Br. J. Pharmac. (1977), **59**, 425–432

INHIBITION BY GLUCOCORTICOIDS OF PROSTAGLANDIN RELEASE FROM ADIPOSE TISSUE *in vitro*

J. CHANG, G.P. LEWIS & PRISCILLA J. PIPER

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

1 When rabbit chopped adipose tissue was incubated with a lipolytic agent (adrenocorticotrophic hormone, $ACTH^{1-24}$, 0.1 μg/ml) in Krebs solution, prostaglandin E_2 was formed in the tissue and about the same amount was found in the medium.

2 In the presence of indomethacin (1 μg/ml) the appearance of prostaglandin E_2 was almost abolished both in the tissue and in the medium.

3 When the incubation was carried out in the presence of hydrocortisone or betamethasone (1–10 μg/ml) the concentration of prostaglandin E_2 leaking or carried into the medium was significantly reduced, whereas that remaining in the tissue was significantly increased. This action of the steroids was not reversed by increasing substrate (arachidonic acid) concentration in the medium.

4 The steroids did not affect lipolysis, nor did they influence prostaglandin metabolism since such activity was not detectable in the adipose tissue.

5 Anti-inflammatory steroids therefore did not reduce prostaglandin formation but increased the tissue/medium ratio, which supports the view that they inhibit the release of prostaglandins after these have been synthesized.

Introduction

The functional vasodilatation which accompanies lipolysis in rabbit subcutaneous adipose tissue is mediated by a prostaglandin which is probably prostaglandin E_2 (Lewis & Matthews, 1970; Bowery, Lewis & Matthews, 1970). During lipolysis it was possible to detect prostaglandin formation within the fat tissue and its identification as prostaglandin E_2 has been confirmed by mass spectroscopic analysis (Dalton & Hope, 1974). Later, Lewis & Piper (1976) found that small amounts of a prostaglandin-like substance were released during lipolysis from the fat tissue into the venous blood. Following the discovery that non-steroid anti-inflammatory agents prevent prostaglandin formation (Vane, 1971), it was shown that such anti-inflammatory agents inhibit prostaglandin formation and functional vasodilatation in subcutaneous fat but do not prevent lipolysis (Bowery & Lewis, 1973). Therefore, it seems likely that inhibition of the vasodilatation was the result of inhibition of prostaglandin synthetase activity in adipose tissue.

Lewis & Piper (1975) have subsequently shown that like non-steroid anti-inflammatory drugs, anti-inflammatory steroids such as hydrocortisone, betamethasone and prednisolone inhibit the prostaglandin-mediated functional vasodilatation in subcutaneous adipose tissue. However, the mechanism of action of the steroids is different. Like indomethacin, hydrocortisone inhibits the vasodilatation and does not antagonize the direct vasodilator action of prostaglandins or reduce lipolysis. However, whereas indomethacin inhibits the formation of prostaglandin in the fat tissue itself, hydrocortisone does not.

We have therefore postulated that anti-inflammatory steroids inhibit the release of prostaglandins after they have been formed in the fat cells.

We have now confirmed the *in vivo* experiments by an *in vitro* study of the changes taking place during lipolysis in chopped fat. A preliminary account of this work was given to the British Pharmacological Society (Chang, Lewis & Piper, 1976).

Methods

Preparation of chopped fat

Female New Zealand white rabbits weighing 3.0 to 3.5 kg were killed by a blow on the head. The epigastric fat pads were dissected out, rinsed in Krebs

bicarbonate solution and divided in 5 g portions. Each portion was chopped into pieces about 2 mm^3 and washed three times with fresh Krebs solution. The fat was then pre-incubated for 20 min at room temperature with or without the anti-inflammatory drug before lipolysis was induced by the addition of adrenocorticotrophic hormone[1-24] (ACTH). The final volume of the incubation medium was 10 ml and total incubation time was usually 2 hours.

Preparation of isolated fat cells

Isolated fat cells were prepared from rabbit epigastric fat pads by the method of Rodbell (1964). The standard isolation and incubation medium was Krebs bicarbonate solution containing 2% bovine serum albumin. The medium was kept at pH 7.4 by bubbling with 95% O_2 and 5% CO_2. The two epigastric fat pads were pooled, cut into small pieces and 5 g was transferred to an incubation vial containing 10 mg of collagenase (Worthington Biochemicals) in 4 ml of the incubation medium. Cells were washed three times with 5 ml of the standard medium and finally suspended in this solution. All vessels used in the preparation were made of plastic or siliconized glass. Incubation of cells was carried out with 1 ml of the cell suspension. The dry weight of 1 ml of cells was determined by the difference between the weight of 1 ml of dried cell suspension and the weight of 1 ml of medium dried overnight at 70°C.

