaxis or blood vessels constricted with noradrenaline (Gryglewski Panczenko, Korbut, Grodzinska & Ocetkiewicz, 1975).

Christ & Nugteren (1970) had earlier observed prostaglandin synthesis in isolated fat cells and had suggested that the release of arachidonic acid (AA) from triglycerides during lipolysis was sufficient to account for the prostaglandin formed. On the other hand it had been thought for some time that the main source of prostaglandins was membrane phospholipids (Vogt, Suzuki & Babilli, 1966; Bartels, Kunze, Vogt & Willie, 1970; Kunze, 1970; Vargaftig & Dao Hai, 1972; Flower & Blackwell, 1976).

In a recent discussion of the mode of action of glucocorticosteroids on the prostaglandin system in adipose tissue (Lewis & Piper, 1978), it was concluded that prostaglandins can be synthesized from the AA derived from both phospholipids and neutral lipid triglyceride. Furthermore, it seems likely that glucocorticoids affect the system by inhibiting phospholipases and through some mechanism still unknown preventing the release of prostaglandins formed from the AA released from the triglyceride fraction.

In an attempt to clarify the mechanism of action of the steroids, we decided to use the simpler system represented by fat cell ghosts from which the fat droplets and much of the cytoplasm has been removed.

Methods

Preparation of fat cell ghosts

Adult female New Zealand white rabbits were killed by a blow on the head, fat tissue was removed and the

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fat cells isolated by the method of Rodbell (1964). Fat cell ghosts were prepared by lysis in hypotonic medium followed by resealing in hypertonic Krebs solution (Rodbell, 1967a, b).

Lipid extraction and fractionation

A whole lipid extraction was carried out by the method of Bligh & Dyer (1959). The neutral lipids and phospholipids were separated by silicic acid column chromatography (Hirsh & Ahrens, 1958). Separation of free fatty acids from neutral lipids was carried out on a florisil column (Carol, 1961) or by thin layer chromatography (t.l.c.). The phospholipids were fractionated into their subclasses by t.l.c. on a 20 × 20 cm glass plate coated with Kieselguhr H of 1 mm thickness, and then developed in chloroform: methanol:water, 65:25:4 (v/v/v) and visualised with iodine vapour. The neutral sub-classes were separated by t.l.c. on a 20 × 20 cm plate coated with Kieselguhr G, 0.25 mm thickness developed in petroleum ether (60° to 80°C): diethyl ether:acetic acid 80:20:1 (v/v/v).

Phospholipid and neutral lipid quantification

The phospholipids were quantified either by phosphate analysis after digestion of the lipid in 60% perchloric acid at 200°C for 2 h (King, 1932) or by fatty acid analysis by gas liquid chromatography (g.l.c.) using as internal standard pentadecanoic acid. The neutral lipids were quantified by fatty acid analysis by g.l.c. using pentadecanoic acid as internal standard.

Fatty acid analysis by gas liquid chromatography

Analysis of the fatty acyl chains of the phospholipids was carried out by g.l.c. of their corresponding methyl esters. These were obtained by taking the sample to dryness under N_2 and transesterifying with 2 ml boron trifluoride:methanol (14%). The anti-oxidant, butylated hydroxy toluene, and internal standard pentadecanoic acid were added before the esterification. The transesterification (more than 95% complete, Morrison & Smith, 1964) was carried out by incubating the samples at 70°C for approximately 15 to 30 min and the methyl esters extracted in petroleum ether (40° to 60°C) and washed twice with an equal volume of water. The ether was dried over anhydrous sodium sulphate, taken to dryness with N_2 and the methyl esters resuspended in a small volume of petroleum ether (Vigo, Goni, Quinn & Chapman, 1978).

The fatty acid methyl esters were separated on a column of 10% polyethelene glycol adipate coated on 100 to 120 mesh celite at 196°C with N_2 flow rate of 40 ml/min in a Pye Unicam 104 gas liquid chromatograph, using a flame ionisation detector.

Incorporation of [^{14}C]-arachidonic acid into the lipids

Fresh preparations of ghosts were incubated for 2 h with [^{14}C]-AA (10 μg/ml) with 10 μg/ml dexamethasone or 50 μg/ml hydrocortisone or without steroids. The steroids were added in Krebs medium and [^{14}C]-AA, initially in hexane, was dried down with N_2 and resuspended in Krebs containing 0.9 mg/ml bovine serum albumin (BSA). After 2 h, excess label was removed by precipitating the ghosts by centrifugation and resuspending in fresh Krebs-BSA. ACTH 1 μg/ml was then added. Control incubation received an equal volume of 0.9% w/v NaCl.

