the first group of enzymes is induced by one or more of the substrates and the later groups of enzymes are induced by intermediate metabolites. This allows growth on intermediates of the pathway, since they are effective as inducers of subsequent enzymes; the earlier enzymes would not be synthesized. For several of the enzymes involved in the metabolism of aromatic compounds the inducer is the product of the enzyme and not its substrate. These enzymes are also subject to repression by products of the pathways, so that the rates of synthesis are balanced according to the metabolic demands of the culture and the nutrients available in the environment.

The aliphatic amidase produced by *Ps. aeruginosa*, and those produced by other species of *Pseudomonas*, exhibit differences in inducer and substrate specificities that are reflected in differences in the amide growth range. Various classes of amidase mutants that have been used to investigate the regulatory controls for the synthesis of amidase include constitutive mutants, altered-inducibility mutants and mutants producing amidases with altered substrate specificities (Clarke, 1970).

- Chen, B., de Crombrughe, B., Anderson, W. B., Gottesman, M. E., Pastan, L. & Perlman, R. L. (1971) *Nature New Biol. (London)* **233**, 67
- Clarke, P. H. (1970) *Advan. Microb. Physiol.* **4**, 179
- Gilbert, W. & Müller-Hill, B. (1966) *Proc. Nat. Acad. Sci. U.S.A.* **56**, 1891
- Greenblatt, J. & Schleif, R. (1971) *Nature New Biol. (London)* **233**, 166
- Ippen, K., Miller, J. H., Scaife, J. & Beckwith, J. (1968) *Nature (London)* **217**, 825
- Jacob, F. & Monod, J. (1961) *J. Mol. Biol.* **3**, 318
- Ornston, L. N. (1971) *Bacteriol. Rev.* **35**, 87
- Perlman, R. L. & Pastan, L. (1968) *J. Biol. Chem.* **243**, 5420
- Riggs, A. D., Newby, R. F. & Bourgeois, S. (1970) *J. Mol. Biol.* **51**, 303
- Silverstone, A. E., Magasanik, B., Reznikoff, W. S., Miller, J. H. & Beckwith, J. R. (1969) *Nature (London)* **221**, 1012
- Zubay, G., Gielow, L. & Englesberg, E. (1971) *Nature New Biol. (London)* **233**, 164

Analysis of Microsomal Hydroxylase Induction in Cell Culture

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The mammalian microsomal enzyme system aryl hydrocarbon hydroxylase is highly inducible in a variety of cells in culture. On the addition of inducer there is a 35min lag, after which the enzyme activity increases up to 100-fold during a 16h period. The half-life of the induced enzyme is 3–4h, and the presence of the inducer has a stabilizing effect on the

enzyme and increases its half-life by about 50%. The rise in enzyme activity during induction is prevented by cycloheximide throughout the induction and by actinomycin D only when added within 1–2h after the inducer. After 2h the further rise in enzyme activity for 6h is insensitive to actinomycin D. Thus RNA synthesis is required only very early in induction. The cells pass from actinomycin D-sensitivity to -insensitivity in the presence of cycloheximide, indicating that the induction-specific RNA may be synthesized in the absence of protein synthesis. The synthesis of the induction-specific RNA is also entirely dissociable from rRNA synthesis, since a low concentration of actinomycin D that inhibits the latter by more than 90% has no effect on enzyme induction. The induction-specific RNA is most probably present in the heterogeneous cytoplasmic RNA, and its synthesis and transfer to the cytoplasm are not dependent on rRNA synthesis. Also, sequential suppression of rRNA synthesis by cycloheximide and actinomycin D does not prevent enzyme induction.

In other studies intraspecific hybrids were made between cells that differ many-fold in base-line activity and inducibility of aryl hydrocarbon hydroxylase. Two hamster cell lines (OBP and BHK), which differ 15-fold in both base-line activity and inducibility, yielded hybrids whose basal and induced activities were as low as or lower than the less-active parent (BHK). Similar results were obtained in hybrids of human cell lines JEG-3 and VA-2. The hybrids of these cells exhibited activity similar to that of the low-activity parent.

Hybrids were formed between the mouse fibroblast line A9, which has no detectable base-line or induced aryl hydrocarbon hydroxylase activity, and 3T3.4C2, which has moderate basal and induced activities. In the A9–3T3.4C2 hybrids both the basal and induced activities were two- to five-fold higher than in the 3T3.4C2 parent. Thus in this hybrid there are increases in both control and induced enzyme activities. No examples were found of a dissociation of basal and induced aryl hydrocarbon hydroxylase activities, suggesting a linkage between the regulation of the basal and induced enzyme activities.

Regulation of Enzyme Amounts in Developing Liver

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The main purpose of our studies of enzyme inductions in immature organs has been to elucidate some of the mechanisms involved in biochemical differentiation. The first step was to identify a series of

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.* **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