Glycerol assay

Glycerol was assayed by the method of Eggstein & Kreutz (1966). Glycerol release was taken as the index of lipolysis since the rate of re-utilization of glycerol by fat cells is minimal owing to the lack of glycerolkinase (Margolis & Vaughan, 1962).

Prostaglandin assay and thin layer chromatography

Immediately after incubation, fat was filtered from the incubation medium and an aliquot of ice-cold Krebs bicarbonate solution containing indomethacin (10 μg/ml) was added to the fat which was then filtered a second time. The two filtrates were combined. The chopped fat was then placed in ice-cold 4% ethanolic solution (brought to pH 3–3.5 with 1 N HCl) which also contained indomethacin. The tissue was homogenized and centrifuged at 10,000 *g* at 0°C. Prostaglandins were extracted from the supernatant with ethyl acetate at pH 3. The percentage recovery of prostaglandins from the incubation medium and fat tissue was monitored by adding 5,000 d/min per 100 μl of [^{3}H]-prostaglandin E_2 before extraction. Identification of the prostaglandin(s) released from the adipose tissue was carried out by thin layer chromatography using the AI and AII systems of Gréen & Samuelsson (1964).

The prostaglandin content of the tissue and supernatant (i.e. incubation medium) was measured by radioimmunoassay (Hennam, Johnson, Newton & Collins, 1974) with modifications (Jose, Niederhauser, Piper, Robinson & Smith, 1976). Tritiated prostaglandin E_2 (sp. act. 160 Ci/mmol; 20,000 d/min ≡ 20 pg) and an anti-serum to prostaglandin E_2 (diluted to bind 50–60% of [^{3}H]-prostaglandin E_2 in the absence of additional prostaglandin E_2) were used in the assay system. Ammonium sulphate solution was used to precipitate the antibody-bound material and this fraction was resuspended in distilled water, mixed with Instagel and the radioactivity determined.

The standard curve covers the range 0–1000 pg but only the linear part of the curve (60–400 pg) was used. The method blank was routinely determined by extraction of 10 ml of Krebs-albumin solution. The values were often lower than the linear range of the curve but corresponded approximately to 40–60 pg/ml.

The cross-reactions with other prostaglandins are as follows: prostaglandin E_1:43.8%; $F_{2\alpha}$:1.3%; $F_{1\alpha}$:0.7% A_2:0·6%; D_2:0.1%; 15-keto-prostaglandin E_2:18.8%; 13, 14 dihydro-15-ketoprostaglandin E_2: 0.2%; 13, 14 dihydro prostaglandin E_2:14.5%. (Determined from mass required to give 50% displacement of [^{3}H]-prostaglandin E_2.) The estimates of prostaglandin content obtained by radioimmunoassay were checked by bioassay on superfused rat stomach strip, chick rectum and rat colon in the presence of suitable antagonists (Piper & Vane, 1969).

Crude preparation for examination of prostaglandin metabolism

Fat tissue was rinsed in ice-cold buffer and homogenized in two volumes (w/v) of ice-cold 100 mM Tris buffer pH 8.0. The supernatant obtained from successive centrifugation at 10,000 *g* for 30 min and 100,000 *g* for 60 min was divided into 5 ml aliquots. The aliquots were stored at −20°C and kept no longer than a week. Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

To 5 ml aliquots were added 2 μmol NAD and prostaglandin E_2 (100 ng). The mixtures were incubated for 2 h at 37°C and the reaction terminated by plunging the tubes into boiling water. Control samples of boiled enzyme with or without added prostaglandin E_2 were also extracted as described above and bioassayed on the superfused rat stomach strip together with the experimental samples.

Partitioning of [^{3}H]-prostaglandin E_2 in fat tissue

The epigastric fat pads were removed from the rabbits and fragments of tissue not exceeding 200 mg were incubated in Krebs bicarbonate solution (pH 7.4) containing [^{3}H]-prostaglandin E_2. In all experiments the tissues were incubated for 2 h and the medium

kept at 37°C. Sufficient [^{3}H]-prostaglandin E_2 was used in each experiment to give approximately 4,000 counts per minute (ct/min) in 100 µl. This amount of radioactivity was determined accurately each time by counting a 100 µl aliquot of the standard [^{3}H]-prostaglandin E_2 solution. At the end of the incubation period the tissue fragments were removed and blotted dry on filter paper. The tissues were then transferred to counting vials containing 2 ml of Soluene 350 (Packard) and left overnight for digestion. After digestion the sample was counted in 10 ml of Instagel. Previous experiments have shown that the samples were thoroughly quenched after 18 h and quenching in each sample was monitored by the external standard method.

