After the placenta is lost at parturition the concentration of placental lactogen in the blood declines rapidly (Grumbach et al., 1968), whereas that of prolactin shows a compensatory increase. I assume that this switch in lactogenic hormone results in a fall in ‘anti-insulin’ activity, a situation which would occur if either the serum concentration of prolactin were lower than that of placental lactogen (Hwang et al., 1971) or if prolactin were inherently less like growth hormone (Niall et al., 1971). Hence the restraint on carbohydrate metabolism is removed and milk synthesis proceeds.

The idea of negative rather than positive control of the initiation of milk synthesis has previously been considered for progesterone, whose concentration also falls at parturition (Kuhn, 1971). Although progesterone probably regulates the switch from placental to pituitary lactogen, the possibility that it has a direct inhibitory effect on the mammary gland seems less likely. Thus although progesterone does inhibit the induction of α-lactalbumin in vitro, its effect appears to be specific to this protein, other proteins being unaffected (Turkington & Hill, 1969; Leader & Barry, 1969). In contrast, the hypothesis I propose suggests a way in which a single hormone, placental lactogen, through its separate biochemical effects on carbohydrate metabolism and specific protein synthesis might exert a general restraint on the synthesis of milk constituents, while simultaneously promoting growth and differentiation of the milk-producing tissue.

Hales, C. N. (1967) Essays Biochem. 3, 73-104

Effects of Prolactin Withdrawal on Activity of Pyruvate Dehydrogenase of Rat Mammary Gland
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It has been shown in an earlier publication (Coore & Field, 1974) that pyruvate dehydrogenase of rat mammary gland is regulated by a phosphorylation–dephosphorylation cycle similar to that shown to operate on the enzyme from other mammalian tissues (reviewed by Wieland et al., 1973). The former study also showed that, during the first half of lactation, the total enzyme activity (after dephosphorylation in vitro) increased sevenfold whereas the fraction of the enzyme which was dephosphorylated in vivo increased threefold. Both parameters altered little until weaning at 21 days after which both fell steeply within 3 days to pre-lactation values.

Prolactin is known to exert a major influence on the development of the mammary
W F

Table 1. Effect of 2-bromo-a-ergocryptine with or without prolactin on rat mammary pyruvate dehydrogenase

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days lactating</th>
<th>Total pyruvate dehydrogenase activity (μunits/mg of DNA)</th>
<th>Initial pyruvate dehydrogenase activity (μunits/mg of DNA)</th>
<th>Initial/total pyruvate dehydrogenase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (solvent injections only)</td>
<td>4</td>
<td>123 ± 19 (5)</td>
<td>105 ± 13 (4)</td>
<td>0.83 ± 0.09 (4)</td>
</tr>
<tr>
<td>2-Bromo-a-ergocryptine injected</td>
<td>12</td>
<td>196 ± 10 (6)*</td>
<td>189 ± 18 (5)</td>
<td>0.98 ± 0.05 (5)*</td>
</tr>
<tr>
<td>2-Bromo-a-ergocryptine and 2mg of prolactin injected 24h before</td>
<td>19-21</td>
<td>235 ± 28 (6)*</td>
<td>227 ± 14 (6)*</td>
<td>0.95 ± 0.05 (5)*</td>
</tr>
<tr>
<td>Control (solvent injections only)</td>
<td>4</td>
<td>105 ± 21 (3)</td>
<td>97 ± 8 (3)</td>
<td>0.92 ± 0.08 (3)*</td>
</tr>
<tr>
<td>2-Bromo-a-ergocryptine injected</td>
<td>12</td>
<td>169 ± 10 (6)*</td>
<td>152 ± 17 (6)*</td>
<td>0.95 ± 0.05 (6)*</td>
</tr>
<tr>
<td>2-Bromo-a-ergocryptine and 2mg of prolactin injected 2h before</td>
<td>19-21</td>
<td>216 ± 15 (6)*</td>
<td>191 ± 14 (6)*</td>
<td>0.90 ± 0.07 (6)*</td>
</tr>
</tbody>
</table>

In all cases 2-bromo-a-ergocryptine was injected 24h before extraction of the enzyme. Freeze-clamped mammary gland was extracted in either 25mM-triethanolamine-7mM-2-mercaptoethanol, pH 7.0, or 20mM-triethanolamine-1mM-EDTA, pH 7.0. The EDTA extract was assayed directly; initial pyruvate dehydrogenase activity. The extract without EDTA was incubated for 15min at 30°C in the presence of 10mM-MgCl₂ and more than 0.5 unit of pyruvate dehydrogenase phosphatase/ml and then assayed, total pyruvate dehydrogenase activity. For further details see Coore & Field (1974). Values are expressed as means ± S.E.M. with numbers of animals in parentheses.

* P<0.05 versus appropriate control.
gland and its concentration in blood rises steeply during the first 4 days of lactation in rats (Amenomori et al., 1970; Simpson et al., 1973). To investigate the effect of prolactin withdrawal on mammary pyruvate dehydrogenase we have used the drug 2-bromo-α-ergocryptine, which depresses blood prolactin concentration in rats (Seki et al., 1974). Since the drug may have other effects we have also performed experiments involving simultaneous administration of 2-bromo-α-ergocryptine and prolactin.

The selection and treatment of rats, operation procedure for sampling the mammary tissue, methods of assay and expression of results were as in Coore & Field (1974). 2-Bromo-α-ergocryptine (CB 154) and bovine prolactin were the generous gifts from Sandoz Ltd., Basle, Switzerland, and N.I.H., Bethesda, Md., U.S.A., respectively. Both agents were injected subcutaneously; 1mg of 2-bromo-α-ergocryptine was dissolved in 0.2ml of 40% (v/v) ethanol and 0.5 or 2mg of prolactin were dissolved in 0.2–0.4ml of 154mM-NaCl, pH 10.0.

