Meetings

Hepatic Microsomal Mixed Function Oxidase

In order to assess the processing of drugs by liver cells, an International Symposium on Microsomes and Drug Oxidations was held at the Eberhard-Karls University of Tübingen, in Tübingen, Germany, on 19 to 22 July 1969.

The first topic discussed in the symposium was concerned with the characteristics of the terminal oxidase cytochrome P-450. This hemoprotein is believed to function as the oxygen activating component, partially reducing molecular oxygen to a more reactive state. D. Y. Cooper (Philadelphia), described his studies with the reconstituted 11-β-hydroxylase system of adrenocortical mitochondria which contain cytochrome P-450; this system when combined with a large excess of nonheme iron protein will hydroxylate steroids. Cooper reported that the hemoprotein has a very high redox potential (−400 to −419 mv), and he found that, in the absence of oxygen, the nonheme iron must be 100 percent reduced before cytochrome P-450 can be even 40 percent reduced. This differs from the mixed function oxidase of liver microsomes, since liver microsomes have not yet been shown to contain a nonheme iron protein.

Several other speakers also described studies on the reduction of cytochrome P-450. Since the hemoprotein has a very poorly defined difference spectrum between reduced and oxidized forms, and since it is very readily reoxidized, the anaerobic form bound to carbon monoxide is usually measured. J. B. Schenkman (New Haven) reported that under anaerobic conditions the microsomal hemoprotein was reduced by NADPH (nicotinamide adenine dinucleotide phosphate reduced form) to little more than 50 to 60 percent in 15 seconds, at 20° to 30°C. The rest of the hemoprotein required 5 to 8 minutes for reduction, giving a biphasic appearance to the kinetic curve.

He suggested that this latter phase of reduction was due to a leakage of reducing equivalents to the remaining hemoprotein. Similar findings were shown by V. Ullrich (Giessen), who suggested that only the substrate-bound form of cytochrome P-450 turns over or is reduced; this would represent the rapid phase of reduction and would indicate that normally almost 50 percent of the hemoprotein is bound to substrate in prepared microsomes. The implication of these observations is that cytochrome P-450 in microsomes exists in two forms, one free and one bound to substrate, and that their physical properties differ. The suggestion of J. R. Gillette (NIH) that the reduction of cytochrome P-450 might be rate limiting in the mixed function oxidase reaction appeared to be confirmed by Schenkman who reported that Arrhenius plots of P-450 reductase and aminopyrine demethylase activities indicate that there is a common rate-limiting step for both reactions, and that they are not the result of activation of oxygen or some later step. In addition, amount of the rapidly reduced fraction of the hemoprotein in microsomes was directly related to the rate of cyclohexane oxidation. Ullrich suggested that under normal conditions the slowly reduced fraction of microsomal cytochrome P-450 does not contribute appreciably to drug oxidation. On the contrary, S. Orrenius (Stockholm) reported that, whereas prior treatment of rats with 3,4-benzopyrene elevated both the microsomal content of cytochrome P-450 and the benzopyrene hydroxylase activity, it did not increase the NADPH–cytochrome P-450 reductase activity. This indicated that either the reductase activity may not be the rate limiting step (at least for all known substrates), or that the benzopyrene hydroxylase system is different from the drug oxidase system. A question left unanswered was whether cytochrome P-450 is really involved in benzopyrene hydroxylase activity; unlike other known substrates of the mixed function oxidase, prior treatment of animals with benzopyrene does not elevate the rates of oxidation of all other substrates, but only that of a select few substrates. D. Nebert (Bethesda) stated that although benzopyrene addition to liver cells in culture elevated the content of cytochrome P-450 and shifted the absorption peak of its CO complex to 448 nm, he did not see any alteration of the extinction coefficient from the Omura value of 91 mM^{-1} cm^{-1} for the CO complex. Removal of the benzopyrene from the cell cultures did not cause reversion of the 448 nm peak to 450 nm. The interpretation of this finding ranged from suggestions (i) that benzopyrene induced formation of a new hemoprotein; (ii) that the shift of the CO absorption peak and the new peak seen in absolute spectrum are due to an allosteric binding (the latter can be converted back to the usual absolute spectrum by excess substrate addition); and (iii) that a new polypeptide is formed which binds to the hemoprotein; this last possibility is supported by evidence for new protein synthesis after benzopyrene had been administered to rats.

In addition to the terminal oxidase cytochrome P-450, the hepatic microsomal mixed function oxidase system appears to contain a flavoprotein (NADPH–cytochrome c reductase), necessary for electron transport to cytochrome P-450. This enzyme is generally thought to be the enzyme activity measured as, and termed, NADPH–P-450 reductase above. M. J. Coon (Ann Arbor) has suggested, on the basis of his study with isolated fatty acid omega oxidase, that NADPH–cytochrome c reductase and NADPH–cytochrome P-450 are different enzymes, because the activity of the NADPH–cytochrome P-450 is more labile than that of NADPH–cytochrome c reductase in his semipurified preparation. However, T. Omura (Osaka) reported that antibodies against NADPH–cytochrome c reductase inhibit cytochrome P-450 reduction. It is possible that cytochrome c reductase involves a different part of the enzyme, but the possibility of a closely associated enzyme responsible for cytochrome P-450 reduction cannot be ruled out by either investigator's studies.

