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).

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Analysis of Microsomal Hydroxylase Induction in Cell Culture

By HARRY V. GELBOIN, FRIEDRICH J. WIEBEL and HAYDEN G. COON (*Chemistry Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014, U.S.A.*)

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

By OLGA GREENGARD (*Department of Biological Chemistry, Harvard Medical School, and the Cancer Research Institute, New England Deaconess Hospital, Boston, Mass. 02215, U.S.A.*)

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