Progress report

Mechanisms and prediction of drug-induced liver disease

The hepatic microsomal enzyme system plays an outstanding role in the metabolism and detoxication of most drugs^{1,2}, whereas the frequency of drug-induced liver disease increases steadily as 'a penalty for progress'³. Two main questions therefore arise: (1) Why do some drugs, ie, by which cellular mechanism(s), provoke side effects upon the liver, and (2) how might we predict them? The purpose of this review is to define the relations between drugs and the liver. The recent metabolic and molecular aspects of drug hepatotoxicity will be emphasized, as well as the limits of animal testing.

Hepatic Response to Drugs

The response of the liver to drugs can be classified into four main types: (1) idiosyncrasy, which might in fact correspond either to hypersensitivity or abnormality of drug metabolism; (2) drug interactions, including enzyme induction; (3) direct hepatotoxicity; (4) interferences with bilirubin disposal. Animal testing appears to be of help in predicting the three latter types as well as many abnormalities of drug metabolism. Unfortunately, it provides no information concerning hypersensitivity, which probably yields in man the highest incidence of liver injuries^{4,5}, sometimes severe. In addition, the incidence of such side effects caused by a certain drug is usually low, so that they may be missed for years. As an example, a convincing report of hepatic disorder due to papaverine⁶ came as a surprise more than half a century after its first use in man⁷. Moreover, as far as hypersensitivity is concerned, there is no definite relation between the chemical structure of a compound and its effects on the liver. Yet the existence of a few cross sensitivities may be of some help in prediction: sensitivity to chlorpromazine may be associated with sensitivity to promethazine⁸; sensitivity to halothane⁹ could resemble that due to other halogenated anaesthetics, eg, methoxyflurane¹⁰ and fluroxene¹¹. Such facts, however, could sometimes be better explained on the basis of possible metabolic peculiarities which will be considered further.

FACTORS INFLUENCING THE HEPATIC RESPONSE TO DRUGS

There are considerable individual variations in the response to drugs to be considered before any conclusion can be reached from animal testing^{2,12,13}. They mainly depend on dose, species, sex, age, nutrition, associated drugs, preexisting liver function, and genetic factors.

According to species, the hepatic disposal of a given compound may be entirely different. For example, rifampicin is highly toxic in the dog, which is unable to desacetylate it efficiently, compared with man whose liver excretes more than 80% of rifampicin as desacetyl-metabolites¹⁴. Another example is

that of methotrexate, which the rabbit metabolizes, whereas other species, such as mouse and man, do not metabolize the drug; based on the known toxicity of non-metabolized methotrexate, its enzymatic cleavage was used successfully in the mouse in order to prevent the toxicity of the drug¹⁵.

The biological half-lives of most drugs and hormones are genetically determined. Studies with ¹⁴C-chlordiazepoxide revealed plasma half-lives of 22 to 24 hr in man as against 10-14 hr in the dog and four to six hr in the rat¹⁶. Metabolic breakdown of chlordiazepoxide is, however, extensive in all three of these species, and the major metabolite formed is the same in man and dog, although different in the rat. In addition, large differences in half-lives of various drugs have been found according to the strain in rats^{17,18} or rabbits¹⁹. In man, a thirty-fold variation in the plasma level is possible for many drugs²⁰, whereas comparable concentrations are regularly achieved in identical twins^{20,21}. Such facts may greatly influence the pharmacological responses and the possible toxic effects of drugs.

Age is also of importance: newborns in most species, including man, have an inefficient hepatic microsomal enzyme system which in adults metabolizes a wide variety of drugs^{22,23}; this makes it impossible to calculate for a newborn a drug dosage based on fractions of an adult dose.

