Effect of Glucocorticoid Hormones on Fatty Acid Mobilization and Re-esterification in Rat Adipose Tissue

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1. The effect of dexamethasone and cortisol on fatty acid mobilization and re-esterification has been studied in intact adipose tissue and isolated fat cells of the rat. 2. Dexamethasone added *in vitro* inhibited both the re-esterification of mobilized free fatty acids and the esterification of palmitate in the medium. 3. Under several conditions (low concentrations of dexamethasone, cortisol at a high concentration, with tissue from starved animals), steroid-induced release of free fatty acids could be accounted for by decreased re-esterification only, overall lipolytic activity remaining unmodified. At higher concentrations of dexamethasone, however, stimulation of lipolytic activity also occurred. 4. Decreased re-esterification produced by dexamethasone was observed in the total absence of glucose from the incubation medium. Further, dexamethasone stimulated the disappearance of prelabelled [^{14}C]glycogen from the tissue. 5. The evidence presented suggests that mobilization of free fatty acids induced by glucocorticoid hormones under physiological conditions is primarily due to a decrease of the re-esterification rate rather than to lipase activation.

Experiments in this Laboratory (Jeanrenaud & Renold, 1960) have established that glucocorticoids *in vitro* stimulate FFA* mobilization from isolated rat epididymal adipose tissue, an observation that is consistent with the finding, *in vivo*, of a major effect of the adrenal cortex on lipid mobilization and ketogenesis, as described in pancreatectomized rats (Scow & Chernick, 1960) and in pancreatectomized baboons (Gillman, Gilbert, Epstein & Allan, 1958). These experiments *in vitro* have been confirmed and extended by several authors (Leboeuf, Renold & Cahill, 1962; Munk, 1962; Fain, Scow & Chernick, 1963), who have further shown that the effect on fatty acid mobilization is accompanied by decreased uptake and utilization of glucose. The decreased utilization of glucose became more apparent when incubation was prolonged over several hours (Herrera, Philipps & Renold, 1962, 1965). With adipose tissue from rats starved for 96 hr. and by using incubation periods of 9–24 hr., Philipps, Herrera & Renold (1965) have shown that cortisol added *in vitro* at a concentration of 0.025 $\mu\text{g./ml.}$ significantly inhibited the oxidation of labelled glucose to carbon dioxide, as well as the incorporation of glucose carbon into fatty acid and protein. This is well within the expected range of physiological concentrations of free cortisol in plasma.

* Abbreviation: FFA, free fatty acids.

The existence of this inhibitory effect of adrenal-cortical hormones on the metabolism of glucose, and more specifically on its conversion into glyceride glycerol, suggested decreased esterification as the mechanism responsible for the increased FFA release. The present experiments were designed to find out whether this was the case, by using as the glucocorticoid hormone a synthetic steroid of greater potency and solubility, dexamethasone. A preliminary report of these experiments has been published (Jeanrenaud & Renold, 1965).

MATERIALS AND METHODS

Animals. Male albino rats (140–160 g.), derived from a Wistar stock and bred in this Laboratory, were used throughout these studies. Unless stated otherwise, they were fed *ad libitum* with Altromin 'R' Laboratory chow (Kunath Co., Aarau, Switzerland).

Chemicals. [^{14}C]Glucose and [^{14}C]palmitate were obtained from The Radiochemical Centre, Amersham, Bucks. They had a chromatographic purity of 99% or greater. Glycerokinase, glycerophosphate dehydrogenase, ATP and NAD^+ preparations were purchased from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Dexamethasone of tested purity was a gift of Ciba Co., Basle, Switzerland; pig insulin was obtained from Novo Research Institute, Copenhagen, Denmark. Dexamethasone was dissolved in 50% ethanol, resulting in a final

ethanol concentration in the incubation media of 0.5% (v/v), a concentration that had no effect on control tissues.