The results are presented as the tissue to medium ratio (T/M). The ratio is calculated by the equation:

$$T/M = \frac{\text{ct/min per 100 mg tissue}}{\text{ct/min per 100 µl medium}}$$

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

In an initial experiment, a time course for incorporation of [^{14}C]-arachidonic acid was measured. It was found that incubation for 4 h was required for maximum incorporation. Thereafter the chopped fat was labelled for this period. Immediately following labelling, excess label was removed from the fat by several washings. The efficiency of removal of labelled material was checked by counting the radioactivity in aliquots of the fluid used for each wash; only 0.1–0.3% of the radioactivity remained after the fourth wash. After uptake of [^{14}C]-arachidonic acid the fat tissue was placed in fresh incubation medium containing ACTH (1 µg/ml) and incubated for 1 hour.

Effect of steroid on ACTH-induced [^{14}C]-prostaglandin synthesis

The fat was incubated in an incubation medium containing hydrocortisone (100 µg/ml) for 2 h and then [^{14}C]-arachidonic acid was added and incubation continued for 4 hours. Immediately following the uptake of [^{14}C]-arachidonic acid the fat was stimulated with ACTH in the presence of hydrocortisone. Control incubations of fat tissue received an equivalent volume of 0.9% w/v NaCl solution (saline).

In another set of experiments, 'cold' arachidonic acid complexed to bovine serum albumin was added to the medium after the uptake of [^{14}C]-arachidonic acid. The complex was formed in the following way. An aliquot of arachidonic acid in *n*-hexane was taken down to dryness. The dried sample was redissolved in 100 µl of ethanol and added to a solution of 1 g of albumin in 10 ml saline at 30°C. The solution took approximately 30 min to clear. This solution was then adjusted to give the required concentration of arachidonic acid. The final concentration of the arachidonic acid was 10 µg/ml. ACTH and hydrocortisone were added as described above. Levels of ^{3}H or ^{14}C in extracts were counted in a Tricarb liquid scintillation counter.

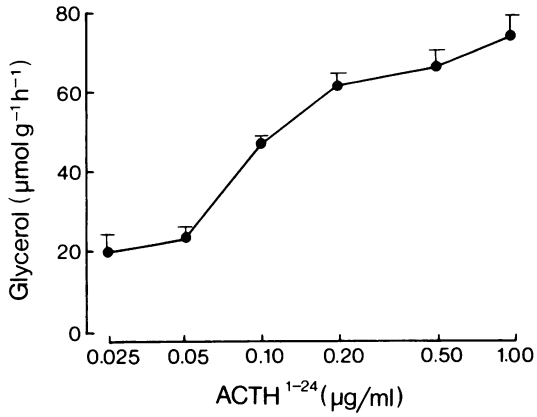

Figure 1 ACTH$^{1-24}$-induced lipolysis in chopped epigastric adipose tissue of rabbit. Results are µmol of glycerol formed per g of chopped fat in 1 h when incubated with increasing concentrations of ACTH$^{1-24}$. Vertical lines show s.e. means. Basal level = 10.24 ± 0.41 µmol g^{-1} h^{-1}.

Materials used

Drugs used were: ACTH$^{1-24}$ (Synacthen, CIBA); bovine serum, albumin (Sigma); arachidonic acid (Sigma); [^{14}C]-arachidonic acid (58 mCi/mmol, Radiochemical Centre, Amersham); betamethasone sodium phosphate (Glaxo Laboratories); hydrocortisone 21-sodium succinate (Sigma); indomethacin (Merck, Sharpe & Dohme).

The Krebs bicarbonate solution had the following composition in g/l (mM): NaCl 6.9 (118), KCl 0.35 (4.7), $CaCl_2.6H_2O$ 0.55 (2.5), KH_2PO_4 0.16 (1.2), $MgSO_4$ $7H_2O$ 0.29 (1.17), glucose 1.0 (5.6) and $NaHCO_3$ 2.1 (25.0).

