Tissue composition was measured as described in the text on rats fed for 30 days on a protein-free diet supplemented or not with 0.3% methionine and in control rats at the beginning of the experiment of 150g on a normal diet (A) or fed for 30 days owing to non-esterified fatty acids themselves, prevented adipocyte suspensions were stirred rather than reciprocated to increase until their molar ratio to that required to compensate for fatty acid esterification, was maintained over the entire period; in phase II it exceeded the rate of lipolysis, so that the concentration of non-esterified fatty acids in the medium declined. In the presence of combinations of adenosine deaminase (which eliminates extracellular adenosine; Schwabe & Ebert, 1974; Fain & Wieser, 1975) and noradrenaline, a different sequence of events occurred, as shown in Fig. 1: although a high rate of fatty acid esterification occurred in phase I, it was virtually eliminated in phase II. When dibutyryl cyclic AMP (N\textsubscript{6},O\textsubscript{2}-dibutyryl-adenosine 3':5'-phosphate) was substituted for the combination of noradrenaline and adenosine deaminase, an almost identical pattern was obtained.

The results in the present paper were obtained by using an experimental format which permits the standardization of non-esterified fatty acid concentrations and the determination of changes may occur in the relationship between cyclic AMP concentration and lipolysis. Furthermore, whereas a non-esterified fatty acid:albumin molar ratio of 3:1 in the blood of the intact animal may be an approximate upper limit, it seems likely that in order to sustain such volumes in the general circulation against the rapid rate of consumption of non-esterified fatty acids that occurs during food deprivation, their concentration in the immediate vicinity of adipocytes may be considerably higher.

Table 1. Effect of methionine addition to a protein-free diet on cell composition

<table>
<thead>
<tr>
<th></th>
<th>DNA (mg/g)</th>
<th>Protein DNA</th>
<th>Serum protein</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein deprived</td>
<td>2.0 ± 0.15</td>
<td>56 ± 5</td>
<td>4.49 ± 0.11</td>
<td>2.33 ± 0.06</td>
</tr>
<tr>
<td>Protein deprived + Met</td>
<td>1.8 ± 0.10*</td>
<td>80 ± 6**</td>
<td>6.38 ± 0.28***</td>
<td>2.56 ± 0.02**</td>
</tr>
<tr>
<td>Control A</td>
<td>1.6 ± 0.04</td>
<td>98 ± 7</td>
<td>8.74 ± 0.32</td>
<td>2.88 ± 0.05</td>
</tr>
<tr>
<td>Control B</td>
<td>1.3 ± 0.02</td>
<td>131 ± 9</td>
<td>9.26 ± 0.24</td>
<td>3.57 ± 0.06</td>
</tr>
</tbody>
</table>

with and without methionine (k\textsubscript{a} = 43 and 46 respectively) as compared with the 150g control rats (k\textsubscript{a} = 77).

Profiles of membrane-bound polyribosomes of control rats and of depleted rats with and without methionine (Fig. 1) showed that the protein-free diet induced a disaggregation of polyribosomes with increase of monomers and dimers, whereas methionine addition resulted in a higher proportion of heavier aggregates than in control rats.


Lipolytic agents as regulators of fatty acid esterification in rat adipose tissue

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In an earlier communication (Souness & Davies, 1977), it was speculated that the local regulatory effect of adenosine in adipocytes contributes to the characteristic response of the latter to the 'fasting-and-refeeding' cycle. The release of non-esterified fatty acids from adipocytes is largely dictated by the fluctuating balance between the processes of lipolysis and fatty acid esterification. The manner in which the latter is regulated is difficult to analyse, partly because its rate is influenced in a complex manner by the concentration of non-esterified fatty acids present, which represent an uncontrolled variable in many experiments.

The results in the present paper were obtained by using an experimental format which permits the standardization of non-esterified fatty acid concentrations and the determination of esterification by the 'balance' method of Vaughan (1962). Adipocytes (20mg of triacylglycerol/ml) were incubated in Krebs–Ringer bicarbonate buffer containing 1% bovine serum albumin at 37°C and repeatedly sampled for up to 120 min. The adipocyte suspensions were stirred rather than reciprocated so as to prevent the development of a distinguishable pericellular zone (method validated by M. F. Grahn, J. E. Souness & J. I. Davies, unpublished work). During the initial period of the incubation (designated phase I), L-noradrenaline caused non-esterified fatty acids to increase until their molar ratio to albumin was 7:1 (approx.). Feedback regulation, principally owing to non-esterified fatty acids themselves, prevented further accumulation of them, so that lipolysis could not exceed that required to compensate for fatty acid esterification (phase II). In the absence of glucose, release of non-esterified fatty acids and glycerol was eliminated in phase II and fatty acid esterification was minimal throughout. By contrast, in the presence of glucose (5.5mm) a high rate of esterification was maintained over the entire period; in phase II it exceeded the rate of lipolysis, so that the concentration of non-esterified fatty acids in the medium declined. In the presence of combinations of adenosine deaminase (which eliminates extracellular adenosine; Schwabe & Ebert, 1974; Fain & Wieser, 1975) and noradrenaline, a different sequence of events occurred, as shown in Fig. 1: although a high rate of fatty acid esterification occurred in phase I, it was virtually eliminated in phase II. When dibutyryl cyclic AMP (N\textsubscript{6},O\textsubscript{2}-dibutyryl-adenosine 3':5'-phosphate) was substituted for the combination of noradrenaline and adenosine deaminase, an almost identical pattern was obtained.

