physiological stimuli that are responsible for triggering the biosynthesis of particular enzymes in rat liver during development. With the application of such stimuli to animals at appropriate ages, it is now possible to evoke the premature appearance of at least a dozen enzymes in liver. However, a variety of complexities emerged that preclude simple conclusions about the mode of action of these stimuli on the biosynthetic machinery. In many cases the effectiveness of a stimulus is restricted to a given age or to a physiological state preconditioned by other regulatory factors.

Tryptophan oxygenase and glucokinase, which normally emerge in the third postnatal week, have been shown to require more than one stimulus to evoke them prematurely, i.e. at least a week before the scheduled time (Jamdar & Greengard, 1970; Greengard & Dewey, 1971). Injections of glucocorticoids followed by those of substrate (tryptophan and glucose respectively) constitute the necessary treatment. For tryptophan oxygenase the early actinomycin-sensitive step (effected by cortisol) could be clearly separated from the period of actual enzyme synthesis (triggered by tryptophan), which was not sensitive to this inhibitor. For glucokinase the substrate-mediated step of the induction process was also inhibited by actinomycin. It is possible, however, that this inhibition was due to a side effect of actinomycin, such as its interference with glucose absorption (Hanoune & Chambaut, 1971). In normal adult rats cortisol or glucose alone or in combination does not raise the specific activity of glucokinase, indicating that the synthesis of glucokinase is not limited at the same reaction sites as in developing rats. The behaviour of tryptophan oxygenase changes with age in a different manner. In adult rats, even if adrenalectomized, its induction does not require sequential treatment with cortisol and tryptophan; either is effective. An enzyme of the neonatal cluster, tyrosine aminotransferase, requires glucagon for its first appearance, but once enzyme biosynthesis is initiated either artificially (with glucagon administration to the foetus) or naturally (at birth) cortisol becomes an effective inducer. Several other enzymes are known to respond to different regulators in immature and mature liver (Greengard, 1970).

For enzymes that are not part of the soluble cytoplasm there is the additional problem that, even if synthesized, they might not persist unless attached to the appropriate subcellular organelle. We recently began to quantify developmental changes in organelle content (mitochondria and endoplasmic reticulum) and in the concentration of enzymes within the organelles. The results obtained so far show that the extent to which the accumulation of an enzyme is related to the simultaneous enlargement of the appropriate compartment depends on the stage of differentiation as well as on the particular enzyme investigated. As

with the soluble enzymes, the effectiveness of inducers varies with age.

One general conclusion from the variety of observations accumulated so far is that any categorical 'one enzyme-one regulator' hypothesis is untenable. During differentiation the series of reactions required for the appearance of a given gene product become operative in an unpredictable time-sequence. A stimulus that finally evokes the enzyme must have facilitated the last 'missing' reaction. Depending on whether this reaction (which might be involved in transcription, peptide assembly or integration into a compartment) happens to be rate-limiting or not in the adult organ the same stimulus will or will not act as an inducer.

Greengard, O. (1970) in *Biochemical Actions of Hormones* (Litwack, G., ed.), pp. 53-87, Academic Press, New York

Greengard, O. & Dewey, H. K. (1971) *Proc. Nat. Acad. Sci. U.S.A.* **68**, 1698

Hanoune, J. & Chambaut, A. M. (1971) *Biochim. Biophys. Acta* **252**, 212

Jamdar, S. C. & Greengard, O. (1970) *J. Biol. Chem.* **245**, 2779

Mechanism of Action of Cortisol in Rat Liver

By M. SLUYSER (*Antoni van Leeuwenhoek-Huis, The Netherlands Cancer Institute, Amsterdam, The Netherlands*)

Cortisol is thought to induce the synthesis of various liver enzymes by activating specific genes. The hormone penetrates the liver nucleus in the form of a 4S complex with cytoplasmic macromolecules. Within the cell nucleus this complex interacts with other macromolecular components to yield a 9S complex. A part of the cortisol is ultimately bound to the chromatin (Beato *et al.*, 1970). Cortisol alone also interacts with isolated rat liver nuclei *in vitro*, and this leads to a stimulation of the DNA-dependent RNA synthesis in these nuclei. However, unphysiologically high concentrations of the hormone are required to obtain this effect (Lukács & Sekeris, 1967). The latter finding suggests that the interaction of the steroid molecule itself with the chromatin is sufficient to trigger RNA synthesis, although the types of RNA synthesized in this case might not necessarily be hormone-specific. The role of the cytoplasmic receptor would primarily be to select the hormone and transport it from the cytoplasm to a specific site on the chromatin in order to induce the synthesis of specific types of RNA.

Structural similarities between steroids and DNA base pairs suggest a model by which steroid hormones might be able to influence the binding of a gene-regulatory protein to DNA. We have proposed that

the protein recognizes certain clusters of nucleotides on the DNA. Steroid molecules might, by interacting with the recognition sites on the protein, cause a partial dissociation of the latter from the DNA (Sluyser, 1971).