Results

Lipolysis and prostaglandin formation

When subcutaneous adipose tissue was taken from the epigastric region of rabbits, immediately chopped and incubated with ACTH for 2 h, lipolysis could be measured by estimation of glycerol release. Figure 1 shows the relationship between lipolysis and concentration of ACTH over a range of 0.01–1.0 µg/ml. It can be seen that ACTH 0.1 µg/ml produced about 50% of the maximum formation of glycerol. This con-

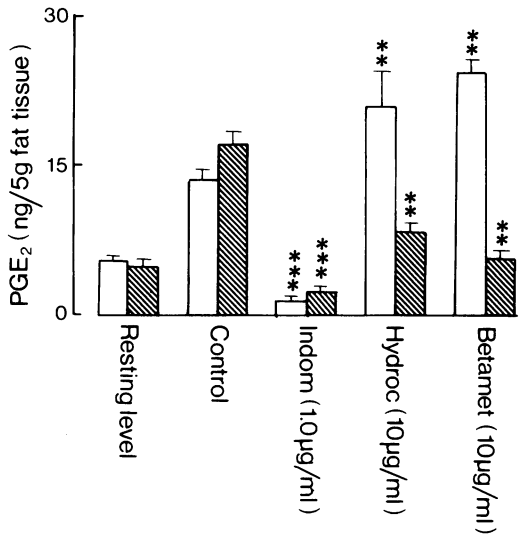

Figure 2 Prostaglandin content (ng/5g tissue) measured by radioimmunoassay, in chopped fat tissue (open columns) and supernatant obtained after separation of the 2 phases (hatched columns). The resting level was obtained by incubating the chopped fat alone and the control level by incubating with 0.1 µg/ml ACTH$^{1-24}$ for 2 hours. The effect of anti-inflammatory drugs was examined by carrying out the incubations in the presence of indomethacin (Indom) 1.0 µg/ml, hydrocortisone (Hydroc) 10 µg/ml or betamethasone (Betamet) 10 µg/ml. Each column represents the mean of 4–6 experiments. Vertical lines show s.e. means. $^{**}P<0.01$; $^{***}P<0.001$.

centration was therefore used in subsequent experiments in which prostaglandin was estimated by radioimmunoassay during lipolysis. Incubation of chopped fat in Krebs solution alone resulted in a small formation in the fat tissue and supernatant of a prostaglandin-like material which co-chromatographed with prostaglandin E_2 in the AI and AII solvent systems. The concentration in the fat was 1.1 ± 0.4 ng/g and in the supernatant 1.0 ± 0.5 ng/ml.

On incubation with ACTH 0.1 µg/ml for 2 h there was a significant increase in the prostaglandin content of both the fat tissue (2.7 ± 0.7) ng/g and the supernatant (3.4 ± 1.3) (Figure 2). These results are somewhat lower than those reported earlier using ACTH 1 µg/ml (Bowery *et al.*, 1970) but the ratio of prostaglandin in resting to stimulated fat is of the same order. During ACTH-stimulated lipolysis prostaglandin synthesis also occurs in isolated fat cells. The amount of prostaglandin E_2 found after incubation with ACTH 0.1 µg/ml was 2–3 ng/g fat cells which accounted almost wholly for the prostaglandin formed in the chopped fat preparation.

Effect of anti-inflammatory agents

In the presence of prostaglandin synthetase inhibitors represented here by indomethacin, or anti-inflammatory steroids such as hydrocortisone and betamethasone, ACTH 0.1 µg/ml continued to cause the release of glycerol, indicating that lipolysis still occurred as shown in Table 1.

However, when the effect of anti-inflammatory agents on prostaglandin in fat tissue and supernatant was examined the result was quite different. In the presence of indomethacin 1 µg/ml the prostaglandin content of both tissue and supernatant was reduced to a value which was even lower than that found after incubation with Krebs solution alone, as shown in Figure 2. On the other hand, when the incubation was carried out in the presence of hydrocortisone or betamethasone although the amount of prostaglandin released into the supernatant was reduced, the tissue prostaglandin content was significantly higher than that found after incubation with ACTH alone (Figure 2). This effect of the steroid was dose-dependent and Figure 3 shows the effect of betamethasone over a dose range of 1–10 µg/ml. Thus, whereas indomethacin reduced the overall amount of prostaglandin, the steroids altered the ratio of prostaglandin concentration in adipose tissue to prostaglandin concentration in the supernatant. The increase in the tissue/medium ratio of prostaglandin with increasing concentrations of hydrocortisone and betamethasone is illustrated in Figure 4.

