Effects of Insulin, Glucocorticoids and Adrenaline on the Activity of Rat Adipose-Tissue Lipoprotein Lipase

Peter ASHBY and Donald S. ROBINSON

Department of Biochemistry, University of Leeds, Leeds LS2 9LS, U.K.

(Received 13 August 1979)

The lipoprotein lipase activity of epididymal fat-bodies from starved rats was measured during incubations at 37°C *in vitro*. Protein-synthesis-independent activation of the enzyme, previously observed during incubations at 25°C, also occurs at 37°C. Protein-synthesis-dependent increases in the activity of the enzyme occur in the presence of insulin and are markedly potentiated by glucocorticoids. The effects on the activity of the enzyme of insulin alone, or in the presence of glucocorticoids, are correlated with its effects on total protein synthesis in the tissue. Adrenaline antagonizes the increase in activity of the enzyme brought about by insulin and abolishes the potentiation of insulin action by glucocorticoids. These changes may be due, at least in part, to its stimulation of inactivation of the enzyme in the tissue. It is suggested that changes in adipose-tissue lipoprotein lipase activity that occur with changes in nutritional status *in vivo* result from the combined effects of changes in plasma insulin and glucocorticoid concentrations.

The removal of triacylglycerol fatty acids from the bloodstream by adipose tissue depends on the action of the enzyme lipoprotein lipase (EC 3.1.1.34) (Robinson, 1970). Although this action is expressed at the luminal surfaces of the capillary endothelial cells of the tissue, the enzyme is synthesized within the adipocyte (Robinson & Wing, 1971). Initially, such synthesis probably yields a proenzyme that is then activated in a protein-synthesis-independent step before being secreted and transported to its functional site of action at the endothelial cell surface (Ashby *et al.*, 1978b).

The activity of lipoprotein lipase in adipose tissue, and hence the rate of uptake of circulating triacylglycerol fatty acids, varies with changes in nutritional status: both are high, for example, in animals in the fed state and both decline on starvation (Robinson, 1970). There is now good evidence that such changes in activity are due, at least in part, to effects of insulin on the rate of synthesis of the enzyme in the adipocyte. Thus the fall in the activity of the enzyme on starvation is associated with a decline in the amount of immunotitratable enzyme in the tissue (Jansen *et al.*, 1978) and is correlated with changes in the plasma insulin concentration (Cryer *et al.*, 1976). Moreover, studies involving the incubation of rat epididymal fat-bodies *in vitro* have shown, on the one hand, that insulin can promote a progressive increase in the activity of lipoprotein

lipase in the tissue over a period of several hours (Salaman & Robinson, 1966) and, on the other, that the effects of insulin on the activity of the enzyme are correlated with its stimulatory action on protein synthesis in the tissue (Hollenberg *et al.*, 1970; Vydelingum *et al.*, 1978).

Additional hormonal regulation of the activity of the enzyme in adipose tissue is probably exerted by adrenaline and noradrenaline (Wing *et al.*, 1966; Davies *et al.*, 1974). Such control is likely to be short-term, and we (Ashby *et al.*, 1978b) have presented evidence suggesting that these hormones lower the activity of the enzyme through its inactivation before release from the adipocyte.

Hormones other than insulin and catecholamines have also been implicated in the control of the enzyme's activity in adipose tissue and, of these, the glucocorticoids are of particular interest in relation to regulation over a longer time scale. The activity of the enzyme in adipose tissue has been shown to be increased after glucocorticoid injection *in vivo* (de Gasquet & Pequignot, 1973; de Gasquet *et al.*, 1975). However, such studies cannot distinguish a direct effect of the hormone from an indirect one (due, for example, to actions on other tissues or on the plasma concentrations of other hormones). Although glucocorticoids promote the induction of lipoprotein lipase during the culture of heart cells from newborn rats (Friedman *et al.*, 1978), there

have been no reports to date of effects of glucocorticoids on the activity of the adipose-tissue enzyme in studies *in vitro*.

In this paper we present evidence, derived from studies involving the incubation of rat epididymal fat-bodies *in vitro* at 37°C, consistent with an action of insulin on the synthesis of the enzyme that is markedly potentiated by glucocorticoids. The results also support our earlier suggestion that adrenaline controls the activity of the enzyme by promoting its inactivation and show that protein-synthesis-independent activation of the enzyme occurs during incubations of fat-bodies at 37°C as well as at 25°C.