Incubation technique. The rats were killed by decapitation without prior anaesthesia. To minimize the effects of differences in glycerol and FFA content between the proximal and the distal parts of the epididymal adipose tissues, each of the two pads from several animals was cut into two to six pieces, which were randomly distributed among the incubation flasks. The incubations were carried out in 25 ml. Erlenmeyer flasks containing 3 ml. of a Krebs-Ringer bicarbonate buffer to which was added dialysed human albumin (3.5 g./100 ml.; Swiss Red Cross, Berne, Switzerland). The buffer was equilibrated with $O_2 + CO_2$ (95:5). The details of the incubation technique have been described elsewhere (Winegrad & Renold, 1958). The glucose, dexamethasone and insulin concentrations, as well as the duration of the incubation, are specified for each experiment. All results have been expressed as μ moles/g. wet wt. of adipose tissue, except for a few experiments with fat cells isolated by the procedure of Rodbell (1964), in which the results have been expressed in terms of μ moles/g. of total lipids contained in the cell suspension.

Glycerol-free fatty acid balance. Estimation of lipolysis and re-esterification in intact adipose tissue was carried out according to the method of Vaughan (1962). Glycerol and FFA were measured in tissues and media before and after incubation. Assuming that the glycerol formed during the incubation was derived from triglycerides, and that the glycerol utilization and FFA oxidation were negligible, the total amount of FFA released from ester form in the system (tissue + medium) was taken as three times the amount of glycerol formed (in μ moles). The difference between total lipolysis (i.e. net glycerol production $\times 3$), and the net amount of FFA actually accumulating (by direct titration), was considered to represent the amount of FFA re-esterified into glycerides. Results thus obtained are in error to the extent that these assumptions are in error. Previous experiments have indicated, however, that free glycerol is little utilized by intact adipose tissue and that glycerol formation from non-glyceride sources does not occur to any significant extent (Steinberg & Vaughan, 1965). Although FFA are taken up readily by adipose tissue, FFA oxidation to CO_2 is known to be small (Bally, Cahill, Leboeuf & Renold, 1960). In the present study, for instance, palmitate oxidation during a 5 hr. incubation did not exceed 0.05μ mole/g. of tissue, and was little influenced by the addition *in vitro* of dexamethasone (0.1μ g./ml.). No significant changes in the content of lower glycerides have been observed *in vitro* in untreated tissues or in tissues incubated with adrenocorticotrophic hormone (Vaughan & Steinberg, 1963), which is in contrast with what has been reported for perfused tissue (Scow, 1965). There is, however, no evidence to rule out the possibility that glycerol might be derived from diglycerides without any net change in the concentration of diglycerides in the tissue.

Analyses. The $^{14}CO_2$ was liberated from the medium by the addition of 0.2 ml. of $N-H_2SO_4$ and collected in 0.2 ml. of Hyamine hydroxide (Packard Instrument Co. Inc., La Grange, Ill., U.S.A.), which was injected into a plastic cup suspended in the flask. FFA were extracted and titrated by the method of Dole & Meinertz (1960). Glycerol was determined enzymically by the method of Wieland (1963). Measurement of $[^{14}C]$ glycogen was performed as follows. The adipose tissue was washed three times in Krebs-

Ringer bicarbonate buffer, and then extracted for 12 hr. in 20 ml. of chloroform-methanol (2:1, v/v). The extracted tissue was dried and dissolved in 0.5 ml. of hot 30% (w/v) KOH by heating for 10 min. at 95° . After cooling, 1 mg. of carrier glycogen and 0.2 ml. of 2% (w/v) Na_2SO_4 were added. Total glycogen was precipitated as the fraction insoluble in 66% (v/v) ethanol. After washing the precipitate twice with 66% ethanol, it was dissolved in water and the precipitation in 66% ethanol, followed by washing, was repeated. The final precipitate was dissolved in water and a portion was counted in a scintillation mixture prepared as described by Bray (1960).