Table 1 Effects of anti-inflammatory drugs on the lipolytic action of ACTH$^{1-24}$

Drug	(n)	*(µg/ml)*	*Glycerol levels following subsequent incubation with ACTH*
Control	(4)	—	33.54 ± 1.05
Indomethacin	(4)	1.0	32.98 ± 0.6 (NS)
Hydrocortisone	(4)	10.0	33.67 ± 0.73 (NS)
Betamethasone	(4)	10.0	34.36 ± 0.55 (NS)

Significance between control and test drug was calculated using Student's *t* test: NS = $P > 0.05$.

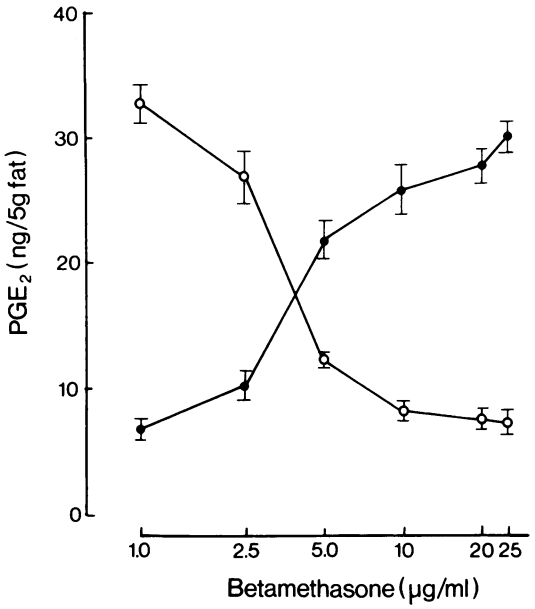

Figure 3 Effect of betamethasone on the prostaglandin content of tissue (●) and supernatant (medium) (○). Incubations were carried out for 2 h in the presence of $ACTH^{1-24}$ 0.1 μg/ml and various concentrations of betamethasone. Prostaglandin concentration was estimated by radioimmunoassay. Each point is the mean of 4–6 experiments. Vertical lines show s.e. means.

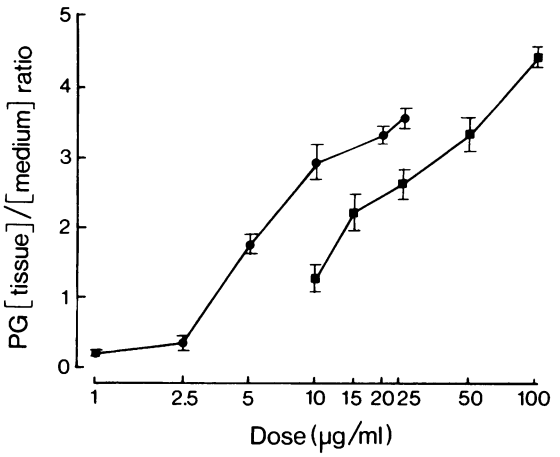

Figure 4 Ratio of prostaglandin content of tissue/prostaglandin content of supernatant (medium). Incubations of chopped fat tissue were carried out in the presence of $ACTH^{1-24}$ 0.1 μg/ml and various concentrations of hydrocortisone (■) or betamethasone (●). Each point is the mean ratio of 4–6 experiments. Vertical lines show s.e. means.

Effect of arachidonic acid

It has been suggested (Gryglewski, Bogumila, Korbut, Grodzinska & Ocetkiewicz, 1975) that steriods inhibit the appearance of prostaglandins in some tissues by interfering with the availability of the substrate precursor, arachidonic acid, and that addition of arachidonic acid reverses the effect of the steroid. When [^{14}C]-arachidonic acid (10 μg/ml) was incubated with chopped adipose tissue for up to 4 h there was a progressive uptake of 70–80% of [^{14}C]-arachidonate into the fat. Incubation of the adipose tissue with ACTH resulted in the release of [^{14}C]-prostaglandin-like material. The percentage conversion of [^{14}C]-arachidonic acid to prostaglandin-like material was 0.2–0.5%. When fat tissue was incubated with hydrocortisone or betamethasone prior to incubation with [^{14}C]-arachidonate and then stimulated with ACTH, the ratio of prostaglandin in the tissue to supernatant was increased as in the experiments without arachidonic acid. This ratio was the same in other experiments in which the substrate concentration was increased by the addition of arachidonic acid complexed to albumin.

