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How the Liver Metabolizes Foreign Substances

Among the most significant of the liver's chemical transformations are the inactivation of drugs, the detoxification of environmental pollutants and the activation of chemicals that can cause cancer

by Attallah Kappas and Alvaro P. Alvarez

The intensity and duration of the action of most drugs is determined

In large part by their rate of metabolism. If nothing else happened to a drug after it entered the body and reached its target organ, for example, it might continue to act indefinitely. Something does happen, however: most drugs are transformed into inactive substances and then excreted. The biotransformation can occur in any of several tissues and organs. Some drugs are transformed chemically in the intestine, some in the lung, the kidney or the skin. By far the greatest number of these chemical reactions are carried out in the liver, which metabolizes not only drugs but also most of the other foreign chemicals to which the body is exposed. Biotransformation in the liver is therefore a critical factor not only in drug therapy but also in defending the body against the toxic effects of a wide variety of environmental chemicals such as insecticides, herbicides, dyes, food preservatives and a number of substances that are suspected of inducing cancer. The central step in the metabolism of most of these agents involves an oxidation reaction mediated by a complex of enzymes that has come under intensive study in recent years in our laboratory at the Rockefeller University Hospital and in other laboratories.

The liver is the largest organ in the body (it weighs about three pounds in an adult) and has diverse functions. It serves, first of all, as the primary receiving depot, chemical-processing plant and distribution center for almost everything that enters the body through the walls of the alimentary canal. All the blood that has absorbed digested food and other substances from the intestines en-

ters the liver through the large portal vein, which ramifies into fine channels through which the blood perfuses slowly among the liver cells. Here nutrients and other foreign substances are removed, metabolized, in some cases stored and then released into the general circulation. Amino acids, for example, are made into proteins and other nitrogenous compounds; glucose is converted into glycogen and stored, to be converted back into glucose and released as required. And drugs and other toxic substances are detoxified. Not everything is metabolized on the first passage of blood through the liver, of course; drugs, for example, are given in doses such that a sufficient amount of the drug moves through the liver to its site of action and is transformed later, on return visits to the liver [see illustration on page 25]. The liver also produces bile, which is a secretion that aids in the digestion of fats when it is released into the small intestine and is also a vehicle for the excretion of transformed substances and other waste products of metabolism.

The biotransformation of drugs and other foreign compounds in the liver is accomplished by several remarkable enzyme systems that can metabolize a wide variety of structurally unrelated drugs, toxic agents and environmental pollutants, which enter the body primarily through ingestion but also through the lungs and the skin. The enzyme systems are built into the membranes of the endoplasmic reticulum of the liver cells, a network of interconnected channels that is present in the cytoplasm of most animal cells. There are two kinds of endoplasmic reticulum, rough and smooth, and they differ in both form and function. The surfaces of the rough mem-

branes are studded with ribosomes, small granules that translate the genetic code into the sequences of amino acids that constitute proteins. The smooth membranes have no ribosomes. In the liver a major function of both kinds of membrane is to assemble the enzymatic complexes that transform foreign substances and then to serve as the site of these transformations. Unlike some other cellular subsystems, the endoplasmic reticulum cannot be separated from cells as an intact structure. If liver cells are homogenized and then centrifuged, the tubular reticulum breaks up and bits of the membranes are sealed off to form the tiny vesicles, or sacs, called microsomes. The microsomal fraction thus obtained from liver cells is a convenient natural source of enzymes for laboratory studies of liver-cell metabolism.

Drugs and other foreign compounds are metabolized in the liver by a rather small number of reactions: oxidation, reduction, hydrolyses and conjugations. Their essential effect is to convert lipophilic, or fat-soluble, compounds into hydrophilic, or water-soluble, ones. The hydrophilic compounds are the more readily removed from the blood by the kidneys and excreted.