Liquid scintillation counting procedure

The radioactivity of samples was estimated with a Packard Tricarb liquid scintillation counter. The scintillation counting fluid was 5 g 2,5-diphenyloxazole (PPO), 0.25 g (1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene) (dimethyl POPOP) and 1 litre toluene. Counting efficiency was calculated from a sample channels ratio and all results were converted to d/min.

Prostaglandin extraction and assays

Immediately after incubation indomethacin 10 μg/ml was added, the medium acidified to pH 3.0 to 3.5 with 1 N HCl and prostaglandins were extracted. The labelled and endogenous prostaglandins were extracted in diethyl ether and the endogenous $PGF_{2\alpha}$ and E_2 assayed by radioimmunoassay. The radioimmunoassay was carried out by a method modified from Hennam & Johnson, Newton & Collins (1974) by Jose, Niederhauser, Piper, Robinson & Smith (1976). [^{14}C]-prostaglandins from ghosts which had been incubated with [^{14}C]-AA (10 μg/ml) were extracted as above and separated by t.l.c. 20 × 20 cm silica gel plate (100 μm layer) using ethyl acetate/isooctane/acetic acid/water (110:50:20:100) as developing solvent. In this system phosphatides remained at the origin. Prostaglandin $F_{2\alpha}$ had an R_F value (× 100) of approximately 18, AA an R_F of about 72, PGD_2 53 and Thromboxane B_2 (TxB_2) 33. Zones containing radioactivity were estimated by scintillation counting.

Table 1 Lipid fractions of fat cell ghosts and the percentage distribution of arachidonic acid (AA) in each

Lipid class	*% total w/w*	*% total AA*
Free fatty acids	4.9	1.6
Neutral lipids	32.9	8.5
Phospholipids	62.2	89.9

All solvents were analar reagents. Authentic standards of prostaglandins E_2 and $F_{2\alpha}$, TxB_2 and AA were run simultaneously. The dried plates were cut into 0.5 cm strips which were eluted with 1 ml methanol for 1 h before addition of 8 ml of scintillant used.

Materials used

Drugs were: $ACTH^{1-24}$ (Synacthen, Ciba-Geigy); bovine serum albumin, essentially fatty acid free (Sigma); [^{14}C]-AA (58 Ci/mol, Radiochemical Centre, Amersham); dexamethasone sodium phosphate (Glaxo); hydrocortisone 21-sodium succinate (Sigma); indomethacin (Merck, Sharp & Dohme); butylated hydroxy toluene (Sigma); pentadecanoic acid (Sigma); boron trifluoride/methanol (14%) (Sigma). Prostaglandins E_2 and $F_{2\alpha}$ were a gift from Dr J. E. Pike, Upjohn, Kalamazoo.

Results

Characterisation of fat cell ghosts

Light and electron microscopy and freeze fracture of the fat cell ghosts revealed closed structures essentially depleted of fat droplets and about 50% were still nucleated. It has been reported by Rodbell (1967) that the ghosts contained about 25% of the mitochondria, 2% of the soluble glycolytic enzymes (glucose 6-phosphate dehydrogenase, fructose 4,6-diphosphate, aldolase, lactic acid dehydrogenase), 15% of the hexokinase and 75% of the adenyl cyclase activity which can be stimulated by ACTH or glucagon.

Fatty acid composition of fat cell ghosts

When the whole lipid was extracted and separated into neutral lipids (NL), phospholipids (PL) and free fatty acids (FFA) and subsequently estimated quantitatively by g.l.c. or phosphate analysis, it was found that the ratio of neutral lipids to phospholipids was approximately 1:2 as shown in Table 1. The table also shows the distribution of AA in the different fractions. It is clear from these results that the largest proportion of AA is present in the phospholipid fraction.

The fatty acid composition of the two fractions is shown in Table 2. The saturated acids, e.g. palmitic (16:0) and stearic (18:0) and the unsaturated, oleic (18:1) and linoleic (18:2) make up 70% to 80% of the total in both fractions. The unsaturated precursor of prostaglandins with 2 double bonds (AA 20:4) made up about 1% in the NL fraction and 5% in the PL fraction, while the percentage of those precursors of PGE_1 or E_3 series (i.e. dihomo-γ-linoleic acid 20:3 and eicosapentanoic acid 20:5 respectively) were present in much lower proportions.