The $[1-^{14}C]$ palmitic acid was added to the incubation medium as the sodium salt. Its incorporation into adipose-tissue triglycerides was measured as follows. At the end of the incubation, the adipose tissue was washed three times with Krebs-Ringer bicarbonate buffer containing albumin and extracted with chloroform-methanol (2:1, v/v) as described above. Samples of the lipid extracts were fractionated by thin-layer chromatography, by using a modification of the method of Brown & Johnston (1962). Glass plates (20 cm. $\times$ 20 cm.) were coated with a suspension of 50 g. of silica gel G (E. Merck A.-G., Darmstadt, Germany) in 85 ml. of water. The plates were dried and then activated at 105° for 30 min. The samples were applied as one line approx. 6 cm. long. The developing solvent was light petroleum (b.p. $55-75^\circ$)-diethyl ether-acetic acid (85:15:0.5, by vol.). The triglyceride spots could be seen easily without staining and were scraped into a mound on the plate. A scintillation vial was placed over the mound and the plate and the vial were simultaneously inverted to allow the silica gel to be transferred into the vial. Then 20 ml. of a naphthalene-dioxan liquid scintillator, prepared as described by Bray (1960) and containing 3% of Cab-O-Sil (Packard Instrument Co. Inc.), was added to the vials. The samples were checked for self-quenching by the internal-standard method, and no quenching was observed. Further, when a $[1-^{14}C]$ palmitate standard was added to adipose-tissue extracts (0.05μ Ci/ml.), no significant amount of radioactivity was recovered in the triglyceride spots.

All radioactive samples were counted in a Packard EX-2 model 314 liquid-scintillation spectrometer. The counting efficiency for ^{14}C -labelled substrates was 71%. The initial specific activity of the labelled substrates was selected in such a way that the number of counts/min. contained in subsequent samples was always at least 50 times that of the background.

RESULTS

Effect of dexamethasone on glycerol-free fatty acid balance in intact adipose tissue. Table 1 shows the effect of dexamethasone (0.1μ g./ml.) on the glycerol-FFA balance in adipose tissue from fed rats. Both net FFA production and total lipolysis were significantly increased above control values but the stimulatory effect was more pronounced on net FFA production. As a consequence, there was proportionally less FFA re-esterified in the presence of dexamethasone.

In the experiments described in Table 2, three concentrations of dexamethasone were used. At a concentration of 0.1μ g./ml., dexamethasone again

had a stimulatory effect on both total lipolysis and net FFA production, but, when the dexamethasone concentration was lowered to 0.01 and 0.001 $\mu\text{g./ml.}$, it stimulated only net FFA production. Decreased re-esterification therefore appeared, under these conditions, to be the main mechanism by which the FFA output was raised.

It should be noted that the net FFA production observed in control tissues varied markedly from experiment to experiment, although total lipolysis appeared to be more stable (Tables 1 and 2). Although the rats had free access to food at all times, it is very likely that these differences were due to changes, from one experiment to another, in their precise nutritional state, which would cause differences in the handling of carbohydrates by the isolated adipose tissues. Comparison between control and hormone-treated tissues is therefore clearly valid only within the same experiment.

When adipose tissue from rats, starved for 48 hr., was used in similar experiments, the basal release of both glycerol and FFA was higher than with the tissue from fed animals (Table 3). Dexamethasone did not alter total lipolysis significantly, but exerted a definite stimulatory effect on the net FFA output, with the result that re-esterification decreased from 28% in the controls to 9% in the hormone-treated tissues.