Table 1 shows the experimental results. At mid lactation 2-bromo-α-ergocryptine treatment diminished the 'initial activity' of the enzyme extracted in the presence of EDTA. This we consider to represent the activity of the enzyme at the moment of sampling in vivo. The ‘total enzyme activity’, however, increased and hence the fraction inactivated by phosphorylation in vivo had increased considerably. Adequate dosage with prolactin prevented both effects of 2-bromo-α-ergocryptine. These findings suggest a major role of prolactin in regulating the synthesis and phosphorylation of the enzyme at mid lactation.

In late lactation 2-bromo-α-ergocryptine treatment again diminished the ‘initial activity’ of pyruvate dehydrogenase but now the ‘total activity’ was also decreased. Again prolactin injection appeared able to reverse these effects. The relatively small effect of 2-bromo-α-ergocryptine in late compared with mid lactation may be due to the fact that in late lactation prolactin concentrations in blood have returned to pre-lactation values (Amenomori et al., 1970; Simpson et al., 1973). Hence at that time additional factors may support pyruvate dehydrogenase activity and further lowering of prolactin concentration in the blood might be of smaller consequence.

In early lactation the large effects of 2-bromo-α-ergocryptine injection on ‘initial’ and ‘total’ pyruvate dehydrogenase activity were undisturbed by prolactin, given either with the drug or 2h before tissue extraction. This suggests that in early lactation some factor other than prolactin was affected by 2-bromo-α-ergocryptine treatment and this factor was of decisive importance in regulating the enzyme activity. Since it has been stated that luteinizing-hormone concentrations in the blood are also decreased by injection of 2-bromo-α-ergocryptine (Seki et al., 1974) it will be of interest to see whether dosage with luteinizing hormone with or without prolactin can reverse the effects of the drug during early lactation. Injection of 0.5mg of prolactin/rat in normal 4 day lactating rats was without any effect on pyruvate dehydrogenase activity.

It is recognized (Turkington, 1972) that several hormones are involved in the development of the mammary gland. Pyruvate dehydrogenase of mammary gland may be a useful test system for studying hormonal interactions and may also reveal a ‘final common pathway’ by which hormones act on this enzyme.

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Changes in Monoamine-Related Enzymes in Cobalt-Induced Epilepsy

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The evidence that points to the involvements of aminergic neurons in epilepsy can be divided into two sets. First, the work that indicates that drugs acting on amine metabolism can modify seizures, including data on the use of reserpine to lower brain amine concentrations in animals (Schlessinger et al., 1968) and man (Pallister, 1959), and p-chlorophenylalanine to decrease brain 5-hydroxytryptamine in rats (De La Torre & Mullan, 1970). These studies all demonstrated that a general decrease of amines tended to increase susceptibility to epileptic seizures. This has been the subject of a review by Carlsson (1974).

Conversely, drugs that potentiate the activities of brain amines, including monoamine oxidase inhibitors (Prockop et al., 1959) or tricyclic anti-depressants (Lehmann, 1967), have at least moderate protective effects. Recently this series of data has been refined into an analysis of the roles of the different amines; dopamine (3,4-dihydroxyphenethylamine) and noradrenaline are now thought by some workers to exert opposing influences on an epileptic focus, inasmuch as dopamine and its agonists can be shown to decrease ‘spiking’ in the cobalt model, whereas noradrenaline agonists have the reverse effect (Ashcroft et al., 1974).

The second set of information consists of work that indicates that an alteration in the concentrations of brain amines, presumably parameters of a functional change in specific cortical circuits, occurs in the development of audiogenic seizures (Schlessinger et al., 1967) and in cobalt epilepsy (Emson & Joseph, 1975). However, other workers have found no changes in brain amines related to epilepsy (Hansen et al., 1973), so the case cannot be said to be by any means complete.

In an attempt to clarify the problem of amine involvement in epilepsy, a study was undertaken of the following amine-related enzymes in the cobalt model (Dow et al., 1972): tyrosine hydroxylase, catecholamine O-methyltransferase and monoamine oxidase. γ-Aminobutyrate aminotransferase was also investigated. The purpose was to use these enzymes as cell markers to study the integrity of aminergic circuitry in the cortex around a cobalt focus, and to ascertain whether these specific neurons could be demonstrated to react differentially.

Tyrosine hydroxylase was assayed by the method of Hendry & Iversen (1971), it being assumed that this would be the most specific marker for noradrenaline neurons, and the catechol O-methyltransferase (Axelrod & Tomchick, 1958) and monoamine oxidase (McCaman et al., 1965) assays were mainly corroborative. γ-Aminobutyrate aminotransferase was examined (Hall & Kravitz, 1967) in the hope that it would provide data on the integrity of local inhibitory gabaminergic fibres and their postulated role in the eventual suppression of a focus, and also as an index of the glial reaction that is associated with the cobalt implant.

Histological studies of the cobalt model done in our laboratory (Emson & Joseph, 1975) by using the Fink–Heimer (Fink & Heimer, 1967) technique have demonstrated degenerating terminals, axons and cells around the primary lesions after 4 days, extending to the 29th day after implant, at which time spikes are still present in the electrocorticogram. Other enzymes so far studied, including glutamate decarboxylase, choline acetyltransferase and aromatic amino acid decarboxylase, tend to follow a similar pattern, reaching their lowest activities in the primary (1°) focus at around day 8 and re-