Materials and Methods

Materials

Horse serum was supplied by Oxoid Ltd., Basingstoke, Hants., U.K. A tritiated amino acid mixture, which contained L-[4,5-³H]leucine, L-[4,5-³H]lysine, L-[2,4,6-³H]phenylalanine, L-[2,3,4,5-³H]proline and L-[2,3,4,6-³H]tyrosine, each at 60–100 Ci/mmol, and insulin immunoassay kits were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Bovine insulin was supplied by Duncan, Flockhart and Co., London E2 6LA, U.K. Steroids were purchased from Sigma Chemical Co. Other materials were as specified by Ashby *et al.* (1978b) or were supplied by BDH, Poole, Dorset, U.K.

Incubation of epididymal fat-bodies

The fat-bodies were removed from specific pathogen-free male rats of the Wistar strain (body wt. 170–180 g) which were maintained as described by Ashby *et al.* (1978a) and starved for 24 h from 09:00 h before the start of the experiments. They were kept at 37°C in gassed Krebs–Henseleit bicarbonate buffer (Krebs & Henseleit, 1932) until they had all been removed from the rats and were then randomly distributed in groups of four into flasks containing 10 ml of the appropriate incubation medium at 37°C.

The incubation medium was Krebs–Henseleit bicarbonate buffer gassed with O₂/CO₂ (19:1) at 37°C. It was supplemented with glucose (10 mM), amino acids at the concentrations used for HeLa-cell culture (Eagle, 1955), radioactive amino acids (0.4 μCi/ml) and dialysed bovine serum albumin adjusted to pH 7.4 with NaOH and to a final concentration of 2% (w/v). Other additions to this basic medium were as specified in the text.

In many of our earlier studies, for example, Salaman & Robinson (1966) and Wing & Robinson (1968), serum and heparin were components of the media in which the fat-bodies were incubated at 37°C. Heparin extracts lipoprotein lipase from the

fat-bodies into the incubation medium, and a large proportion of any increases in the activity of the enzyme that occur during incubations in its presence at 37°C is due to a rise in the enzyme activity in the medium. Serum was included in the earlier studies because it increased the stability of the extracted enzyme at 37°C (Robinson & Wing, 1971). Heparin is now known to have multiple effects on the stability and activity of lipoprotein lipase (Olivecrona *et al.*, 1977), and serum could contain other hormones, which would complicate interpretation of the results. For these reasons, heparin and serum were omitted in the present study and virtually no lipoprotein lipase activity was therefore released into the medium.

Assay of lipoprotein lipase

The enzyme was assayed in acetone/ether-dried preparations of the fat-bodies and incubation medium combined, prepared as described by Ashby *et al.* (1978b). The assay for enzyme activity was carried out as described by Ashby *et al.* (1978b), except that the volume of the assay was reduced to 1 ml and horse serum, rather than rat serum, was used as a source of apoprotein CII. The measured activities were similar to those observed with rat serum, and the assay was linear with respect to enzyme concentration and time over the same range. One unit of enzyme activity is defined as that amount of enzyme that releases 1 μmol of fatty acid/h in the assay.

Incorporation of tritiated amino acids into fat-body proteins

This was determined as described by Ashby *et al.* (1978b). Incorporation of radioactivity into the fat-body protein was linear for up to 8 h under the conditions of the experiments. The results are expressed in c.p.m. because of the difficulty of obtaining meaningful estimates of the counting efficiency for radioactive samples precipitated on to filter discs. The constancy of the sampling procedures and of the sample geometry ensures identical efficiency of counting within each experiment (Mans & Novelli, 1961).

Results

Analysis and presentation of results

Lipoprotein lipase is now recognized to be an enzyme that is normally turning over rapidly, and its half-life in a variety of tissues at 37°C appears to be between 20 min and 1 h (Wing & Robinson, 1968; Patten, 1970; Hollenberg *et al.*, 1970; Chajek *et al.*, 1978; Wise & Green, 1978). The measured lipoprotein lipase activities of epididymal fat-bodies at the time of their removal from the rats represent therefore a balance between particular rates of

enzyme synthesis and activation and particular rates of inactivation or degradation of the enzyme, established as a result of the hormonal influences operating on the tissue *in vivo*. After incubation for several hours *in vitro*, a new level of activity will be achieved that will represent the balance of the effects of the hormones in the medium on these factors. In the light of the above, it has been appropriate in several of the experiments reported to compare directly the enzyme activities achieved after incubation of fat-bodies for fixed times in different hormonal environments, rather than to relate them to the initial activities.