Since an inhibitory effect of dexamethasone on FFA re-esterification might be related to decreased glucose uptake and α -glycerophosphate formation, it was decided to test whether dexamethasone influenced the glycerol-FFA balance when glucose was not added to the incubation medium. The results in Table 4 show that, with tissue from fed rats, both total lipolysis and net FFA production were significantly enhanced by the hormone (0.1 $\mu\text{g./ml.}$) in the absence of glucose from the

Table 1. *Effect of dexamethasone on the glycerol-free fatty acid balance in adipose tissue from fed rats*

Incubations were for 4 hr. in Krebs-Ringer bicarbonate buffer containing albumin (3.5 g./100 ml.) and glucose (5 mm). Each fat pad was divided longitudinally into four pieces, two pieces for initial glycerol and FFA, one piece for incubation as control, and one piece for incubation as dexamethasone-treated tissue. All results are expressed as $\mu\text{moles/g.}$ of wet tissue. Each value is the mean $\pm$ s.e.m. of 12 values. Significance of difference from controls: * $P < 0.0025$; † $P < 0.005$.

	Total lipolysis (net glycerol $\times 3$)	Net FFA production	FFA re-esterified	Re-esterification as % of total lipolysis
Controls	19.7 (± 1.3)	8.2 (± 1.5)	11.5 (± 1.1)	58
Dexamethasone (0.1 $\mu\text{g./ml.}$) added	23.2* (± 1.5)	15.2† (± 1.3)	8.0 (± 0.7)	35
Dexamethasone-induced effect	+3.5 (± 0.27)	+7.0 (± 0.96)		

Table 2. *Effect of different concentrations of dexamethasone on the glycerol-free fatty acid balance in adipose tissue from fed rats*

Incubations were carried out as described in Table 1. Each fat pad was divided into six pieces, i.e. 12 pieces/animal. These pieces were randomly distributed, four for initial glycerol and FFA, two for incubation as control and two for incubation at each of the three dexamethasone concentrations. Six animals were used to produce enough tissue for each incubation flask. All results are expressed as $\mu\text{moles/g.}$ of wet tissue. Each value is the mean $\pm$ s.e.m. of six values. Significance of difference from controls: * $P < 0.0025$; † $P < 0.025$; ‡not significant.

	Total lipolysis (net glycerol $\times 3$)	Net FFA production	FFA re-esterified	Re-esterification as % of total lipolysis
Controls	19.7 (± 1.0)	3.6 (± 0.8)	16.1 (± 0.4)	82
Dexamethasone (0.1 $\mu\text{g./ml.}$) added	25.3† (± 1.4)	11.6* (± 1.2)	13.7 (± 0.6)	54
Dexamethasone (0.01 $\mu\text{g./ml.}$) added	22.3‡ (± 1.2)	9.3* (± 0.9)	13.0 (± 0.7)	58
Dexamethasone (0.001 $\mu\text{g./ml.}$) added	19.5‡ (± 1.0)	5.5* (± 0.8)	14.0 (± 0.8)	72

Table 3. *Effect of dexamethasone on the glycerol-free fatty acid balance in adipose tissue from rats starved for 48 hr.*

Incubations were carried out as described in Table 1. All results are expressed as $\mu\text{moles/g.}$ of wet tissue. Each value is the mean $\pm$ s.e.m. of six values. Significance of difference from controls: * not significant; $\dagger P < 0.0025$.

	Total lipolysis (net glycerol $\times 3$)	Net FFA production	FFA re-esterified	Re-esterification as % of total lipolysis
Controls	30.6 (± 4.0)	22.0 (± 2.6)	8.6 (± 1.9)	28
Dexamethasone (0.1 $\mu\text{g./ml.}$) added	33.5* (± 4.5)	30.5 $\dagger$ (± 2.4)	3.0 (± 0.9)	9
Dexamethasone-induced effect	+2.9 (± 1.20)	+8.5 (± 0.92)		

Table 4. *Effect of dexamethasone on the glycerol-free fatty acid balance in adipose tissue from fed rats*

Incubations were carried out as described in Table 1, but without glucose in the medium. All results are expressed as $\mu\text{moles/g.}$ of wet tissue. Each value is the mean $\pm$ s.e.m. of 12 values. Significance of difference from controls: * $P < 0.025$; $\dagger P < 0.005$.