The mechanism responsible for eliminating fatty acid esterification in the presence of dibutyryl cyclic AMP, and combinations of noradrenaline and adenosine deaminase, remains uncertain. The presence of high concentrations of non-esterified fatty acids may be a necessary prerequisite: in experiments where adenosine deaminase was added at various times during incubation of fat-cells with noradrenaline, the interval between the addition of the enzyme and the elimination of esterification was diminished. Nevertheless the presence of high concentrations of non-esterified fatty acids is clearly not in itself sufficient to initiate the response.

A common feature of the two treatments that prevent esterification in phase II is that they cause large and sustained increases in cellular cyclic AMP. Even though evidence has been provided that enzymes involved in esterification are inhibited by cyclic AMP-dependent mechanisms (Nimmo & Houston, 1978; Saggerson et al., 1979) a direct effect by this substance would seem unlikely in view of the delayed response observed.

Although the changes in the rate of fatty acid esterification were observed under conditions in which the concentration of cyclic AMP and the ratio of non-esterified fatty acids:albumin were both high, they may nevertheless be of significance in the context of the 'fasting–refeeding' cycle where, for instance, changes may occur in the relationship between cyclic AMP concentration and lipolysis. Furthermore, whereas a non-esterified fatty acid:albumin molar ratio of 3:1 in the blood of the intact animal may be an approximate upper limit, it seems likely that in order to sustain such volumes in the general circulation against the rapid rate of consumption of non-esterified fatty acids that occurs during food deprivation, their concentration in the immediate vicinity of adipocytes may be considerably higher.

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Fig. 1. Effect of adenosine deaminase on noradrenaline-stimulated lipolysis (a) and esterification (b)

For details of incubation conditions, see the text. (Q) Noradrenaline (1 µM) only; (Δ) noradrenaline and adenosine deaminase (1 µg/ml; calf intestine, from Boehringer; sp. activity 200 units/mg, where 1 unit of activity is the amount of enzyme required to transform 1 µmol of substrate/min under optimal conditions). The points represent the means ± S.E.M. of three replicates.

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Xanthine oxidase in adipose tissue: potential effects on lipolytic activity

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The products of the activity of xanthine oxidase (EC 1.2.3.2) include such reactive substances as H₂O₂, singlet oxygen and the superoxide and hydroxyl free radicals (Kellogg & Fridovich, 1975), which are currently receiving considerable attention not only because they lead to the formation of lipid hydroperoxides, but also because the latter may promote the formation of prostaglandins (Hemler et al., 1979) and the free radicals themselves may stimulate cyclic GMP synthesis (Mittal & Murad, 1977).

It has been shown that xanthine oxidase is present in isolated adipocytes and that it can oxidize the appreciable quantity of purine derivatives released during their incubation in Krebs–Ringer bicarbonate buffer containing bovine serum albumin (Fain, 1979; M. F. Grahn, J. E. Soussen & J. I. Davies, unpublished work). This activity is clearly a potential source of H₂O₂ and its free-radical relatives.

One mode of action that is open to the purine-related substrates of xanthine oxidase was discovered by Raben & Matsuaki (1966), who showed that, while diminishing the lipolytic response to L-adrenaline, adenosine, inosine and hypoxanthine added to incubation media containing adipose-tissue pieces also enhanced the degradation of the hormone. Both of these effects were reversed by allopurinol (4-hydroxy-pyrazolo[3,4-d]pyrimidine), an inhibitor of xanthine oxidase.

It became apparent that the degradation of the hormone by products of xanthine oxidase activity cannot adequately account for the effects of purine derivatives on lipolysis when it was shown that these substances also inhibit the lipolytic action of corticotropin (Davies, 1968). However, a more general anti-lipolytic effect might be expected if the free radicals generated by xanthine oxidase cause changes, not only in the hormone, but also in its target cell. Such changes in adipocyte properties caused by exposure to H₂O₂ have been depicted as ‘insulin-like’ effects (Czech, 1977).

Table 1 shows that a combination of xanthine oxidase and xanthine, a substrate of the enzyme, has a potent inhibitory action against the lipolytic response of isolated rat adipocytes to