Partitioning of prostaglandins

A possible alternative to the hypothesis that steroids prevent the release of prostaglandins from fat cells could be that steroids influence the uptake of prostaglandin into the fat tissue. Table 2 shows that when fat tissue was incubated with Krebs solution containing [^{3}H]-prostaglandin E_2, about 30% was taken up into the tissue. This distribution of [^{3}H]-prostaglandin E_2, between tissue and fluid is in good

Table 2 Proportion of [^{3}H]-prostaglandin E_2 taken up by the chopped fat preparation, expressed as tissue to medium ratio

	Tissue/Medium (T/M)
Control (without ACTH)	0.37 ± 0.02
ACTH	0.38 ± 0.03 (NS)
ACTH + hydrocortisone	0.38 ± 0.03 (NS)
ACTH + betamethasone	0.36 ± 0.03 (NS)
Betamethasone	0.36 ± 0.05 (NS)
Hydrocortisone	0.38 ± 0.06 (NS)

T = counts per minute corrected for 100 mg fat tissue; M = counts per minute corrected for 100 μl medium.
Significance between control and test drug was calculated by Student's *t* test: NS = $P > 0.05$. All ratios are expressed as means ± s.e. mean; $n = 4$ in all cases.

agreement with the findings of Bito (1972) for fat tissue. When the incubation was carried out in the presence of hydrocortisone (10 μg/ml) or betamethasone (10 μg/ml) this distribution was unaltered.

Inactivation of prostaglandins

When prostaglandin E_2 was incubated for up to 2 h with the supernatant obtained by centrifuging homogenized adipose tissue at 100,000 *g* no inactivation of prostaglandin E_2 could be detected. However, when prostaglandin E_2 was incubated with the high speed supernatant of guinea-pig or rabbit lung, prepared under the same conditions, metabolism and inactivation occurred as has been described by many workers.

Discussion

The experiments described have shown that whereas a non-steroid anti-inflammatory agent, indomethacin, reduced the total amount of prostaglandin formed during lipolysis in chopped fat, anti-inflammatory steroids did not reduce the total formation but increased the tissue/medium ratio. In retrospect, further evidence of this inhibition of prostaglandin release by steroids was provided by Eakins, Whitelocke, Bennett & Martenet (1972). They detected the release of prostaglandin-like activity into the aqueous humour of patients with anterior uveitis. After local steroid treatment, prostaglandin-like activity was no longer found in the aqueous humour.

Since the discovery that non-steroid anti-inflammatory drugs produce at least some of their anti-inflammatory effects by inhibiting prostaglandin synthetase and therefore preventing the formation of prostaglandins, it has been difficult to explain similar anti-inflammatory activities of corticosteroids since it has been demonstrated by several groups of workers that anti-inflammatory steroids do not inhibit prostaglandin synthetase (Vane, 1971; Flower, Gryglewski, Herbaczynska-Cedro & Vane, 1972; Greaves, Kingston & Pretty, 1975; Lewis & Piper, 1975). In conditions where prostaglandin formation might occur extracellularly or where the cells are damaged so much that their membranes no longer provide a barrier to the diffusion of materials, prostaglandin synthetase inhibitors would be expected to prevent the appearance of prostaglandins whereas corticosteroids would not. Anti-inflammatory steroids did not prevent the release of prostaglandins from isolated strips of rabbit jejunum or perfused spleen, whereas prostaglandin synthetase inhibitors prevented their appearance (Lewis & Piper, 1975; Ferreira, Moncada & Vane, 1971). In both cases cells may have been altered or damaged; in the jejunum, prostaglandin formation was probably due to cell breakdown and in the spleen, cell membranes may have been altered by prolonged perfusion with Krebs solution and dextran. However, inhibition of prostaglandin release could well provide an explanation for the local vasoconstrictor activity of glucocorticoids in skin (McKenzie, 1962). During lipolysis there is no damage to the fat cells and release of prostaglandins from these cells is therefore probably a carrier-mediated process rather than leakage. As steroids do not alter the uptake of prostaglandins into the chopped adipose tissue, it seems to be this carrier-mediated release which is inhibited by these drugs. It is not yet known whether glucocorticoids act in other tissues as they appear to do in adipose tissue, i.e. inhibiting the release of prostaglandin, but there are some indications that this might be so.