	Total lipolysis (net glycerol $\times 3$)	Net FFA production	FFA re-esterified	Re-esterification as % of total lipolysis
Controls	21.3 (± 1.3)	11.9 (± 1.3)	9.4 (± 0.6)	45
Dexamethasone (0.1 $\mu\text{g./ml.}$) added	25.9* (± 1.8)	17.6 $\dagger$ (± 1.5)	8.3 (± 1.0)	32*
Dexamethasone-induced effect	+4.6 (± 0.6)	+5.7 (± 0.9)		

medium, and the re-esterification fell from 45% to 32%.

A separate experiment was performed to test whether adipose tissue remained functional when incubated for a prolonged period of time in a medium containing no glucose. Tissue that had been washed in Krebs-Ringer bicarbonate buffer containing no glucose was incubated for 5 hr. in the absence of glucose. It was next transferred to a new medium containing unlabelled glucose (5 mM) and incubated for a further 1 hr. [$U\text{-}^{14}\text{C}$]Glucose, with or without insulin (1 milliunit/ml.), was then added to each flask by injection through the rubber stopper and the incubation was continued for a further 2 hr. Basal glucose oxidation and insulin responsiveness were measured as the $^{14}\text{CO}_2$ production during this last 2 hr. incubation period.

After 5 hr. in the absence of glucose, adipose tissue could still metabolize [$U\text{-}^{14}\text{C}$]glucose at the expected rate (Jeanrenaud, 1961), and respond to insulin, as evidenced by the following values: basal glucose oxidation to carbon dioxide $12.3 \pm 1.9 \mu\text{g.}$ -atoms of glucose carbon/g. wet wt.; glucose oxidation to carbon dioxide in the presence of insulin, $42.6 \pm 3.4 \mu\text{g.}$ -atoms of glucose carbon/g. wet wt.

As tissue glycogen may represent a source of α -glycerophosphate for triglyceride ester formation, it was decided, in view of the finding that re-esterification was decreased by dexamethasone even in the absence of added glucose, to test the influence of this hormone on the disappearance of prelabelled tissue glycogen. Adipose tissue utilized [^{14}C]glycogen when incubated for 5 hr. in the absence of glucose and, in the presence of dexamethasone, this utilization was significantly enhanced (Table 5). This observation is consonant with the report of Shafrir & Kerpel (1964) that the glycogen content of adipose tissue is lowered by the administration of cortisone *in vivo*.

Effect of dexamethasone on glycerol-free fatty acid balance of isolated fat cells. As shown in Table 6, dexamethasone at a concentration of 0.01 $\mu\text{g./ml.}$ did not alter the net glycerol output of isolated fat cells that were incubated for 4 hr., but did have a stimulatory effect on net FFA release. When cortisol (30 $\mu\text{g./ml.}$) was used instead of dexamethasone, reproducible effects were obtained only with fat cells prepared from tissue exhibiting maximum esterification activity, i.e. tissue from rats that were starved for 54 hr. and then re-fed for 18 hr. Under

Table 5. *Effect of dexamethasone on the disappearance of [¹⁴C]glycogen in adipose tissue from fed rats.*

Adipose tissue was preincubated for 1 hr. in Krebs-Ringer bicarbonate buffer containing albumin (3.5 g./100 ml.) and [¹⁴C]glucose (1.25 mM; 5 μ C/ml.). The tissue was then quickly washed three times with unlabelled medium of the same composition. Part was analysed for [¹⁴C]glycogen without further incubation ('initial tissue'). The rest was incubated for 4 hr. in fresh Krebs-Ringer bicarbonate medium containing albumin (3.5 g./100 ml.) but no glucose, and with or without dexamethasone. At the end of this second incubation, [¹⁴C]glycogen analysis was again carried out. Each fat pad was divided longitudinally into four pieces, two for 'initial tissues', one for incubation as control, and one for incubation with dexamethasone. Each value is the mean $\pm$ s.e.m. of 12 values. Significance of difference from controls: **P* < 0.01.