Further fractionation of the different lipid classes was carried out by t.l.c. using the appropriate solvent systems. The triglyceride fraction accounted for 98% of the fatty acids present in the neutral lipids. The remainder were present in monoglycerides, diglycerides and cholesterol esters. The distribution of different phospholipid subclasses is shown in Table 3. The

Table 2 Fatty acid composition of neutral lipids and phospholipids in fat cell ghosts

*Type of fatty acid**	*Neutral lipid fraction %*	*Phospholipid fraction %*
16:0	32.00 ± 1	20.96 ± 0.5
16:1	7.06 ± 2	4.27 ± 0.3
18:0	10.23 ± 3.7	17.23 ± 1.8
18:1	25.06 ± 2	17.13 ± 1.6
18:2	16.65 ± 0.6	18.33 ± 0.0
18:3	2.61 ± 0.4	1.32 ± 0.2
20:0	1.0 ± 0.6	0.17 ± 0.0
20:1	1.0 ± 0.5	0.2 ± 0.1
20:2	1.17 ± 0.7	0.3 ± 0.1
20:3	0.13 ± 0.0	0.5 ± 0.2
20:4	0.8 ± 0.2	5 ± 0.9
20:5	0.55 ± 0.1	1.26 ± 0.4
22:0	0.65 ± 0.2	0.23 ± 0.0
22:3	0.0	0.44 ± 0.2
22:4	0.3 ± 0.0	0.34 ± 0.0
22:5	0.2 ± 0.1	1.03 ± 0.0
22:6	1.07 ± 0.42	8.83 ± 0.6
24:0	0.0	7.6 ± 9.5

* The numerals indicate numbers of C atoms and double bonds.

results show that the main constituents are phosphatidylethanolamine and phosphatidylcholine, which also contain the largest proportion of AA.

Distribution of [^{14}C]-arachidonic acid incorporated into fat cell ghosts

[^{14}C]-AA 10 μg/ml, in a total of 3 ml was incubated with the ghosts; after 2 h 13% of the total was incorporated into the fat cell ghosts. After 2 h incubation excess label was removed by centrifugation and after washing the ghosts, whole lipid extraction was carried out and the different classes analysed. It was found that the [^{14}C]-AA was distributed in a different pattern from that of endogenous arachidonic acid. The neutral lipids were found to contain 10%, the phospholipids 23%, while 67% of the labelled AA remained free. The incorporation was therefore non-specific in that it was taken up equally into neutral and phospholipid, i.e. in proportion to the percentage of each present.

Effect of lipase activating agents on the release of arachidonic acid

(a) *Endogenous*: A number of compounds known to activate lipases or phospholipases in different organs or tissues were tested in fat cell ghosts.

Bradykinin (4 μg/ml) which has been found to stimulate phospholipase activities in lung and skin (Vargaftig & Dao Hai, 1972; Juan & Lembeck, 1976) also stimulated AA release in fat cell ghosts.

ACTH (1 μg/ml), dibutyryl cyclic adenosine 3',5'-monophosphate (1 mM) and theophylline (1 mM) were found to mobilise AA in fat cell ghosts. All these compounds are lipolytic agents in adipose tissue where the hormone-dependent triglyceride lipases are activated by cyclic adenosine 3',5'-monophosphate (cyclic AMP) (Butcher, Baird & Sutherland, 1968; Mosinger & Vaughan, 1969). ACTH stimulates cyclic AMP by activating adenyl cyclase, theophylline by inhibiting the phosphodiesterases.

(b) *Exogenous*: [^{14}C]-AA was incorporated into fresh ghost preparations as previously described. After removal of excess label, ACTH was added and the ghosts incubated for 2 h and then centrifuged and the total lipid extracted. The lipids were fractionated into the subclasses which were then analysed for radioactivity. It was found that ACTH caused the release of AA from both neutral lipid and phospholipid fractions. The amount of [^{14}C]-AA taken up into the triglycerides was (125 ± 13 μg AA/μg triglyceride) $\times 10^{-6}$ and was reduced to (80 ± 20 μg AA/μg triglyceride) $\times 10^{-6}$ when incubated with ACTH 1 μg/ml. The corresponding amount taken up by phospholipids was (250 ± 7 μg AA/μg phospholipid) $\times 10^{-6}$ which was reduced to (125 ± 10 μg AA/μg phospholipid) $\times 10^{-6}$ by ACTH.