Gryglewski *et al.* (1975) found that hydrocortisone and dexamethasone inhibited the release of prostaglandins from isolated blood vessels caused by the action of catecholamines, and from guinea-pig perfused lungs during anaphylaxis. These authors also reported that the steroid inhibition of prostaglandin release was reversed by the prostaglandin precursor, arachidonic acid, and concluded that the steroids prevented prostaglandin release by impairing the availability of substrate for prostaglandin synthetase. The uptake of arachidonate into lung tissue in such a way that it can be converted to prostaglandin is a slow process (Crutchley, Piper & Seale, unpublished observations). It seems unlikely that in the experiments of Gryglewski *et al.* (1975) the perfused arachidonic acid reached the site at which endogenous arachidonic acid is released during lipolysis. It might remain in or around the pulmonary vasculature where steroids do not inhibit the conversion of arachidonic acid to prostaglandins (Dawson & Tomlinson, 1974). Such a situation might explain the apparent removal of steroid inhibition by infusion of arachidonic acid. In experiments with rabbit adipose tissue *in vivo* (Lewis & Piper, unpublished observations) arachidonic acid failed to restore the prostaglandin-mediated vasodilatation during lipolysis after inhibition by steroids. In the present experiments arachidonic acid did not reverse the effect of the glucocorticoids *in vitro*, although the [^{14}C]-arachidonic acid administered was available for conversion into prostaglandins. We therefore conclude that, at least in adipose tissue, steroids do not prevent release of prostaglandins by blocking the availability of substrate to the enzyme but by some other mechanism.

The authors of two recent papers have concluded that anti-inflammatory steroids inhibit synthesis of prostaglandins rather than their release, but do not provide very firm evidence for specific inhibition of prostaglandin synthetase. In the first paper Kantrowitz, Robinson, McGuire & Levine (1976) found that relatively low concentrations of anti-

inflammatory steroids appeared to inhibit prostaglandin synthesis in transplants of rheumatoid synovia. In this paper prostaglandins were identified and estimated by radioimmunoassay alone; this would have been more satisfactory if supported by evidence of chemical extraction or biological assay of prostaglandins, since difficulties are common particularly in the case of antiserum to prostaglandin E_2 and unsupported results often unreliable. It appears more likely that the steroids were producing some sort of toxic effect on the cells as suggested by the authors. Their findings from one experiment, based on a routine histological examination of haematoxylin and eosin-stained sections, that there were no apparent differences between control and dexamethasone-treated cells do not exclude a possible biochemical lesion in the transplants. Such a toxic effect might well lead to a non-specific inhibition of enzyme activity in the transplants.

In the second of these reports, Tashjian, Voelkel, McDonough & Levine (1976) examined the amount of prostaglandin formed during incubation of mouse fibrosarcoma cells. The experimental results support our working hypothesis and it is only the authors' interpretation which is in contention since they found that there was a dose-related inhibition of prostaglandin release into the incubation medium by low concentrations of hydrocortisone (5×10^{-9} to 5×10^{-7}M) while formation of prostaglandins in the cells continued and was not significantly different from control incubations without steroid. We conclude from their findings that the release of prostaglandins formed within the cells was inhibited by the corticosteroids since the tissue/medium ratio increased from 0.4 to 2.0 (rough calculation from data in Tashjian *et al.*, 1976). The authors' findings that there was no increase in intracellular prostaglandin in the presence of hydrocortisone cannot be regarded as evidence against this conclusion. Firstly, synthesis was not inhibited and secondly, no analysis was made to find why prostaglandin accumulation did not occur. For example, unlike rabbit fat cells, mouse fibrosarcoma cells might contain prostaglandin metabolizing enzymes that would tend to keep the intracellular concentration low.

We therefore conclude that in rabbit adipose tissue, and possibly also in other tissues, anti-inflammatory steroids prevent the release of prostaglandins, probably by interfering with a transport mechanism, but do not affect the synthesis of prostaglandins.

We thank the Medical Research Council and the Arthritis and Rheumatism Council for supporting this research and Ciba/Geigy for supplying $ACTH^{1-24}$.

References

BITO, L.Z. (1972). Accumulation and apparent active transport of prostaglandins by some rabbit tissues *in vitro*. *J. Physiol., Lond.*, **221**, 371–387.

BOWERY, BARBARA & LEWIS, G.P. (1973). Inhibition of functional vasodilatation and prostaglandin formation in rabbit adipose tissue by indomethacin and aspirin. *Br. J. Pharmac.*, **47**, 305–314.

BOWERY, N.G., LEWIS, G.P. & MATTHEWS, J. (1970). The relationship between functional vasodilatation in adipose tissue and prostaglandin. *Br. J. Pharmac.*, **40**, 437–445.

CHANG, J., LEWIS, G.P. & PIPER, PRISCILLA J. (1976). The effects of anti-inflammatory steroids on levels of prostaglandin in adipose tissue *in vitro*. *Br. J. Pharmac.*, **56**, 342–343P.