	Tissue [¹⁴ C]glycogen (counts/min./g. of wet tissue)	
'Initial tissues'	16444	($\pm$ 2176)
Control tissues	6904	($\pm$ 793)
Dexamethasone-treated (0.1 μ g./ml.) tissues	4470*	($\pm$ 620)

Table 6. *Effect of dexamethasone and cortisol on glycerol and free fatty acid release by isolated fat cells*

Dexamethasone experiment: fat cells were isolated from fed rats and incubated for 4 hr. in 2 ml. of Krebs-Ringer bicarbonate buffer containing albumin (3.5 g./100 ml.) and glucose (2.5 mM). Cortisol experiment: fat cells were isolated from rats that had been starved for 54 hr. and re-fed for 18 hr. They were incubated for 4 hr. in 2 ml. of Krebs-Ringer bicarbonate buffer containing albumin (3.5 g./100 ml.) but no glucose. Each value is the mean $\pm$ s.e.m. of six values. Significance of difference from controls: **P* < 0.01.

Source of cells	Addition to medium	Total lipolysis (glycerol $\times$ 3)	Net FFA release (μ moles of FFA/g. of total lipid)
Fed rats	None	25.3 ($\pm$ 3.1)	5.2 ($\pm$ 1.8)
	Dexamethasone (0.01 μ g./ml.)	22.9 ($\pm$ 2.8)	13.8* ($\pm$ 2.1)
Starved-re-fed rats	None	65.4 ($\pm$ 10.5)	42.3 ($\pm$ 3.5)
	Cortisol (30 μ g./ml.)	65.6 ($\pm$ 8.0)	57.7* ($\pm$ 2.4)

these conditions, cortisol did not alter total lipolytic activity, but it significantly stimulated the release of FFA into the incubation medium even in the absence of glucose.

Effect of dexamethasone on palmitate incorporation into intact adipose tissue. The inhibitory effect of

Table 7. *Effect of dexamethasone on the incorporation of [1-¹⁴C]palmitate into adipose-tissue triglycerides from fed rats.*

Paired epididymal fat bodies were incubated for 5 hr. in Krebs-Ringer bicarbonate buffer containing albumin (3.5 g./100 ml.) and [1-¹⁴C]palmitate (910 μ equiv./l.), either with or without hormone. Glucose (2.5 mM) was present as indicated. Each value is the mean $\pm$ s.e.m. of six values. Significance of difference from controls: **P* < 0.005.

Additions		[1- ¹⁴ C]Palmitate incorporated (μ mole/g. of wet tissue)
Glucose (mM)	Dexamethasone (μ g./ml.)	
0	0	0.58 ($\pm$ 0.03)
0	0.1	0.56 ($\pm$ 0.03)
2.5	0	0.96 ($\pm$ 0.05)
2.5	0.1	0.68* ($\pm$ 0.05)

dexamethasone on the esterification of FFA was also studied in experiments in which paired samples of adipose tissue from fed rats were incubated with [1-¹⁴C]palmitic acid in the presence or the absence of added dexamethasone. Account was taken of the rise in medium FFA concentration during the incubation period by averaging the initial and final specific activities of each medium. No influence of dexamethasone on the incorporation of palmitate into triglycerides could be detected in the absence of glucose (Table 7). However, when glucose was present (2.5 mM), dexamethasone caused a significant decrease of palmitate incorporation into triglycerides.

DISCUSSION

From the data presented it is apparent that the re-esterification of mobilized FFA by adipose tissue as well as the esterification of palmitate added to the incubation medium, was inhibited in the presence of dexamethasone. With low concentrations of dexamethasone and with tissue from starved animals, the steroid-induced mobilization of FFA could be accounted for by decreased re-esterification alone. Although inhibition of re-esterification was present in all instances, and although it was of definite quantitative importance, it did not appear to be the only mechanism by which FFA output was accelerated by dexamethasone. When the concentration of hormone was sufficiently high, true lipolytic activity was observed. This observation suggests that glucocorticoids may well have more than one site of action in adipose-tissue metabolism. Further, the decreased re-esterification produced by

dexamethasone did not appear to be necessarily linked to decreased glucose transport through the cell membrane, since it was also observed when glucose was absent from the incubation medium.