Oxidation accounts for most of the transformations, largely because there are so many different ways in which a compound can be oxidized [see illustration on pages 26 and 29]. The alkyl side chains of barbiturates and some other drugs, for example, are oxidized to form alcohols. In the case of compounds incorporating aromatic rings, including polycyclic hydrocarbons (such as those in cigarette smoke) and many drugs, a hydroxyl group is inserted into the ring.

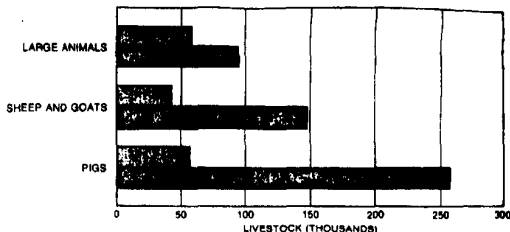
accomplished as a result of the establishment of certain new rural practices.

The first of these is related to household economics at the production-team level. Individual household members receive a share (in money and produce) of the annual earnings of their team; the share is proportional to the number of work points each has earned during the year. For example, a 70-year-old grandmother in a suburban Shanghai commune told me that she and nine of the other 10 members of the household worked regularly in the fields. The only nonproducer was a five-year-old child. The family received a monthly advance of the equivalent of \$80 against its joint annual earnings. In 1973 its share of the team's income after operating expenses and taxes were deducted was \$2,100, or about \$200 per person.

Withholding for taxes and expenses and to provide funds for reinvestment absorbs about half of the gross income of a commune. For example, the manager of the suburban Rainbow Bridge Commune in Shanghai told us that 30 percent of the commune's gross income was required to meet the cost of its agricultural endeavors. Another 11 percent went into a fund for capital reinvestment, 4 percent was paid to the government as tax and 3 percent went into a welfare fund for the support of new mothers and the elderly. That left 32 percent of the gross income for distribution to individuals in proportion to the work points earned.

Such a work-point system simultaneously accomplishes two objectives. It provides a strong incentive for increased overall output in order to maximize the amount that is divided among the individuals in a household. At the same time it encourages householders and production teams alike to keep their numbers from increasing, so that the maximum earnings are divided into the fewest possible shares. The latter constraint, of course, places the disadvantages of population growth squarely before each individual. This economic consideration must generate substantial social pressure in favor of small families.

Other growth-limiting factors are at work. As one example, it is said that improved health care has greatly reduced infant mortality, thus undercutting the validity of the traditional view that many births are needed to ensure the survival of even one offspring to maturity. Another example is the current program of providing care for the aged, one of the objectives of the 3 percent welfare withholding reported by the Shanghai commune. We visited one such residence for



LIVESTOCK IN CHINA are compared according to categories: estimates, made by the U.S. Department of Agriculture, are for 1969 (gray) and 1973 (black). Sheep and goats are largely confined to grasslands and mountains in the west and northwest. That is also true of rangeland cattle, lumped together here with large draft animals: horses, mules, donkeys and water buffaloes. Pigs are by far the most numerous of all the domestic mammals in China.

the elderly, maintained by a commune near Peking. Normally, we were told, each family cared for its own elders. Nonetheless, some individuals inevitably lost their families or became separated from them; that was the case with the 75 residents we saw. They were encouraged to engage in handicrafts; meanwhile they were housed, clothed and fed at commune expense, and a clinic attached to the residence looked after their health needs. Custodial care of this kind, if it were widely practiced, would diminish the validity of another traditional view that favors multiple births: the view that one needs to have many children in order to ensure one's welfare in old age.

A third growth-limiting factor that may even now be undergoing a crucial test is the continued existence of private landholdings throughout rural China. We were told that when the commune system was established in the late 1950's, between 5 and 7 percent of the arable land in each commune was set aside for private use. The land was parceled out at a fixed rate: each adult in a household was assigned one 150th of a hectare. The household children received private land too, but only the two eldest children in each household were eligible. If a household had more than two children at the time of the land assignment, or if more children arrived later, those children received no share. In such a family there would simply be less private land per person. In its early days the allocation system probably had relatively little effect on rural family planning. Today, nearly a generation later, the system must act as a powerful social force favoring small families.