DALTON, C. & HOPE, W.C. (1974). Cyclic AMP regulation of prostaglandin biosynthesis in fat cells. *Prostaglandins*, **6**, 227–242.

DAWSON, W. & TOMLINSON, R. (1974). Effect of chromoglycate and eicosatetraynoic acid on the release of prostaglandins and SRS-A from immunologically challenged guinea-pig lungs. *Br. J. Pharmac.*, **52**, 107–108P.

EAKINS, K.E., WHITELOCKE, R.A.F., BENNETT, A. & MARTENET, A.C. (1972). Prostaglandin-like activity in ocular inflammation, *Br. med. J.*, **3**, 452–453.

EGGSTEIN, M. & KREUTZ, F.H. (1966). A new method of determining neutral fats in blood serum and tissue. *Klin. Wschr.*, **44**, 262–267.

FERREIRA, S.H., MONCADA, S. & VANE, J.R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature, New Biol.*, **231**, 237–239.

FLOWER, R.J., GRYGLEWSKI, R., HERBACZYNSKA-CEDRO, KRYSTYNA & VANE, J.R. (1972). The effects of anti-inflammatory drugs on prostaglandin biosynthesis. *Nature, New Biol.*, **238**, 104–106.

GREAVES, M.W., KINGSTON, W.P. & PRETTY, KATHLEEN (1975). Action of a series of non-steroid and steroid anti-inflammatory drugs on prostaglandin synthesis by the microsomal fraction of rat skin. *Br. J. Pharmac.*, **53**, 470.

GRÉEN, K. & SAMUELSSON, B. (1964). Prostaglandins and related factors XIX. Thin layer chromatography of the prostaglandins. *J. Lipid Res.*, **5**, 117–120.

GRYGLEWSKI, R.J., BOGUMILA, P., KORBUT, R., GRODZINSKA, L. & OCETKIEWICZ, A. (1975). Corticosteroids inhibit prostaglandin release from perfused mesenteric blood vessels of rabbit and from perfused lungs of sensitised guinea pig. *Prostaglandins*, **10**, 343–355.

HENNAM, J.F., JOHNSON, D.A., NEWTON, J.R. & COLLINS, W.P. (1974). Radioimmunoassay of prostaglandin in peripheral venous plasma from men and women. *Prostaglandins*, **5**, 531–542.

JOSE, P., NIEDERHAUSER, U., PIPER, PRISCILLA, J., ROBINSON, CHRISTINE & SMITH, A.P. (1976). Degradation of prostaglandin $F_{2\alpha}$ in the human pulmonary circulation. *Thorax* (in press).

KANTROWITZ, F., ROBINSON, D.R., McGUIRE, M.B. & LEVINE, L. (1975). Corticosteroids inhibit prostaglandin production by rheumatoid synovia. *Nature, Lond.*, **258**, 737–739.

LEWIS, G.P. & MATTHEWS, J. (1970). The mechanism of

functional vasodilatation in rabbit epigastric adipose tissue. *J. Physiol., Lond.*, **207**, 15–30.

LEWIS, G.P. & PIPER, PRISCILLA J. (1975). Inhibition of release of prostaglandins as an explanation of some of the actions of anti-inflammatory corticosteroids. *Nature, Lond.*, **254**, 308–311.

LEWIS, G.P. & PIPER, PRISCILLA J. (1976). The action of corticosteroids on the prostaglandin system. In *The Role of Prostaglandins in Inflammation,* Proceedings of a Workshop held during the VIIIth European Rheumatology Congress, Helsinki, 1975, ed. Lewis, G.P. pp. 148–160. Bern, Stuttgart and Vienna: Hans Huber.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

MARGOLIS, S. & VAUGHAN, M. (1962). α-glycerophosphate synthesis and breakdown in homogenate of adipose tissue. *J. biol. Chem.*, **237**, 44–48.

McKENZIE, A.W. (1962). Percutaneous absorption of steroids. *Archs Derm.*, **86**, 611–614.

PIPER, PRISCILLA J. & VANE, J.R. (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature, Lond.*, **223**, 29–35.

RODBELL, M. (1964). Metabolism of isolated fat cells. (1) Effects of hormones on glucose metabolism and lipolysis. *J. biol. Chem.*, **239**, 375–380.

TASHJIAN, A.H. Jr., VOELKEL, K.F., McDONOUGH, J. & LEVINE, L. (1975). Hydrocortisone inhibits prostaglandin production by mouse fibrosarcoma cells. *Nature, Lond.*, **258**, 739–741.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231**, 232–235.

(Received October 6, 1976.
Revised November 11, 1976.)