It is generally assumed that the acidic proteins of chromatin are specific regulators of gene activity, and that the histones are involved in a more general role in preserving nucleoprotein structure. This assumption is based on the belief that the number of different types of histones per cell is too small to allow histones to act as regulators of single genes. However, chromatographic studies have revealed that the lysine-rich histones are complex mixtures of structurally closely related molecules (Stellwagen & Cole, 1969). It seems possible that refined fractionation studies may also reveal such a micro-heterogeneity in some other types of histones as well.

In view of the possibility that histones are involved in steroid-hormone action we have investigated whether histones can bind steroid molecules. [^3H]-Cortisol was injected into rats and histones were isolated from liver nuclei by various methods. These histone preparations were found to contain a low but significant amount of radioactivity. The specific radioactivity of the liver histones increased linearly with increasing doses of injected [^3H]cortisol. The histone-[^3H]cortisol complexes could be dissociated in 40% guanidinium chloride, indicating non-covalent binding. When rat liver nuclei were incubated with receptor-[^3H]cortisol complex, radioactivity became bound to the histones. On the other hand, practically no radioactivity was bound to these histones when the nuclei were incubated with [^3H]cortisol in the absence of cytoplasmic receptor (M. Sluyser & Y. Hermes unpublished work).

The binding of steroids to rat liver histones *in vitro* is of the low-specific non-saturable type (Sluyser, 1969, 1971). However, *in vivo* specificity might be conferred on this interaction by the receptor protein that transports the hormone to the chromatin. Although it is not clear whether the binding of receptor-[^3H]cortisol complex to rat liver histones is physiologically important, these studies encourage further work to investigate the possible involvement of histones in the mechanism of steroid-hormone action.

Beato, M., Brändle, W., Biesewig, D. & Sekeris, C. E. (1970) *Biochim. Biophys. Acta* **208**, 125

Lukács, I. & Sekeris, C. E. (1967) *Biochim. Biophys. Acta* **134**, 85

Sluyser, M. (1969) *Biochim. Biophys. Acta* **182**, 235

Sluyser, M. (1971) *Biochem. Soc. Symp.* **32**, 31

Stellwagen, R. H. & Cole, R. D. (1969) *Annu. Rev. Biochem.* **38**, 951

Enzyme Activity Changes Relevant to the Control of Tryptophan Metabolism

By G. CURZON (*Department of Neurochemistry, Institute of Neurology, London WC1N 3BG, U.K.*)

A number of important biochemical processes are under the influence of tryptophan concentration. Among these are the synthesis of NAD through a series of reactions occurring subsequent to the action of hepatic pyrrolase on tryptophan and the synthesis of the putative neurotransmitter 5-hydroxytryptamine. The rate-limiting step in the synthesis of the latter substance is the conversion of tryptophan into 5-hydroxytryptophan by tryptophan hydroxylase. Also, tryptophan concentrations appear to have a limiting role in ribosomal aggregation and protein synthesis, and administration of tryptophan is followed by increased synthesis of many enzymes (for a review see Brown, 1971).

In these circumstances the synthesis and activities of the two enzymes tryptophan pyrrolase and tryptophan hydroxylase are of special importance. Tryptophan pyrrolase has been studied in great detail, but with most attention being paid to the mechanisms by which its synthesis or activity are increased by adrenocortical hormones or substrate. Much less work has been done on the relationships between induced tryptophan pyrrolase activity and the control of physiological concentrations of tryptophan and its metabolites. The existence of specific influences on plasma tryptophan concentrations is suggested by their diurnal rhythm being out of phase with the rhythms of those of other amino acids (Fernstrom *et al.*, 1971). However, a clear relationship between the rhythms of tryptophan concentration and of tryptophan pyrrolase activity is not apparent (Harde-land & Rensing, 1968; Rapoport *et al.*, 1966). Also, although adrenalectomy abolished the rhythm of tryptophan pyrrolase activity it altered but did not abolish that of the tryptophan concentration (Rapoport *et al.*, 1966).

Major difficulties in determining the physiological significance of pyrrolase changes derive from: (i) most work on the metabolism of tryptophan *in vivo* on the tryptophan pyrrolase pathway having concerned the handling of large tryptophan loads; (ii) most measurements of tryptophan pyrrolase activity having been done *in vitro* on liver homogenates or preparations. Although activities measured by these two methods were found to correlate positively (Altman & Greengard, 1966), both are of limited physiological relevance, and two studies suggest that the apparent induction by corticoids of tryptophan pyrrolase as determined *in vitro* may not indicate altered tryptophan metabolism. Thus Powanda & Wannemacher (1971) find that tryptophan but not cortisol administration is followed by increased liver