was centrifuged at 20000g for 20 min and the supernatant filtered through a column (3.5 cm × 0.85 cm) of Sephadex G-25 (fine grade) to remove all molecules of mol. wt. less than 5000. Cell extracts were incubated at 37°C with [3H]thymidine, ATP and MgCl₂, and the phosphorylated products collected on DE-81 (DEAE-cellulose) filters to be assayed for radioactivity. The assay was shown to be linear with added extract protein and with time of incubation. Hypoxanthine/guanine phosphoribosyltransferase and adenine phosphoribosyltransferase activities were determined in the same cell extracts by the methods previously published (Green & Martin, 1973). Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were determined by the method of Glock & McLean (1953).

We found that thymidine kinase activity is increased 2–3-fold by physiological concentrations of oestrogen or insulin and it is decreased by hormones and anti-hormones that inhibit cell division. The dose–response curve for the increase in enzyme activity caused by oestradiol-17β is the same as that for the increase in [³H]thymidine incorporation. The effect on thymidine kinase appears to be relatively specific, since the activities of the purine-salvage-pathway enzymes hypoxanthine/guanine phosphoribosyltransferase and adenine phosphoribosyltransferase and of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are unaffected by hormone treatment.

The effect of oestrogen on [³H]thymidine incorporation cannot wholly be explained by its stimulation of thymidine kinase activity, however. Oestrogen does stimulate division of MCF-7 cells as measured by increase in cell number per dish, although the effect does not become apparent until after 6 days in the presence of the hormone. Stimulation of [³H]thymidine incorporation does not occur before 24 h after the addition of oestradiol, but the increase in thymidine kinase activity is significant by 12 h. This latter result would seem to indicate that thymidine incorporation is regulated at some step beyond thymidine kinase so that this regulatory point is not affected by oestradiol until later times in the response.

Thymidine kinase is a pyrimidine 'salvage' enzyme and as such is not on the direct pathway for synthesis of pyrimidine nucleotides de novo. In view of this it is difficult to see why it should be subject to hormonal regulation; nevertheless, in these cells at least, it seems to be induced by oestradiol and the increase in its activity is one of the earliest responses to the hormone.

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Activation of Hepatic Glycogen Phosphorylase in Anoxic Liver
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It is well established that lack of oxygen in the liver directly causes rapid acceleration of glycogenolysis, manifested by increased degradation of glycogen, and formation of glucose and lactate (Levine, 1965; Burton & Ishida, 1965; Glinsmann et al., 1969; Hems & Brosnan, 1970; Woods & Krebs, 1971; Walli et al., 1974; Seglen, 1974; Jakob
Table 1. Time course of changes in content of phosphorylase a in vivo in ischaemic liver

Rats were either killed by cervical dislocation or anaesthetized fairly deeply with Nembutal. The liver was rapidly removed, and portions were sequentially freeze-clamped. Results are means±S.E.M. for the numbers of observations in parentheses.

<table>
<thead>
<tr>
<th>Duration of ischaemia (s)</th>
<th>Cervical dislocation</th>
<th>Nembutal anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed</td>
<td>48 h-starved</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>3.0±1.5 (6)</td>
</tr>
<tr>
<td>10</td>
<td>11.9±0.9 (6)</td>
<td>—</td>
</tr>
<tr>
<td>30</td>
<td>—</td>
<td>10.7±1.4 (3)*</td>
</tr>
<tr>
<td>60</td>
<td>20.9±0.6 (3)*</td>
<td>12.0±1.4 (3)*</td>
</tr>
<tr>
<td>180</td>
<td>18.6±0.6 (3)*</td>
<td>6.5±1.1 (6)*</td>
</tr>
</tbody>
</table>

* P<0.05, compared with initial value.

& Diem, 1974; Birch et al., 1974). There may be activation of glycogen phosphorylase in hypoxic tissue, as shown by the increase in content of glucose 6-phosphate, compared with that in control samples (Hems & Brosnan, 1970).

Activation of phosphorylase might be expected to occur in hypoxic liver, since glycogenolysis in general is controlled by the amount of active phosphorylase (the a form). Perhaps surprisingly, there are no reports of a rapid increase in the amount of phosphorylase a in liver, in response to lack of oxygen, although an increase after a period of cyanide treatment has been shown (Jakob & Diem, 1974).