The individual variations in the lipoprotein lipase activities of epididymal fat-bodies from 24 h-starved rats are much greater from one consignment of rats to another than within a consignment (Ashby *et al.*, 1978b). For this reason, effectors of the enzyme activity have only been compared using groups of fat-bodies taken from animals within a single consignment. Determination of the significance of the effects observed requires the pooling of data from separate experiments using different batches of rats. For this purpose, statistical analysis has been carried out by the paired-sample *t* test, which eliminates the effects of variations between consignments (Campbell, 1974).

Protein-synthesis-independent changes in lipoprotein lipase activity

Activation. When epididymal fat-bodies from starved rats are incubated *in vitro* at 25°C in the presence of cycloheximide, their lipoprotein lipase activity increases initially by a protein-synthesis-independent mechanism (Cryer *et al.*, 1973). The activation process appears to be energy-dependent but hormonally independent, and we have interpreted it as representing a post-translational modification of a pool of proenzyme molecules that is present in the fat-bodies at the time of their removal from the rats (Ashby *et al.*, 1978b).

The results in Fig. 1 (curve A) show that a protein-synthesis-independent rise in the activity of the enzyme also occurs during similar incubations in the presence of cycloheximide at 37°C. However, presumably because of the greater instability of the enzyme at 37°C than at 25°C (Robinson & Wing, 1971; Stewart & Schotz, 1971), the magnitude of the increase in activity is less than at 25°C (compare Ashby *et al.*, 1978b) and it is followed after 1–2 h by a decline. In the experiment in Fig. 1, this continued so that at 5 h the total activity was only 1.2 units/fat-body.

The experiments in Fig. 1 were carried out with insulin in the incubation medium. However, we have confirmed (S. M. Parkin, P. Ashby & D. S. Robinson, unpublished work) that, as in incubations at 25°C (Ashby *et al.*, 1978b), the protein-synthesis-

Fig. 1. Time course of changes in total lipoprotein lipase activity during incubations of fat-bodies in the presence of cycloheximide

Groups of four fat-bodies were incubated at 37°C for different time-periods in the incubation medium described in the Materials and Methods section to which insulin (0.2 m-i.u./ml) and cycloheximide (10 µg/ml) had been added (●). Some of the incubation media also contained adrenaline (10 µM) (○). The lipoprotein lipase activity of the fat-bodies and medium combined was determined at the end of each incubation period. Protein synthesis was inhibited by more than 94% in all cases. The results are typical of three experiments.

independent activation process does not depend on the presence of insulin.

Inactivation. The initial phase of the rise in lipoprotein lipase activity during the incubation of fat-bodies in the presence of cycloheximide at 25°C still takes place in the presence of adrenaline (Ashby *et al.*, 1978b). However, it is followed by a rapid decline in activity, which our previous results have suggested may be due to an inactivation process that occurs after the post-translational activation, but before secretion of the enzyme from the adipocyte. We have now shown that a similar sequence of activity changes occurs during incubations in the presence of cycloheximide at 37°C (curve B, Fig. 1). In three paired incubations, the differences between curves A and B were significant from 0.75 h onwards.

Protein-synthesis-dependent changes in lipoprotein lipase activity

Effects of insulin. We have previously shown that insulin promotes protein-synthesis-dependent in-

creases in adipose-tissue lipoprotein lipase activity under two sets of conditions: first, during incubations of epididymal fat-bodies from starved rats at 37°C in media containing heparin (Salaman & Robinson, 1966) and, second, during similar incubations at 25°C in media that did not contain heparin (Robinson & Wing, 1971; Cryer *et al.*, 1973). However, an insulin-dependent increase has not previously been shown when the fat-bodies are incubated at 37°C in the absence of heparin.