One other component of liver microsomes has been suggested as being involved in the mixed function oxidase

activity. G. J. Mannering (Minneapolis) showed that when phospholipase c was added to liver microsomes, about 70 percent of the phospholipid could be removed. Such treatment resulted in a loss of about 40 percent of the drug-metabolizing activity. Aniline hydroxylase activity was decreased to a lesser extent, but the availability of cytochrome P-450 for ferrihemochrome formation with the basic amine aniline was actually increased. Phospholipase A destroyed all of the drug metabolizing activity as did phospholipase D. According to H. Staudinger (Giessen) this treatment did not appear to affect cytochrome P-450 so that not all of the phospholipid may be necessary for the integrity of the hemoprotein. That phospholipid is necessary for mixed function oxidase activity was supported by the observation by S. Narasimhulu (Pennsylvania) that butanol extraction of adrenal cortex microsomes removed all of the 11-β-hydroxylase activity, but addition of phospholipid restored the activity.

The composition of the microsomal membrane and the turnover of components of the membrane received considerable attention. G. Dallner (Stockholm) showed by density gradient experiments that the microsomal fraction is a composite of subfractions of vesicles, differing with respect to enzyme content, hemoprotein content, and sedimentation characteristics. Fractions rich in cytochrome b5 could be separated from fractions rich in cytochrome P-450. In livers of benzopyrene-treated animals, cell fractions containing cytochrome P-448 could be separated from a fraction containing cytochrome P-450. This indicated that the endoplasmic reticulum membrane is not homogeneous, but contains enzymes and components in discrete groups. This would suggest that incorporation of components into the membrane could occur at different times during its synthesis. In agreement with this possibility was the report of T. Gram (Bethesda) who showed that ^{32}P incorporation into phospholipid is the same in smooth and rough microsomes from liver, although the incorporation of labeled amino acids into microsomal protein occurs first in the rough microsomes.

The half-life of the total microsomal protein was reported to be 3 days by T. Omura (Osaka), using labeling with [*guanido*-^{14}C] arginine. Prior treatment with phenobarbital caused a retention of label in the microsomes. The turnover of the protein moiety of cytochrome b5 also ceases after injection of phenobarbital, owing to a cessation of breakdown. This phenomenon also occurred with NADPH–cytochrome c reductase, initially; with continued phenobarbital administration, catabolism began again, and the enzyme became more labile than in control animals. Phenobarbital also affected the equilibration of NADPH–cytochrome c reductase between rough and smooth microsomes; after prior treatment with phenobarbital the appearance of label from amino acids was the same in both microsomal fractions.

Microsomal RNA was also affected by phenobarbital treatment of animals. According to J. Seifert (Prague), ribosomal RNA has a half-life of 5.5 days. The degradation of ribosomal RNA but not of total RNA was blocked by phenobarbital treatment. After cessation of the phenobarbital, the degradation of ribosomal RNA (loss of radioactivity) and microsomal protein had a similar pattern, but both differed from the pattern of loss of label from total cellular RNA. The turnover of microsomal heme differed considerably from that of other microsomal constituents. In a different approach to the study of microsomal heme turnover, H. Greim (Tübingen) separated the microsomal cytochromes by removal of cytochrome b5, after pulse-labeling the hemes with δ-[^{14}C]aminolevulinic acid (ALA), and was then able to determine the turnover of each of the hemoproteins separately. The half-life of cytochrome b5 heme was 45 hours and that of cytochrome P-450 was 22 hours. These rates were considerably faster than those reported for the protein labeled cytochrome b5, the protein of the microsomes, and the microsomal phospholipid. Greim also showed that initially, within 22 hours after injection of phenobarbital, the cause of elevation of cytochrome P-450 concentration in the microsomes is an enhanced rate of synthesis and not a cessation of hemoprotein breakdown. In this type of study, however, injection of too much ALA must be avoided. Normally the cell pool of ALA is very small, and heme synthesis could be easily driven; the ALA would not function just as a label in this case, but also as a substrate.

The meetings were concluded with studies on drug metabolism in man. E. van der Kleijn, in a study on the kinetics of drug distribution and metabolism, mentioned that variation of gastrointestinal tract flora could affect the distribution of drugs in the body. In addition, differences in the ability to concentrate drugs in tissues where they are metabolized might explain some of the species differences in the products formed from different drugs. This conclusion is similar to that reached by W. W. Oppelt (Tübingen) in his study on the effect of inducing agents on drug metabolism in lung microsomes. He found that some compounds selectively elevate liver enzymes, but not lung microsomal enzymes. In testing the effect on the lung of inhalation of ethyl ether, Oppelt found an increase in lung microsomal cytochrome b5 (20 percent) and cytochrome P-450 (40 percent), while the liver microsomal hemoproteins were not affected.

The meeting was organized by Dr. H. Remmer, Director of the Institute for Toxicology, the University of Tübingen, with the help of Dr. H. Schroeder. Major financial support for the meeting was provided by Dr. Karl Thomae GmbH., a pharmaceutical company, of Biberach. Additional financial support was provided by several other pharmaceutical companies.

The symposium was divided into the following subtopics: Mechanism of action of the hepatic microsomal mixed function oxidase; Various types of microsomal oxidations and miscellaneous subjects; Factors affecting the hepatic microsomal mixed function oxidase; Drug metabolism in isolated cells and mechanism of induction; Composition of microsomes and turnover of microsomal components; and Drug metabolism in man and its variation. The proceedings of this symposium probably will not be published.

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Transplant Donation Procedures

Adoption of a simplified Uniform Donor Card (Fig. 1) for indicating gifts of organs and tissues was the subject of a second and final meeting on 7 November 1969 of the Ad Hoc Committee on Medical-Legal Problems, which reports to the Committee on Transplantation of the National Research Council.