Effect of glucocorticoids

The results given in Table 4 show the effect of glucocorticoids on the incorporation of AA into the differ-

Table 3 Distribution of phospholipid subclasses and arachidonic acid (AA)

Phospholipid subclasses	*% total w/w*	*% total w/w AA*
Lysophosphatidylcholine	1	0.8
Phosphatidylinositol	8	12
Phosphatidylserine	11	5
Phosphatidylethanolamine	37	47
Cardiolipin	9	3
Phosphatidylcholine	32	30.8
Phosphatidic acid	2	1.4

Table 4 Distribution of [^{14}C]-arachidonic acid in the lipid fractions of fat cell ghosts

Sample	*Neutral lipids*	*Phospholipids*	*Free fatty acids*
Control	10%	23%	67%
+ Hydrocortisone (50 μg/ml)	10%	7%	83%
+ Dexamethasone (10 μg/ml)	14%	7%	79%

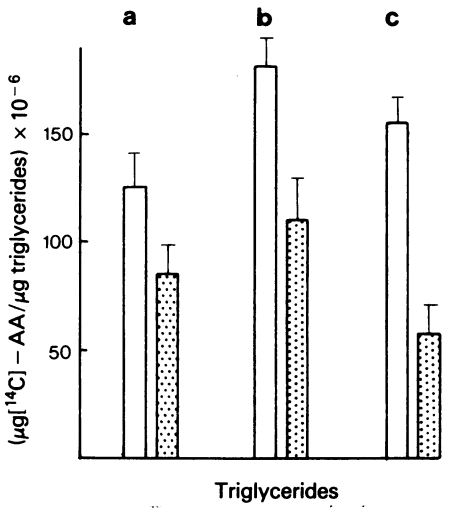

Figure 1 [^{14}C]-arachidonic acid ([^{14}C]-AA) incorporated into the triglycerides was measured (a) in the absence of steroids, (b) in the presence of hydrocortisone (50 μg/ml) and (c) in the presence of dexamethasone (10 μg/ml) before (open columns) and after (stippled columns) stimulation with ACTH (1 μg/ml). The figure shows that hydrocortisone and dexamethasone potentiated the action of ACTH on AA release.

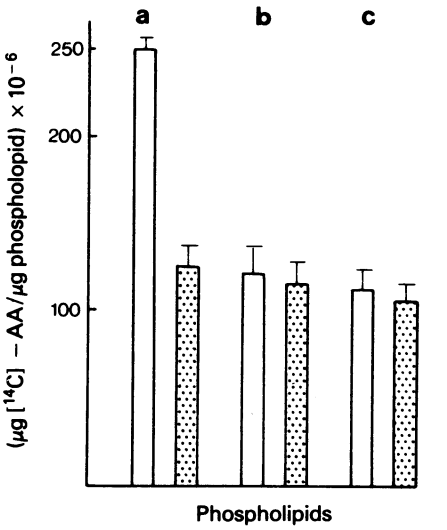

Figure 2 [^{14}C]-arachidonic acid ([^{14}C]-AA) incorporated into the phospholipids was measured (a) in the absence of steroids, (b) in the presence of hydrocortisone (50 μg/ml) and (c) in the presence of dexamethasone (10 μg/ml) before (open columns) and after (stippled columns) stimulation with ACTH (1 μg/ml). The figure shows that hydrocortisone and dexamethasone considerably reduce the AA release.

ent lipid fractions. On the neutral lipid the steroids have no effect or cause some enhancement. In contrast both hydrocortisone and dexamethasone significantly reduce the incorporation of [^{14}C]-AA into phospholipid. In another series of experiments illustrated in Figures 1 and 2, when corticosteroids, either hydrocortisone (50 μg/ml) or dexamethasone (10 μg/ml), were added to the ghosts before incubation with [^{14}C]-AA, the uptake of AA into the phospholipids was reduced while that into the triglycerides was enhanced. Furthermore, the release of AA from the PL fraction was inhibited while that from the NL fraction was increased.