Curve A of Fig. 2 shows the time course of the rise in lipoprotein lipase activity that occurs when fat-bodies from starved rats are incubated at 37°C in the presence of insulin under the conditions of our present experiments. The magnitude of the rise in activity is not dissimilar to that observed during incubations in the presence of cycloheximide (curve A, Fig. 1). However, the crucial difference is that the activity persists at an elevated plateau value for at least 6 h in the absence of cycloheximide and that, as curve A of Fig. 3(a) shows, the plateau activity

Fig. 2. Time course of changes in total lipoprotein lipase activity during incubations of fat-bodies in the absence of cycloheximide

Groups of four fat-bodies were incubated at 37°C for different time-periods in the incubation medium described in the Materials and Methods section, to which insulin (2 m-i.u./ml) had been added (●). Dexamethasone (400 nM) was also present in some of the incubation media (○). The lipoprotein lipase activity of the fat-bodies and medium combined was determined at the end of each incubation period. The results are typical of three experiments.

achieved is dependent on the insulin concentration. Moreover, the effects of insulin on the activity of the enzyme are significantly correlated with its concurrent stimulation of protein synthesis in the tissue [line A of Fig. 3b: correlation coefficient (r) = 0.92; $P < 0.01$].

These results, together with those of other workers (Hollenberg *et al.*, 1970; Vydelingum *et al.*, 1978), support the view that insulin promotes an increase in

Fig. 3. Relation of total lipoprotein lipase activity during incubations of fat-bodies to (a) insulin concentration in the incubation medium and (b) rate of protein synthesis in the tissue

Groups of four fat-bodies were incubated for 5 h at 37°C in the incubation medium described in the Materials and Methods section to which insulin had been added. The insulin concentration was varied as shown in (a), and adrenaline (10 μ M) was either present (○) or absent (●). The lipoprotein lipase activity of the fat-bodies and medium combined and the total tissue protein radioactivity were determined after 5 h. The results are for a single experiment typical of six experiments.

the rate of lipoprotein lipase synthesis in the fat-body at 37°C and that this leads to a new steady-state enzyme activity at which the increased rate of synthesis of the enzyme is again balanced by the loss of activity due to inactivation or degradation (Schimke, 1969).

Effects of glucocorticoids. Curve B of Fig. 2 shows that when dexamethasone (400nM), as well as insulin, is present during incubations of fat-bodies from starved rats at 37°C, the lipoprotein lipase activity again rises to a plateau value that is maintained for several hours. However, the magnitude of the rise is much greater than in the presence of insulin alone. Table 1 shows that several different glucocorticoids are effective in enhancing the response to insulin, although the synthetic compounds, dexamethasone and triamcinolone acetone, are the most potent.

The effect on the plateau lipoprotein lipase activity of varying the concentration of dexamethasone in the incubation medium at various concentrations of insulin is shown in Fig. 4. The results are supplemented by Table 2, which provides a statistical analysis of the results at the optimal concentration of dexamethasone in the medium (400nM). It is evident that dexamethasone alone causes a small increase in lipoprotein lipase activity, but that this effect is powerfully potentiated in the presence of insulin.

Table 1. *Effects of glucocorticoids on lipoprotein lipase activity*

Groups of four fat-bodies were incubated for 6.5h at 37°C in the incubation medium described in the Materials and Methods section to which insulin (2m-i.u./ml) was added. Steroids at a concentration of 40nM were present as shown. The lipoprotein lipase activity of the fat-bodies and medium combined was determined at the end of the incubation period. The number of experiments (*n*) signifies the number of direct comparisons, and the significance of the differences between the means (*P*) was calculated by the paired sample *t* test (see the Materials and Methods section).

Steroid addition to medium	Mean lipoprotein lipase activity (units/fat-body)		Difference (±s.d.)	<i>n</i>	<i>P</i> <
	Steroid absent	Steroid present			
Triamcinolone	4.2	8.7	4.5 ± 1.4	6	0.001
Dexamethasone	6.1	10.2	4.1 ± 0.9	7	0.001
Cortisol	4.0	5.2	1.2 ± 0.5	7	0.001
Corticosterone	4.0	5.3	1.3 ± 0.4	7	0.001
Aldosterone	4.0	5.3	1.3 ± 0.4	7	0.001
Deoxycorticosterone	4.0	4.7	0.7 ± 0.5	7	0.02

The effects of varying the concentration of insulin in the incubation medium at the optimal concentration of dexamethasone (400nM) are shown in Fig. 5(a) (curve A) and again the results are supplemented by the statistical analysis in Table 2. At 2.0m-i.u. of insulin/ml, the mean activity attained after incubation for 6.5 h is 3.6-fold that present after incubation for the same period in the absence of hormones and 2.1-fold that after incubation with insulin alone.