In summary, with respect to agricultural production in China now and in the

near future our group was inclined to accept the government's assertion that self-sufficiency was achieved in 1971, when some 250 million metric tons of rice, wheat and other major foodstuffs were harvested. Furthermore, given a continuation of the present aggressive and co-ordinated effort it seems that China will be able to achieve substantial increases in agricultural production over the next decade. Whether the increase for any particular crop will be as little as 20 percent or as much as 50 percent will probably prove to be a function of present yields. For example, rice yields are already high, so that an increase of 50 percent in the annual harvest will be much harder to achieve than a 50 percent increase in the maize or sorghum harvest.

China nonetheless faces serious long-term problems. If the country's remarkable agricultural advance is to continue, two of these problems must soon be resolved. The first problem lies in the current Chinese policy that emphasizes applied and decentralized agricultural research for the sake of immediate increases in food production. Although the short-term benefits of this policy are obviously important, the policy must be complemented in the near future by similar emphasis on more basic scientific investigation.

The second problem has to do with the government's remarkably comprehensive efforts to retard the growth of China's population. Only if this policy meets with success will the Chinese people continue to receive the benefits of increased agricultural production and the accompanying advances in living standards that so many of China's people now seem to expect.



ENDOPLASMIC RETICULUM of a rat liver cell is enlarged 18,000 diameters in this electron micrograph made by Edward S. Reynolds of the Harvard Medical School. Most of the biotransformations of foreign substances take place in this system of membranous tubules. The "rough" endoplasmic reticulum is covered

with ribosomes, the structures in which proteins are synthesized; it appears here as fairly linear double membranes studded with black dots. The "smooth" endoplasmic reticulum lacks ribosomes and forms a more branching, tubular network; there are patches of it between the mitochondria, the fine-grained gray objects, at right.



SMOOTH ENDOPLASMIC RETICULUM proliferates when the synthesis of the enzyme systems it contains is stimulated by drugs. This micrograph, also made by Reynolds and reproduced at the same scale as the one at the top of the page, is of a cell from the liver of a rat that was treated with phenobarbital. The drug stimulates the synthesis of cytochrome P-450, an enzyme located in the

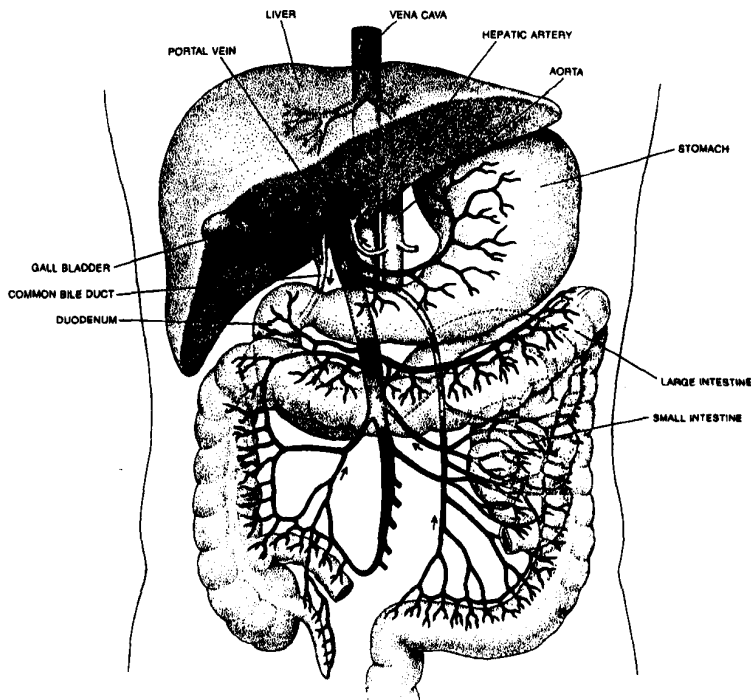
endoplasmic reticulum membranes. The cytoplasm of the cell has expanded because of a striking increase in the smooth endoplasmic reticulum membranes, which now fill most of the space between the more dispersed mitochondria. Enzyme induction by one drug can significantly affect the metabolism, and thus the activity, of other drugs that are metabolized by the same enzymes.