There are surprisingly few data about the influence on drug disposal of preexisting liver dysfunction. This could be related to (1) the absence of a satisfactory method of studying needle biopsy samples, and (2) the fact that such biopsies are usually not performed during severe hepatic disease. Some enzyme activities were, however, measured, and appeared to be only affected in the most severe cases²⁴. Hepatic impairment has been found to alter²⁵ or not²⁶ the plasma disappearance rate of phenylbutazone; to alter that of chloramphenicol²⁷ and of isoniazid²⁵. Plasma levels of rifampicin were higher in cirrhotic patients than in patients with a normal liver²⁸. Half-lives of salicylic acid, aminopyrine, dicoumarol, and antipyrine were not different in cirrhotic and control patients²⁶. Following portacaval shunt in the rat, specific activities of the microsomal enzymes aniline hydroxylase and nitroreductase were found to be low²⁹. To summarize, it is conceivable that liver dysfunction would yield high plasma levels mainly of those drugs being excreted with an important biliary concentration, whereas impairment of drug metabolism would appear only in severe hepatocellular failure.

Many possible drug interactions will also influence the hepatic response to drugs. An impressive number of substances, including hundreds of drugs³⁰, and especially phenobarbitone, are known to induce the non-specific microsomal drug-metabolizing enzyme system¹. These inducing agents can lower considerably the blood levels of most drugs which are normally metabolized by the liver. As the pharmacological activity of drugs is most often due to the parent compound, diminution of therapeutic efficiency will ensue. A striking example is that of phenobarbitone given together with coumarin anti-coagulants: severe haemorrhagic accidents may appear when phenobarbitone therapy is suppressed³¹.

As far as the toxicity of drugs is concerned, there are two possibilities to consider: (1) the drug itself is toxic, as its metabolites are not; (2) toxicity is due to one of the metabolites. One can clearly predict that in the first case, which is the most frequent, enzyme induction will protect, as in the second it will enhance the toxic effects. But a few substances seem able to reduce the hepatic metabolism of various drugs: allopurinol and nortriptyline inhibit

in man the metabolism of antipyrine and bishydroxycoumarin³²; chloramphenicol increases in man the serum levels of unchanged tolbutamide and diphenylhydantoin³³; halothane depresses the metabolism of various barbiturates and aminopyrine in the rat³⁴; progesterone and its metabolites act *in vitro* as inhibitors of the hepatic mixed-function oxidase system³⁵; abnormalities of serum transaminases during combined rifampicin and isoniazid therapy are more likely to appear in those patients who are 'slow acetylators' phenotypes for isoniazid³⁶.

The hepatic disposal of hormones, and especially of steroids, is very similar to that of drugs³⁷. In this respect, it is worth noting that the circadian rhythm which exists in hepatic drug-metabolizing activity in the intact rat was no longer present in adrenalectomized animals³⁸. This circadian cycle influenced the methadone-induced mortality in the rat³⁹. On the other hand, drugs may act on steroid metabolism, for the hydroxylated metabolites of chlorpromazine inhibit the conjugation of hydrocortisone, as the demethylated metabolites of chlorpromazine are able to stimulate it⁴⁰.

Direct Hepatotoxicity

Direct hepatotoxicity can be defined by a constant action of a given compound and a strictly dose-dependent effect. It may lead either to zonal liver cell necrosis or to intrahepatic cholestasis.

CELLULAR INJURY

There are only a few drugs able to induce hepatocellular injury by direct toxicity, because this kind of hepatitis-like reaction is often severe, making many of the causative agents of unacceptable risk. Chloroform, various antineoplastic antibiotics, tannic acid, and the tetracyclines belong to that group. The cellular mechanism of their hepatotoxicity has been mainly studied in animals given carbon tetrachloride (CCl_4) or ethionine. Only the most currently accepted hypothesis will be summarized here.

The intense steatosis that these poisons usually provoke seems to be mainly the result of early impaired protein synthesis^{41,42,43}. The first effect could be a marked decrease of intracellular ATP resulting in a block of m-RNA synthesis, and therefore of protein synthesis as well. Lack of the protein moiety of the lipoproteins ensues, explaining the accumulation of triglycerides; the steatosis is the result of the absence of the carrier protein to which triglycerides must be attached to be moved out of the cell. On the other hand, liver cell necrosis provoked by CCl_4 could be due to metabolites of CCl_4 , such as CCl_3 free radicals^{44,45}. These free radicals, with a highly reactive unpaired electron, would directly attack the mitochondrial membrane, blocking the oxidative phosphorylation.