As in the absence of dexamethasone, the effects of insulin on the lipoprotein lipase activity are correlated with its stimulatory effects on protein synthesis in the tissue [Fig. 5b, line A, correlation coefficient (*r*) = 0.98; *P* < 0.001]. However, the slope of the regression line relating the plateau activity of the enzyme to the rate of protein synthesis is

Fig. 4. *Effects of dexamethasone concentration on total lipoprotein lipase activity during incubations of fat-bodies*
Groups of four fat-bodies were incubated for 6.5 h at 37°C in the incubation medium described in the Materials and Methods section. The dexamethasone concentration was varied as shown. The concentration of insulin in the medium was zero (●), 0.3m-i.u./ml (○) or 2.0m-i.u./ml (■). The lipoprotein lipase activity of the fat-bodies and medium combined was determined at the end of the incubation period.

Table 2. *Effects of dexamethasone and insulin on total lipoprotein lipase activity*

Groups of four fat-bodies were incubated for 6.5 h at 37°C in the incubation medium described in the Materials and Methods section to which insulin and dexamethasone (400 nM) were added as indicated. The lipoprotein lipase activity of the fat-bodies and the medium combined was determined at the end of the incubation period. The results are taken from six experiments similar to those of Fig. 4. The significance of the differences between the means (*P*) was calculated by the paired sample *t* test (see the Materials and Methods section).

Insulin concentration (m-i.u./ml)	Dexamethasone absent (—) or present (+)	Mean lipoprotein lipase activity (units/fat-body)	Difference (± s.d.)	<i>P</i> <
0	—	3.8		
0	+	5.4	1.6 ± 1.0	0.02
0.3	—	4.6		
0.3	+	9.5	4.9 ± 2.0	0.01
2.0	—	6.4		
2.0	+	13.6	7.2 ± 1.9	0.001
0	—	3.8		
0.3	—	4.6	0.8 ± 1.0	NS
0	—	3.8		
2.0	—	6.4	2.6 ± 1.5	0.01
0	+	5.4		
0.3	+	9.5	4.1 ± 2.0	0.01
0	+	5.4		
2.0	+	13.6	8.2 ± 1.0	0.001

increased in the presence of dexamethasone (compare line B, Fig. 5b) showing that, at equivalent rates of protein synthesis, the lipoprotein lipase activity attained is higher in the presence of dexamethasone than in its absence. Although other explanations are possible, this can best be interpreted at the present time as indicating that the effect of dexamethasone is to increase the rate of synthesis of the enzyme relative to the rate of synthesis of other proteins in the tissue, as has been proposed for other systems (Higgins & Gehring, 1978).

Dexamethasone inhibited total protein synthesis in the present experiments by between 19 and 35% at concentrations from 40 pM to 4 μM. However, there was no correlation between the rate of total protein synthesis and the plateau lipoprotein lipase activity when the dexamethasone concentration was varied over the same range at a constant insulin concentration of 2 m-i.u./ml (six experiments). This finding is therefore consistent with the concept of a specific stimulatory action of glucocorticoids on the synthesis of a small number of proteins in the tissue.

Effects of adrenaline. The plateau lipoprotein lipase activity that is achieved during incubations of fat-bodies in the presence of insulin and adrenaline (10 μM) is again dependent on the insulin con-

Fig. 5. *Relation of total lipoprotein lipase activity during incubations of fat-bodies in the presence or absence of dexamethasone to (a) insulin concentration in the incubation medium and (b) rate of protein synthesis in the tissue*

Groups of four fat-bodies were incubated for 6.5 h at 37°C in the incubation medium described in the Materials and Methods section. The insulin concentration was varied as shown in (a) and dexamethasone (400 nM) was either present (○) or absent (●). The lipoprotein lipase activity of the fat-bodies and medium combined and the total tissue protein radioactivity were determined after 6.5 h. The results are typical of six experiments.

centration, but is lower than in the absence of adrenaline at all the concentrations of insulin tested (curve B versus curve A of Fig. 3a). Moreover, although the plateau value remains correlated with the rate of total protein synthesis [line B of Fig. 3b; correlation coefficient (*r*) = 0.97; *P* < 0.01], the regression line is shifted, so that a lower enzyme activity is achieved at any given rate of protein synthesis.