The results are given in Figure 1 for the triglycerides and in Figure 2 for phospholipids. The open columns indicate uptake and the stippled columns the AA remaining after release by ACTH. The results given in Figure 1 show that the uptake of AA into the triglycerides was increased by hydrocortisone and also by dexamethasone although less significantly. In addition the release of AA, i.e. the difference between the open and stippled columns, was increased by hydrocortisone and even more so by dexamethasone. On the other hand the uptake of AA into the phospholipids (open columns of Figure 2) was considerably reduced by the corticosteroids as was the release as shown by the difference between open and stippled columns in Figure 2.

Figure 3 illustrates the effect of dexamethasone on the release of endogenous AA upon ACTH stimulation. As for exogenous AA, we observed that dexamethasone inhibited AA release from the phospholipids, while enhancing its release from the neutral lipids. The net effect which is given by the addition of these two curves was a slight decrease of the AA release as the concentration of dexamethasone was increased.

The prostaglandin system in fat cell ghosts

In view of the fact that the ghosts contain the enzyme systems necessary for the release of AA, it was of interest to establish whether they also contained the enzymes which convert AA to prostaglandins. [^{14}C]-AA was therefore incorporated into the ghosts which were then stimulated with ACTH. The radiolabelled prostaglandins synthesized under these conditions were extracted and analysed by t.l.c.

It was found that the percentage conversion of [^{14}C]-AA to PGE_2 and $PGF_{2\alpha}$ was 0.82 ± 0.44% and 1.37 ± 0.6% ($n = 4$) respectively. Conversion to other unidentified products amounted to 0.5%. Indomethacin inhibited PGE_2 and $PGF_{2\alpha}$-formation but not the

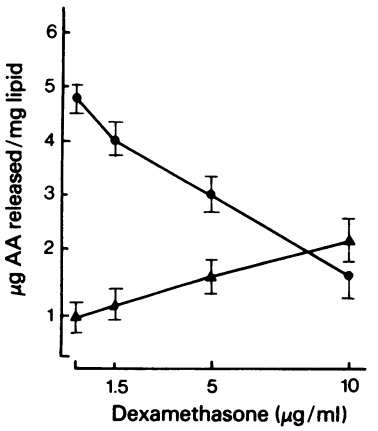

Figure 3 Effect of various concentrations of dexamethasone on the release of arachidonic acid (AA, μg/mg lipid) after stimulation with ACTH (1 μg/ml). This figure shows that dexamethasone increased the release of AA from the neutral lipids (▲) but decreased the release of AA from the phospholipids (●) as the concentration of dexamethasone was increased.

lipoxygenase products, as analysed by radiochromatography. Synthesis of prostaglandins from endogenous AA, determined by radioimmunoassay amounted to 10.3 ± 4.4 ng/mg lipid of $PGF_{2\alpha}$ and 9.8 ± 4.4 ng/ml lipid of PGE_2.

When the ghosts were incubated in the presence of steroids and stimulated by ACTH there was a small reduction in the formation of prostaglandins. Table 5 shows the effect of increasing concentrations of dexamethasone on the synthesis of $PGF_{2\alpha}$ and PGE_2. There was an overall reduction of $PGF_{2\alpha}$ and PGE_2 synthesis from (9.1 ± 5.8 = 14.9 ng/mg) to (6.5 ± 4.9 = 11.4 ng/mg) by dexamethasone 10 μg/ml. These values correspond very favourably with the reduction in AA release by the same concentrations of dexamethasone shown in Figure 3. The overall reduction of AA release by dexamethasone 10 μg/ml was from (4.8 + 0.7 = 5.5 μg/mg) to (2.2 + 1.6 = 3.8 μg/mg).

Discussion

The experiments described show that, as in rabbit adipose tissue *in vivo* or *in vitro*, in the simpler system of fat cell ghosts stimulation with ACTH leads to formation of prostaglandins E_2 and $F_{2\alpha}$. The precursor of these prostaglandins, AA, was found to be mainly present in the phospholipids (90%) while 8.5% was in the neutral lipids and 1.5% was unbound. When the ghosts were stimulated with ACTH in the presence of glucocorticoids these drugs inhibited the mobilisation of endogenous AA from the phospholipids but stimulated its release from the neutral lipids. Steroids also influenced the uptake of exogenous [^{14}C]-AA, inhibiting incorporation into phospholipids but stimulating inclusion into the neutral lipids. These important observations may be used to explain some earlier findings in adipose tissue of various species.