Although all these cellular mechanisms are still not proved to be the only explanation for the action of all poisons on the liver they suggest several lines of enquiry. The role of metabolites of CCl_4 is well supported by the enhancement of CCl_4 toxicity by previous treatment with enzyme inducers^{46,47}. It is interesting to speculate that some similarities might exist between the toxic effect on the liver of CCl_4 and halothane. Although several arguments support the theory that in man the adverse effects of halothane remain unpredictable⁹, this does not exclude true hepatotoxicity in predisposed patients. The metabolism of halothane has been extensively studied^{48,49,50,51},

and includes microsomal dechlorination⁵¹. A toxic metabolite is conceivable, which would explain the increased risk which has been suggested in the case of previous administration of phenobarbitone in man^{52,53} and in the rat⁵⁴. The induction of halothane metabolism by halothane itself has been demonstrated in mice⁴⁸; if this were to happen in man, as has been suggested⁵⁵, it could play some role in the higher incidence of liver necrosis following repeated exposure to the anaesthetic. Very similar mechanisms can be incriminated for methoxyflurane which undergoes a similar metabolic fate⁵⁶ to that of halothane, and induces the dechlorinating system in animals⁵¹. The toxicity of methoxyflurane in man could be dose-dependent, as it is correlated with renal dysfunction^{57,58,59}, and that of fluorene was claimed to be enhanced by the previous administration of enzyme inducers¹¹. Finally, the toxicity of a number of halogenated aromatic hydrocarbons⁶⁰, possibly including chloroform, could at least be partly explained on a metabolic basis. Similar mechanisms have also been suggested for chloramphenicol⁶¹, whereas pretreatment with phenobarbitone has a protective effect against a variety of agents, including toluene and benzene⁶² or aflatoxin⁶³.

INTRAHEPATIC CHOLESTASIS

Various drugs, including mainly such hormones as 17- α alkyl substituted oestrogens or other steroids, are able to provoke intrahepatic cholestasis by direct toxicity. Such cholestasis is highly predictable, and may be studied in any animal species. The drugs which are concerned include the oestrogenic compounds found in contraceptive pills^{64,65,66}. The rarity of jaundice due to oral contraceptives makes it likely that additional factors, possibly genetic, exist in predisposed women.

The cellular mechanisms explaining drug-induced cholestasis have been extensively studied. In the rat at least, an early and striking effect is a diminution in bile flow. This could be related to increased permeability of the biliary tree to water^{67,68}. It could also be explained by a decrease in the so-called bile salt-independent fraction of bile secretion⁶⁹. This last hypothesis is in accordance with the fact that only those oestrogens able to diminish the bile flow were found also to decrease the Na⁺K⁺ATPase in rat liver plasma membrane⁷⁰, as it was suggested that this ATPase could provide the driving force for the bile salt-independent fraction of cholestasis⁷¹. Cholestatic drugs could also interfere with the hepatic disposal of bile salts. This at least was claimed for steroids and chlorpromazine. The former have been shown⁷² to inhibit the conversion of cholate into taurocholate; such impairment could contribute to cholestasis, for the rat at least is unable to excrete cholate in its bile. An alternate interference of cholestatic drugs on bile acid metabolism has been suggested as a result of hypoactive hypertrophic smooth endoplasmic reticulum in the hepatocyte⁷³. This theory refers to an excess of the poorly soluble monohydroxy bile acids resulting from enhanced synthesis of cholesterol, the precursor of bile acids, due to hypertrophic smooth endoplasmic reticulum. Hypoactivity of smooth endoplasmic reticulum would account for reduced enzymatic activity, with impaired ring hydroxylation of cholesterol.