When adrenaline ($10\mu\text{M}$) was present during incubations of fat-bodies in the presence of both dexamethasone (400 nM) and insulin, the potentiating effect of dexamethasone on the plateau lipoprotein lipase activity was completely abolished at all concentrations of insulin. Thus, in seventeen paired experiments, in which fat-bodies were incubated for 6 h, the mean ($\pm\text{s.d.}$) activity in the presence of insulin ($25\text{--}2000\mu\text{ i.u./ml}$), dexamethasone and adrenaline was 5.2 ± 1.0 units/fat-body and that in the presence of insulin and adrenaline was 5.9 ± 0.6 units/fat-body. The difference between paired incubations was not significant.

These results could be accounted for by the postulated action of adrenaline in increasing the rate of inactivation of the enzyme (see the introduction), although the possibility that adrenaline has some relatively specific effect on glucocorticoid-induced lipoprotein lipase synthesis cannot be excluded.

Insulin concentrations in the medium. The insulin concentrations in the medium have been calculated from the stated strengths of stock preparations supplied by the manufacturer. However, it is recognized that insulin in solution in glass containers is adsorbed on the container walls. Immunoassays of the insulin concentrations in the medium in the flasks after incubations for 1 h at 37°C were therefore carried out in four separate experiments. The mean ($\pm\text{s.d.}$) final concentration was only $8.35 \pm 0.14\%$ of that expected on the basis of the dilution of the stock preparations.

This loss of insulin, which is not unprecedented (Bernaert *et al.*, 1977), may be related to the fact that the glass containers used in the present work were routinely cleaned in acid. Separate experiments demonstrated that the maximal response to insulin was obtained at a concentration of about $80\mu\text{ i.u./ml}$ when siliconized glassware was used. This corresponds to the immunoreactive insulin concentrations measured in unsiliconized glassware.

Discussion

The foregoing results demonstrate that glucocorticoids markedly potentiate the effect of insulin in enhancing the activity of adipose-tissue lipoprotein lipase under the conditions of the present experiments. From the work of others, the proximate effects of glucocorticoids are likely to be exerted at the level of RNA synthesis (Higgins & Gehring, 1978). With regard to the action of insulin, previous work has shown that when DNA-directed mRNA synthesis is inhibited by actinomycin D, the rise in enzyme activity remains dependent on the insulin concentration and is enhanced rather than diminished (Wing & Robinson, 1968; P. Ashby &

J. A. Little, unpublished work). This suggests that the insulin effect may be post-transcriptional. The short half-life of lipoprotein lipase allows the possibility that changes in the overall rate of protein synthesis could be disproportionately reflected in lipoprotein lipase activity (Schimke, 1969). Thus the effects of insulin may reflect its general stimulation of adipose-tissue protein synthesis.

The question of the physiological significance of the hormonal effects we have observed is clearly an important one. Mean plasma insulin concentrations for the rats used in the present work have been reported by us previously as being 69 and $24\mu\text{ i.u./ml}$ for fed and 24 h-starved animals respectively (Cryer *et al.*, 1976), and Knox *et al.* (1979) have reported concentrations of between 100 and $200\mu\text{ i.u./ml}$ for rats in a variety of nutritional states. The immunoreactive insulin concentrations used during the fat-body incubations were within this range. Thus our results suggest that rises in plasma insulin concentration similar to those that occur during the transition from the starved to the fed state *in vivo* could bring about increases in adipose-tissue lipoprotein lipase activity. On the other hand, the magnitude of the rise would more closely approach the 6-fold increase that occurs *in vivo* (Ashby *et al.*, 1978a) if there were also a concomitant change in the plasma glucocorticoid concentration. Corticosterone is the principal plasma glucocorticoid in the rat, and its concentration in the free state in rat plasma is reported as ranging from 5 to 500 nM (Westphal, 1971). The concentration rises immediately before the onset of nocturnal feeding and remains high during the fed state when the insulin concentration is also elevated (Peret *et al.*, 1973; Knox *et al.*, 1979). In the present studies significant effects ($P < 0.001$) of corticosterone were observed at a concentration (40 nM) at the lower end of the physiological range. The maximal effects of dexamethasone in enhancing the action of insulin were at 400 nM when the plateau lipoprotein lipase activity achieved after the incubation of the fat-bodies was around 3.6-fold that in the absence of hormones (Table 2). On the present evidence therefore it is possible that much of the change in the activity of the enzyme that is seen *in vivo* during alterations in nutritional state can be attributed to the combination of changes in insulin and glucocorticoid concentrations that occur in the plasma.

