The mixture was diluted to terminate the permeabilization reaction and the acini were washed three times to remove detergent before final resuspension in the same medium. To assess adenylate cyclase activity, the permeabilized cells were incubated at 37°C in the same medium containing ATP, an ATP-regenerating system, Ro 7-2956, and appropriate additions; after 15 min, perchloric acid was added to a final concentration of 6%. The deproteinated extract was neutralized and its cyclic AMP content was determined using the competitive binding assay of Tovey et al. (1974).

In comparison with control acini, the permeabilized cells accumulated less cyclic AMP (around 35% of control) when incubated without added ATP, but more (200% of control) when ATP was present. The permeabilized cells responded markedly to the β-agonist isoprenaline, and in the presence of ATP, an ATP-regenerating system, Ro 7-2956, and appropriate additions; after 15 min, perchloric acid was added to a final concentration of 6%. The deproteinated extract was neutralized and its cyclic AMP content was determined using the competitive binding assay of Tovey et al. (1974).

In further experiments, 8-phenyltheophylline was itself found to inhibit the forskolin/GTP-stimulated adenylate cyclase activity of permeabilized acini. The adenosine analogue, N-ethylcarboxamidoadenosine, which acts via stimulatory R₁ adenosine receptors, had variable effects on enzyme activity. These results suggest that mammary acini membranes are equipped with inhibitory (R₁) adenosine receptors. No evidence for the presence of stimulatory (R₂) adenosine receptors was found.

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Cholesterol synthesis and fatty acid synthesis may be inversely regulated in rat mammary acini

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The lactating rat mammary gland contains a high level of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) activity (Middleton et al., 1981) which shows a diurnal variation (Gibbons et al., 1983; Smith et al., 1986). The expressed activity of the enzyme in the gland is only a fraction of the total available capacity due to inactivation by phosphorylation (Smith et al., 1986). Lipogenesis in the mammary gland was also reported to undergo a marked diurnal rhythm which was the inverse of that observed with cholesterol synthesis (Munday & Williamson, 1983). The activity of acetyl-CoA carboxylase, the rate-determining enzyme in fatty acid synthesis, is also regulated by a phosphorylation/dephosphorylation mechanism (Hardie & Guy, 1980). Since both fatty acid synthesis and cholesterol biosynthesis compete for the same substrates, the inverse relationship in their diurnal variation suggests that they may be mutually regulated. Investigation of this hypothesis using acini prepared from lactating mammary glands would be dependent on the retention of the appropriate enzyme activities in the acini. We have therefore investigated cholesterol synthesis and fatty acid synthesis in acini prepared from rats killed at different points in the light-dark cycle.

Lactating Wistar rats were maintained on a constant lighting schedule (lights on 08:00 h to 20:00 h). Acini were prepared (Robson et al., 1984) from the mammary glands of mid-lactating rats killed at five time points (Table 1) and were incubated in Krebs-Henseleit buffer containing bovine serum albumin (4%, w/v), Ficoll (2%, w/v), glucose (5 mM) and [14C]-labelled acetate (2 μM, 0.5 Ci/mol). Separation of cholesterol and fatty acids was as described (Stansbie et al., 1976) and incorporation of [14C] into the two fractions quantified. Acini were also homogenized (Polytron) in a medium containing KF (100 mM) and proteolytic inhibitors and a microsomal pellet obtained by differential centrifugation. A portion of the pellet was assayed (Balasubramaniam et al., 1976) directly for HMG-CoA reductase activity. The rest was washed free of fluoride and incubated with rat liver phosphoprotein phosphatase before being assayed for HMG-CoA reductase activity.

Cholesterol synthesis in the acini, as measured by incorporation of [14C]-acetate, varied in a cyclical manner (Table 1), reaching a maximum value at 14:00 h, the mid-point of the light phase. The minimum value was observed 9 h into the dark phase (05:00 h) when cholesterol synthesis was reduced to 31% of the maximum. The variation in 'expressed' HMG-CoA reductase activity measured in microsomal fractions prepared at the same time paralleled the change in cholesterol synthesis but showed a greater variation than cholesterol biosynthesis. At 14:00 h enzyme activity was 4.4-fold

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Abbreviation used: HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase.
Total HMG-CoA reductase activity was recorded as that present in microsomes after phosphatase treatment. Expressed HMG-CoA reductase activity was taken as the enzyme activity measured in microsomes obtained from acini homogenized in the presence of fluoride. The results given are the means ± S.E.M. for three animals, except where indicated by the number in parentheses.

<table>
<thead>
<tr>
<th>Time of day (h)</th>
<th>Rate of incorporation (nmol/h per mg of DNA) of [14C]acetate into:</th>
<th>Percentage of [14C]acetate incorporated into:</th>
<th>HMG-CoA reductase activity (nmol of mevalonate formed/min per mg of microsomal protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol</td>
<td>Fatty acids</td>
<td></td>
</tr>
<tr>
<td>00:00h</td>
<td>1.34 ± 0.151</td>
<td>62.63 ± 1.47</td>
<td>0.025 ± 0.002</td>
</tr>
<tr>
<td>11:00h</td>
<td>1.41 ± 0.08(8)</td>
<td>49.68 ± 0.91(5)</td>
<td>0.026 ± 0.008(8)</td>
</tr>
<tr>
<td>14:00h</td>
<td>2.93 ± 0.13</td>
<td>21.73 ± 2.13</td>
<td>0.057 ± 0.003</td>
</tr>
<tr>
<td>20:00h</td>
<td>2.31 ± 0.12</td>
<td>37.83 ± 3.22</td>
<td>0.043 ± 0.004</td>
</tr>
<tr>
<td>05:00h</td>
<td>0.87 ± 0.06(5)</td>
<td>68.56 ± 0.63</td>
<td>0.015 ± 0.001(5)</td>
</tr>
</tbody>
</table>

The use of computer-generated enzyme kinetic data in the undergraduate biochemistry practical course

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Experiments in enzyme kinetics form a part of all practical biochemistry courses at the undergraduate level. However, some of the materials required are expensive and the acquisition of large amounts of data can be tedious and time-consuming. The widespread availability of microcomputers has made the use of simulations an attractive alternative, but this needs to be justified in terms of the objectives of practical work. A series of such objectives have been formulated and it is shown that many of them can be satisfied by simulations; they also lead to clear criteria for assessment.

Some objectives for practical work are as follows: (1) to review and apply concepts previously dealt with in lectures, tutorials, seminars and assigned reading; (2) to acquire knowledge of the applications, limitations and precision of activity and fatty acid synthesis, as was observed in whole tissue (Smith et al., 1976) and in intact animals (Gibbons et al., 1983; Munday & Williamson, 1983) respectively. They therefore retain the phosphorylation status of the enzyme during preparation and are suitable for investigation of the mechanisms regulating the two pathways.


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a wide range of modern techniques and instruments; (3) to acquire relevant manipulative skills; (4) to learn the principles of experimental planning and the use of 'range-finding' experiments; (5) to learn to assess and manipulate experimental data using data transformation, graphical interpretation and statistical analysis; (6) to be able to present data and critically to discuss experimental results. There is an additional objective from the point of view of the instructor: (7) the experiment should involve minimum expenditure, give reproducible results, and be feasible within the time available. All of these objectives except (2) and (3) can be satisfied in part by the use of computer simulations. A simple program has been written for the Apple IlE and BBC microcomputers which generates rates using standard equations with a superimposed random error; such programs are easily written and require only very modest programming ability. A unique set of data is supplied to each student who must input the concentration of substrate and, where relevant, inhibitor, and write down the rate displayed on the screen. This program has been used over...