New insights into the differences between the a and b forms of phosphorylase (Stalmans & Hers, 1975), and into the requirement to rapidly freeze tissues before enzyme assays, have opened the way to more accurate estimation of the hepatic content of phosphorylase a. In this paper, we show an immediate increase in the content of phosphorylase a in rat liver which was anoxic as a result of rapid cessation of blood flow. Phosphorylase a in freeze-clamped liver was determined by the method of Stalmans & Hers (1975) with minor modifications (Hems et al., 1976).

In a first group of experiments, livers were rapidly removed in vivo. An initial sample of the liver was immediately freeze-clamped (with a delay of 3–8 s after severance of blood supply) and then further samples of the same liver were freeze-clamped at intervals (Hems & Brosnan, 1970). There was an increase in the hepatic content of phosphorylase a within 30 s of ischaemia (Table 1).

The values for hepatic phosphorylase a in fed rats which were fairly deeply anaesthetized and allowed to settle were low (Table 1) in agreement with other studies of sampling procedures (Stalmans et al., 1974a). During ischaemia in livers from anaesthetized 48-h-starved rats, a smaller increase in phosphorylase a content was observed (Table 1); thus in the absence of glycogen, which is an inhibitor of phosphorylase a phosphatase (Stalmans et al., 1971; Khandelwal, 1977), a lower maximal phosphorylase a activity was attained during anoxia.

These results establish that the hepatic content of phosphorylase a immediately increases in response to lack of oxygen. This must imply either activation of phosphorylase b kinase or inhibition of phosphorylase a phosphatase. Activation of kinase seems unlikely in anoxic tissue, when the ATP availability is decreasing, and no such activation has been reported. The only simple explanation for the increase in the amount of phosphorylase a in anoxic liver, yet available, is that there is slowing of the dephosphorylation of phosphorylase a, as a result of the modifier action of AMP on this process (Stalmans et al., 1974b; Khandelwal, 1977).

It is well established that there is a rapid increase in the content of AMP in ischaemic liver (e.g. Hems & Brosnan, 1970; Faupel et al., 1972). The possible role of AMP in
Table 2. *Time course of changes in content of phosphorylase a and AMP in ischaemic perfused liver of fed rats*

Livers were removed, and portions sequentially freeze-clamped, after perfusion of the liver with 10mM-glucose for 40min (Hems *et al.*, 1976). Results are means ± S.E.M. for the numbers of observations in parentheses.

<table>
<thead>
<tr>
<th>Duration of ischaemia (s)</th>
<th>Phosphorylase a (µmol/min per g of fresh liver)</th>
<th>AMP (µmol/g of fresh liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5.6 ± 0.6 (8)</td>
<td>0.21 ± 0.05 (6)</td>
</tr>
<tr>
<td>30</td>
<td>9.7 ± 0.7 (4)*</td>
<td>0.51 ± 0.05 (4)*</td>
</tr>
<tr>
<td>60</td>
<td>12.0 ± 0.9 (6)*</td>
<td>0.60 ± 0.05 (4)*</td>
</tr>
<tr>
<td>180</td>
<td>16.2 ± 0.8 (7)*</td>
<td>0.72 ± 0.14 (6)*</td>
</tr>
<tr>
<td>300</td>
<td>15.8 ± 0.4 (4)*</td>
<td>0.98 ± 0.12 (4)*</td>
</tr>
</tbody>
</table>

* P < 0.05, compared with initial value.

hypoxia-induced phosphorylase activation was investigated in the perfused liver, and offers improved access for rapid sampling of the liver, which can be freeze-clamped in less than 2s. In a series of ischaemic samples prepared from perfused livers, an increase in content of phosphorylase a was observed within 30s (Table 2). The hepatic content of AMP increased in parallel with the phosphorylase a. This is compatible with the hypothesis that during anoxia, phosphorylase is activated as a result of inhibition by AMP of the dephosphorylation of the a form.

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**A Binding Assay for Thyroxine-Binding Globulin Based on its Affinity for Thyroxine-Sepharose-4B**

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Many binding assays, such as radioimmunoassay, depend on antibody binding of the substance to be measured. In the case of thyroxine-binding globulin, which functions as a transport protein for thyroxine, we have preferred the use of this physiological binding...