These results should be compared with those of Fain, Kovacev & Scow (1965). Although these authors failed to demonstrate an effect of dexamethasone on intact adipose tissue in the absence of glucose, they observed that the steroid stimulated fatty acid release, but not glycerol release, when glucose was present in the incubation medium. They did not report true lipolytic activity of dexamethasone, probably because high concentrations of the hormone were not tested. Finally, they showed that dexamethasone added together with growth hormone had a marked lipolytic activity, i.e. stimulated both FFA and glycerol release. This combined effect of the two hormones was obtained in the absence of added glucose from the incubation medium, and was inhibited by puromycin, which suggested that it was related to the synthesis of some enzyme protein. Fain *et al.* (1965) proposed that the joint lipolytic action of dexamethasone and growth hormone was not related to the inhibitory effect of dexamethasone on glucose metabolism, since the action was observed in the absence of this substrate. Their conclusion was strengthened by the finding that, although dexamethasone and the aminonucleoside of puromycin (a compound without effect on protein synthesis) had similar effects on glucose metabolism, only dexamethasone potentiated the effect of growth hormone on lipolysis.

Shafir & Kerpel (1964) have previously stressed that glycogen within the adipose cells is an important source of triglyceride glycerol. This could have some relevance to the effect of dexamethasone, particularly in the absence of added glucose. As shown by these experiments, a stimulatory effect of dexamethasone on the disappearance of [^{14}C]glycogen was observed when adipose tissue was incubated without glucose (Table 5). Further, the effect of cortisol on FFA mobilization in isolated fat cells was obtained reproducibly only when the cells were prepared from tissues from rats that were starved for 54 hr. and then re-fed for 18 hr., i.e. from tissues with a high glycogen content.

In relation to the possible function of α -glycerophosphate as a regulator of re-esterification of fatty acids in adipose tissue, the finding that dexamethasone simultaneously increased glycogen breakdown while decreasing FFA esterification was unexpected. However, Denton, Yorke & Randle (1966) have questioned the idea that the supply of α -glycerophosphate may determine the re-esterification rate. They found that alloxan-diabetes, which lowered glucose uptake by fat pads incubated with albumin and insulin, did not lower the glycerophosphate

concentration. It would clearly be of interest to know the α -glycerophosphate concentration in adipose tissue from steroid-treated animals.

The experiments reported above do not define the precise site(s) of action of glucocorticoid hormones on adipose tissue. They suggest, however, that lipase activation by steroid hormones may not play the determinant role under physiological conditions, and that decreased re-esterification may well be due to a direct effect of the steroid at some step of intermediary glucose metabolism within the cell, resulting in a decreased formation of α -glycerophosphate from either glucose or endogenous glycogen. Such an effect might be related to synthesis of some enzyme protein, as proposed by Fain *et al.* (1965). An additional effect at the level of the cell membrane cannot be excluded.

Finally, these findings indicate that the stimulatory effect of glucocorticoids on FFA mobilization from adipose tissue differs from that of adrenaline, adrenocorticotrophic hormone and glucagon. Glucocorticoid-induced FFA release is accompanied by decreased glucose metabolism, little or no increase in true lipolytic activity, and decreased re-esterification, a process that, from the present work, appears to be of prime importance. On the other hand, the FFA mobilization due to adrenaline, adrenocorticotrophic hormone and glucagon is associated with increased glucose uptake, true lipolytic activity and, on the basis of a similar glycerol-FFA balance study, with an increased rate of re-esterification (Vaughan & Steinberg, 1963).

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