It has long been recognised that adipose tissue is a major site of lipid storage and release and that it is sensitive to adrenocortical hormones and catecholamines. The stimulatory effect of ACTH has been well documented (Jeanrenaud & Renold, 1960; Rodbell, 1964; Shaw & Ramwell, 1968; Mosinger & Vaughan, 1969; Rossell, 1969). ACTH acts at the plasma membrane level stimulating adenyl cyclase and producing an increase of intracellular cyclic AMP (Butcher *et al.*, 1968). This in turn leads to activation of the hormone-dependent triglyceride lipases.

In 1968 Shaw & Ramwell, working with rat epididymal fat pads, showed that prostaglandins were synthesized upon nervous or hormonal stimulation. Christ & Nugteren (1970), using isolated fat cells, found that the release of AA from the triglycerides during lipolysis was sufficient to account for the prostaglandins formed. All this previous work on fatty acid release has been restricted to their mobilisation from neutral lipids. The main advantage of studying fat cell ghosts is that hydrolysis of both phospholipids and neutral lipids can be investigated, whereas in the whole fat cell the changes in phospholipids would be masked by those in the more abun-

Table 5 The effect of glucocorticoids on prostaglandin formation in ghosts after stimulation with adrenocorticotrophic hormone (ACTH)

Treatment (μg/ml)		*$PGF_{2\alpha}$ (ng/mg lipid)*	*PGE_2 (ng/mg lipid)*
ACTH	*Dexamethasone*		
—	—	3.3 ± 1	3.6 ± 0.4
1	—	9.1 ± 2	5.8 ± 0.6
1	1.5	8.1 ± 2	4.8 ± 0.25
1	5	7.8 ± 2	5.1 ± 0.65
1	10	6.5 ± 1.4	4.9 ± 0.23

dant neutral lipids. The effect of glucocorticoids has also been well documented. Ashmore, Cahill, Hillman & Renold (1958) demonstrated that lipogenesis in diabetic rats could be restored by the use of corticosteroids. This work was followed by a wide study of the effect of these drugs on glucose accumulation and fatty acid release in this tissue both *in vivo* and *in vitro* (Jeanrenaud & Renold, 1960). These authors observed that the glucocorticoids stimulated the release of non-esterified fatty acids which was later confirmed by Munck (1962) and Munck & Koritz (1962) who proposed that the fatty acids were derived from the triglycerides.

It seems likely that the primary action of glucocorticoids is to decrease glucose utilisation by certain extrahepatic tissues such as adipose tissue, skin, lymphoid tissue and others by controlling glucose metabolism. At the same time they modify the metabolism of lipids and proteins so as to potentiate the action of the adrenocortical hormones which stimulate catabolism. In our present study we used fat cell ghosts from which most of the fat droplets had been removed and the final ratio of neutral lipids to phospholipids was 1:2. Fat cell ghosts are known to contain 75% of adenyl cyclase present in the original cells and can be stimulated by ACTH and glucagon (Rodbell, 1967a, b). In the study of AA release from fat cell ghosts we found that ACTH, theophylline, dibutyryl cyclic AMP and bradykinin were also capable of stimulating its release.

When ACTH was used to stimulate fatty acid release (particularly AA), we observed that fatty acids are not only released from neutral lipids but also from phospholipids and this happened when the release of endogenous or exogenous AA, which had previously been incorporated in the ghosts, was stimulated. This suggests that ACTH can also stimulate phospholipase A_2. Jeanrenaud & Renold (1960), Munck (1962) and Munck & Koritz (1962) showed that glucocorticoids potentiate fatty acid release after the stimulation with ACTH. In fat cell ghosts we observed the same effect on the AA release from the neutral lipids, but the opposite effect on release from the phospholipids was observed. Glucocorticoids inhibited the AA release from this fraction suggesting that phospholipase A_2 may be inhibited.

In whole fat cells 95% of the total weight is neutral lipids and therefore the effect of the glucocorticoids on the fatty acid release from the neutral lipids may be of major importance. This agrees with the observations of Christ & Nugteren (1970) that the AA released from the triglycerides during lipolysis is sufficient to account for the prostaglandins formed and with those of Chang *et al.* (1977) that glucocorticoids do not inhibit prostaglandin formation in adipose tissue.

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