As far as amine-containing drugs, such as chlorpromazine, are concerned, a direct impairment of micelle formation has been suggested: native chlorpromazine could form insoluble complexes with bile salts⁷⁴, as chlorpromazine metabolites would not. In addition, non-metabolized chlorpromazine has caused precipitation of biliary proteins *in vitro*⁷⁵. Bearing in mind a possible

induction of chlorpromazine metabolism by the drug itself, it is interesting to speculate that such an action could account for the so-called 'desensitization' which frequently occurs in man⁷⁶ during prolonged administration of phenothiazines. This again would be an example of a metabolic basis for a liver injury apparently due to hypersensitivity, supposing that the normally occurring adaptation⁷⁷ to the drug could be lacking in the few individuals in whom chlorpromazine causes overt cholestatic jaundice.

Interference of Drugs with the Disposal of Bilirubin

A few drugs are able to interfere with the hepatic disposal of bilirubin and cholephilic dyes. This kind of side effect is highly predictable from animal testing; it is usually of little practical importance in man as creating only moderate and transient unconjugated hyperbilirubinaemia, but its study allowed experimental models to be designed which appeared of value in improving our knowledge of liver physiology. The competition, however, for plasma protein binding between drugs and bilirubin and their role on kernicterus⁷⁸ will not be considered here. Various drugs, which all seem to be organic anions able to be concentrated within the liver cell and bile, interfere with the first stage of liver cell transport, ie, uptake. These drugs include mainly bunamiodyl⁷⁹, male fern extracts^{80,81}, novobiocin⁸², rifampicin²⁸, and rifamycin SV⁸³. Such competition for uptake could be due either to interference with hypothetical membrane carriers, or to competition for binding at the site of cytoplasmic anion-accepting proteins⁸⁴, as has been suggested at least for bunamiodyl and flavaspidic acid⁸⁵.

There have been conflicting reports on drugs which may interfere with the conjugation of bilirubin. In work comparing the effect on glucuronyl-transferase activity *in vitro* of bunamiodyl, rifampicin, and novobiocin⁸⁶, only the last compound inhibited enzyme activity; the assays were performed on liver homogenates, according to a method⁸⁷ using bilirubin as substrate. The lack of inhibition which was found for bunamiodyl is in contradiction to a previous report⁸⁸, in which other methods were used. However, species differences could exist as far as glucuronyl-transferase activity is concerned: with the steroids incriminated as the possible cause of breast milk jaundice different behaviour between human and rat glucuronyl-transferase activity was observed⁸⁹. Finally, all these drugs have also a cholestatic effect with decreased bile flow, impaired biliary excretion of bilirubin, and increased hepatic storage^{81,82,90}. Such increased storage, which is only seen in experiments of long duration, contrasts with the reduced storage which was observed shortly after a single dose of the drug^{79,81}; the former could be explained on the basis of impaired excretion, as the latter would reflect the reduction in hepatic uptake. Such impaired excretion is in any case of interest, for there is a relationship between bile flow and excretory function of the liver⁹¹. This also leads to speculation that the enhanced excretion of various cholephilic anions, which has been observed following chronic administration of phenobarbitone⁹², could be related at least in part to its choleretic effect⁹³.

Concluding Remarks

The most striking development in the field of drug-induced liver disease is the molecular approach to toxicity, including the cellular mechanisms of

steatosis, necrosis, cholestasis, and competition phenomena. Based on this type of approach, and in order to prevent toxicity, new therapeutic possibilities arise, such as those acting on the drug-metabolizing systems; in the near future perhaps the efficacy of various actions of drugs could also be adapted by modifying bile flow. Species differences, however, as well as the number of drug interactions, make valueless many of the conventional ways of testing the response of the liver to drugs. It appears that testing a new compound should never be limited, as it too often has been, to light microscopy of the liver and the determination of a lethal dose which varies greatly from species to species, and even from strain to strain. Animal testing of a new compound, as far as the liver is concerned, should be conducted in acute and chronic experiments in various species and should try to answer at least the following questions: (1) Is the drug metabolized, and if so, how? (2) Is the toxicity due to the parent compound and/or to its metabolites? (3) Does chronic administration of the drug influence its own metabolism? (4) Which are the compounds able to interact with it? For many drugs, old or new, the answers to these questions remain unknown. In such cases, probably the simplest way at the moment to avoid the possible side effects will be to remember that 'a major cause of preventable drug reactions is that too many pills are prescribed'⁶⁴.

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