not additive. The stimulation of activity by tryptophan or cortisol was not inhibited by actinomycin D.

It appears that around the time of weaning L-serine dehydratase synthesis is stimulated after cortisol release, but that then the increased dietary intake of glucose associated with weaning lowers the cortisol-stimulated activity. Subsequent increase of activity is effected by the increased intake of amino acids (perhaps specifically tryptophan) that is also associated with weaning (see Snell & Walker, 1972); this effect may operate at the translational level of enzyme synthesis.

We are grateful to the Wellcome Trust for financial support.

- Evered, D. F. & Roffe, L. M. (1971) *Comp. Biochem. Physiol. B* **39**, 377-381
 Goswami, M. N. D., Boulekbache, H. & Meury, F. (1972) *Comp. Biochem. Physiol. B* **41**, 323-330
 Greengard, O. & Dewey, H. K. (1967) *J. Biol. Chem.* **242**, 2986-2991
 Inoue, H., Kasper, C. B. & Pitot, H. C. (1971) *J. Biol. Chem.* **246**, 2626-2632
 Snell, K. & Walker, D. G. (1972) *Biochem. J.* **128**, 403-413
 Vernon, R. G., Eaton, S. W. & Walker, D. G. (1968) *Biochem. J.* **110**, 725-731
 Yeung, D. & Oliver, I. T. (1971) *Comp. Biochem. Physiol. A* **40**, 135-144

Enzyme Adaptation for Glycerol Utilization in Neonatal Rats

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The changes in hepatic glycerol kinase (EC 2.7.1.30) and glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) activities during the development of the rat were described by Vernon & Walker (1970). Factors that regulate these changes and their relationship to the normal physiological events have now been investigated. Low activities of both enzymes are detected in the foetal liver at 18 days gestation, and these activities rise fourfold during the following 4 days and then continue to rise more rapidly after birth. The effect of starvation on prematurely delivered, full-term and 6h-old pups that have suckled once was examined, the animals being maintained in a humidicrib at 37°C for 24h and the hepatic activities then measured. These were 60% lower in the experimental animals than in normal suckled litter mates.

Increased activity of glycerol 3-phosphate dehydrogenase can be observed 5h after injection of thyroxine, cortisol or glycerol *in utero*, but glycerol kinase activity is increased only by thyroxine. In-

jections of neither glucagon nor dibutyl cyclic AMP (6-*N*,2'-*O*-dibutyladenosine 3':5'-cyclic monophosphate) have any effect on these enzymes. Administration of cortisol or glycerol to 5-day-old animals resulted in similar rises 24h later in both glycerol kinase and glycerol 3-phosphate dehydrogenase activities, but thyroxine had no effect.

Glycerol utilization in liver slices from animals of various ages incubated *in vitro* was examined by measuring the incorporation of ¹⁴C from [U-¹⁴C]-glycerol into CO₂, liver lipids and liver glucose + glycogen. Less than 0.5% incorporation into CO₂ was found for suckling rats, but this rises for weanling animals. Incorporation into glucose is by far the greatest in slices from animals 0-2h old. By 1 day *post partum* this has fallen through a minimum and then rises again to a new maximum at 15 days (Vernon & Walker, 1970). Incorporation of radioactivity into total liver lipids was very low throughout the whole suckling period.

These studies suggest that gluconeogenesis from glycerol may be important in the immediately newborn animal. Blood glycerol concentrations at this time measured by the method of Sheath (1970) are high, then decrease for several hours until normal suckling causes a fresh rise in them.

Prevention of the normal rise in glycerol kinase and glycerol 3-phosphate dehydrogenase activities by starvation and the failure of attempts at prenatal induction with glucagon suggest that postnatal hypoglycaemia is not involved in regulation of these enzymes, and that their development is related to as yet unknown suckling or associated factors.

Sheath, J. B. (1970) *Clin. Biochem.* **3**, 339-341

Vernon, R. G. & Walker, D. G. (1970) *Biochem. J.* **118**, 531-536

Enzyme Synthesis and Adaptation of Hepatic 'Malic' Enzyme

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The adaptive behaviour of rat hepatic 'malic' enzyme (EC 1.1.1.40) has been characterized sufficiently, both in the adult (Wise & Ball, 1964; Tarentino *et al.*, 1966) and in the developing postnatal animal (Vernon & Walker, 1968*a,b*; Hemon, 1968; Lockwood *et al.*, 1970), to indicate that similar physiological stimuli are involved in both adaptive and developmental phenomena.

A radio-immunological technique was developed to monitor changes in rate of hepatic 'malic' enzyme synthesis, relative to total soluble protein (Schimke & Doyle, 1970), during both adaptation and develop-

ment of the rat. 'Malic' enzymes from the livers of normal and hyperthyroid rats were found to be kinetically, electrophoretically and immunologically identical. Hence the increased activity found in hyperthyroid rat liver was due to an increase in the same form of enzyme protein as in normal liver. 'Malic' enzyme was therefore purified from the livers of hyperthyroid rats by a modification of the method of Hsu & Lardy (1967) for pigeon liver 'malic' enzyme. The final preparation (specific activity 21–23 units/mg of protein) was homogeneous as assessed by analytical centrifugation, by electrophoresis (including iso-electric focusing) and by immunological analysis.