This work was supported by a grant from the Medical Research Council. Mrs. D. P. Bennett and Mr. N. M. Johnson are thanked for their expert technical assistance, and Dr. Susan Parkin and Dr. S. Higgins for helpful discussions.

References

- Ashby, P., Grazier, A. M. & Robinson, D. S. (1978a) *Biochem. J.* **171**, 305–311

- Ashby, P., Bennet, D. P., Spencer, I. M. & Robinson, D. S. (1978b) *Biochem. J.* **176**, 865–872
- Bernaert, D., Wanson, J. C., Drochmans, P. & Popowski, A. (1977) *J. Cell Biol.* **74**, 878–900
- Campbell, R. C. (1974) *Statistics for Biologists*, Cambridge University Press, Cambridge
- Chajek, T., Stein, O. & Stein, Y. (1978) *Biochim. Biophys. Acta* **528**, 456–465
- Cryer, A., Foster, B., Wing, D. R. & Robinson, D. S. (1973) *Biochem. J.* **132**, 833–836
- Cryer, A., Riley, S. E., Williams, E. R. & Robinson, D. S. (1976) *Clin. Sci. Mol. Med.* **50**, 213–221
- Davies, P., Cryer, A. & Robinson, D. S. (1974) *FEBS Lett.* **45**, 271–275
- de Gasquet, P. & Pequignot, E. (1973) *Horm. Metab. Res.* **5**, 440–443
- de Gasquet, P., Pequignot-Planche, E., Tonnu, N. T. & Diaby, F. A. (1975) *Horm. Metab. Res.* **7**, 152–157
- Eagle, H. (1955) *Science* **122**, 43–46
- Friedman, G., Stein, O. & Stein, Y. (1978) *Biochim. Biophys. Acta* **531**, 222–232
- Higgins, S. J. & Gehring, V. (1978) *Adv. Cancer Res.* **28**, 313–397
- Hollenberg, C. H., Vost, A. & Patten, R. L. (1970) *Recent Prog. Horm. Res.* **26**, 463–504
- Jansen, H., Garfinkel, A. S., Twu, J.-S., Nikazy, J. & Schotz, M. R. (1978) *Biochim. Biophys. Acta* **531**, 109–114
- Knox, A. M., Sturton, R. G., Cooling, J. & Brindley, D. N. (1979) *Biochem. J.* **180**, 441–443
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Mans, R. J. & Novelli, G. D. (1961) *Arch. Biochem. Biophys.* **94**, 48–53
- Olivecrona, T., Bengtsson, G., Marklund, S.-E., Lindahl, U. & Höök, M. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 60–65
- Patten, R. L. (1970) *J. Biol. Chem.* **245**, 5577–5584
- Peret, J., Macaire, I. & Chanex, M. (1973) *J. Nutr.* **103**, 866–874
- Robinson, D. S. (1970) *Compr. Biochem.* **18**, 51–116
- Robinson, D. S. & Wing, D. R. (1971) *Biochem. Soc. Symp.* **33**, 123–135
- Salaman, M. R. & Robinson, D. S. (1966) *Biochem. J.* **99**, 640–647
- Schimke, R. T. (1969) *Curr. Top. Cell Regul.* **1**, 77–124
- Stewart, J. E. & Schotz, M. C. (1971) *J. Biol. Chem.* **246**, 5749–5753
- Vydelingum, N., Kissebah, A. H. & Wynn, V. (1978) *Horm. Metab. Res.* **10**, 38–46
- Westphal, U. (1971) *Steroid-Protein Interact. Monogr. Endocrinol.* **4**, 164–236
- Wing, D. R. & Robinson, D. S. (1968) *Biochem. J.* **106**, 667–676
- Wing, D. R., Salaman, M. R. & Robinson, D. S. (1966) *Biochem. J.* **99**, 648–656
- Wise, L. S. & Green, H. (1978) *Cell* **13**, 233–242