A specific antiserum to purified 'malic' enzyme was raised in rabbits and the enzyme–anti-enzyme precipitin reaction was subsequently characterized both qualitatively and quantitatively. Dissociation of this antigen–antibody complex with sodium dodecyl sulphate and subsequent electrophoresis yielded three protein components, the subunit of 'malic' enzyme (molecular weight 61000) and the light and the heavy chains of the γ -globulin antibody.

Administration of L-[4,5-³H]leucine *in vivo* to rats aged 30–35 days and isolation of immunoprecipitable hepatic 'malic' enzyme by the specific antiserum indicated that the relative rate of enzyme synthesis was decreased threefold by starvation for 48h or in alloxan-diabetic animals and was increased almost fourfold on treatment with thyroxine, compared with the rate in the normal animal. Rats aged 30 days and maintained on a solid milk (high-fat) diet from weaning also showed a threefold decrease in the synthesis of this enzyme.

The development of hepatic 'malic' enzyme was prematurely evoked in animals throughout the suckling and weaning periods by treatment with thyroxine. Increased synthesis of 'malic' enzyme occurred in both suckling (9 and 15 days old) and weaned (23 days old) animals after treatment with thyroxine. Early weaning (at 16 days) also caused increased synthesis of this enzyme at 23 days of age. Changes in 'malic' enzyme activity in rat liver during adaptation and development can therefore be partially ascribed to a change in the rate of enzyme synthesis [cf. Silpananta & Goodridge (1971) on chick liver].

- Hemon, P. (1968) *Biochim. Biophys. Acta* **151**, 681–683
Hsu, R. Y. & Lardy, H. A. (1967) *J. Biol. Chem.* **242**, 520–526
Lockwood, E. A., Bailey, E. & Taylor, C. B. (1970) *Biochem. J.* **118**, 155–162
Schimke, R. T. & Doyle, D. (1970) *Annu. Rev. Biochem.* **39**, 929–976
Silpananta, P. & Goodridge, A. G. (1971) *J. Biol. Chem.* **246**, 5754–5761
Tarentino, A. L., Richert, D. A. & Westerfeld, W. W. (1966) *Biochim. Biophys. Acta* **124**, 295–309
Vernon, R. G. & Walker, D. G. (1968a) *Biochem. J.* **106**, 321–329

- Vernon, R. G. & Walker, D. G. (1968b) *Biochem. J.* **106**, 331–338
Wise, E. M. & Ball, E. G. (1964) *Proc. Nat. Acad. Sci. U.S.* **52**, 1255–1263

Induction of Ornithine Decarboxylase Activity in Baby-Hamster Kidney Cells

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Ornithine decarboxylase catalyses the first, and probably rate-limiting, step of polyamine biosynthesis. During liver regeneration its activity increases 90-fold and remains elevated for at least 72h (Russell & Snyder, 1968; W. T. Melvin & R. Y. Thomson, unpublished work). The role played by stress during the response in liver regeneration is uncertain (Schrock *et al.*, 1970), and for this reason it was decided to investigate the relationship of the activity of this enzyme to growth in a cell-culture system.

The growth rate of BHK 21/C13 cells in culture can be controlled by varying the serum concentration of the medium. If exponentially growing cells (Eagle's medium + 10% serum) are transferred to medium + 1% serum, DNA synthesis slows down and has almost ceased after 3–4 days. These cells appear to be stationary in the G1 phase. Addition of serum to such cells results in a synchronous wave of DNA synthesis 15h later in about 50% of the cells (D. K. Howard, unpublished work).

Ornithine decarboxylase activity was measured in this system by using a modification of the ¹⁴CO₂-absorption method of Russell & Snyder (1968); activity in exponentially growing cells is comparable with that found in regenerating liver. Serum depletion results in an immediate decrease in activity, and serum replacement in a corresponding increase.

After serum depletion the ornithine decarboxylase activity decays immediately, with a half-life of 55min, although in this short period arginine and thymidine incorporation are not measurably inhibited. Essentially the same half-life (60min) is obtained after the addition of cycloheximide (5 μ g/ml) to exponentially growing cells. This is much longer than the half-life for the rat liver enzyme (11min; Russell & Snyder, 1969), but is nonetheless considerably shorter than the half-lives for almost all other mammalian enzymes studied.

Serum replacement to cells depleted for 5 days induces ornithine decarboxylase activity. A characteristic peak is evident after about 5h, and it appears before increases in DNA synthesis and enzymes such as the deoxypyrimidine nucleoside kinases. This suggests the possibility that ornithine