In other cases alkyl groups are removed from either nitrogen or oxygen atoms, amino groups are removed or sulfoxides are formed. Reduction and hydrolysis are also catalyzed by liver enzymes, but these reactions are less common than oxidation.

Conjugation of a chemical is combination with some natural constituent of the body such as the glucose derivative glucuronic acid, the amino acid glycine or the tripeptide glutathione. In the presence of the appropriate enzyme these

natural agents can combine readily with compounds that have carboxyl (COOH), sulfhydryl (SH), amino (NH₂) or hydroxyl (OH) groups. Some drugs have these groups when they are in their active form and are handled in the liver by conjugation; for most drugs, however, conjugation is a second step that comes after metabolism by oxidation, reduction or hydrolysis. Almost without exception the conjugated compound is devoid of pharmacological (or any biological) activity.

Clearly the enzyme systems that catalyze these reactions were not invented by the mammalian organism in order to cope specifically with drugs or novel pollutants. Their basic physiological role has presumably been to metabolize endogenous substrates: substances normally present in the body. For example, liver microsomal enzymes oxidize steroid hormones, cholesterol and fatty acids. The products of these oxidations may then be conjugated (with glucuronic acid, for instance) and excreted. Bill-



LIVER is a primary site of metabolism of substances entering the body through the alimentary tract. All the fine blood vessels that absorb nutrients and other substances through the wall of the intestines come together and enter the liver through the portal vein. The liver's supply of oxygenated blood from the heart enters

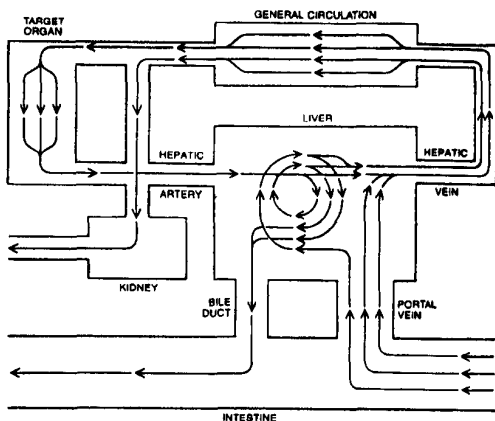
through the hepatic artery. After passing through the sinusoids of the liver (see illustration on page 26) the blood is collected by the hepatic veins, which feed into the vena cava. The liver secretes bile, which is collected by the bile duct, stored in the gall bladder and emptied into the duodenum, the first segment of the small intestine.

rubin, a product of the oxidation of heme, the red pigment of hemoglobin, is an example. It is normally prepared for excretion by glucuronide conjugation. The rate of formation of glucuronides is generally low in newborn infants, however, because of a deficiency of the enzyme glucuronyl transferase. As a result bilirubin may not be conjugated and excreted at an adequate rate. Excessive amounts of it can then accumulate and cause grave damage to the brain, a condition called kernicterus.

It has been apparent for some time that the human fetus and the newborn infant are far more sensitive than adults to many drugs. A number of drugs can pass across the placenta, so that obstetricians need to exert care in administering them to an expectant mother. Barbiturates or morphine given to a woman during childbirth can be stored in the infant's tissues and cause respiratory depression and occasionally death. The explanation for the sensitivity of infants to drugs has emerged from a number of reports in recent years on the maturation of the capacity to metabolize and conjugate drugs. These studies make it clear that the capacity to oxidize and conjugate is negligible in the mammalian fetus and newborn animal and increases after birth at a rate that varies with the species, the type of reaction and the drug. Impaired drug metabolism increases the intensity and duration of drug action. For example, newborn mice treated with a dose of the hypnotic drug hexobarbital equivalent to 10 milligrams per kilogram of body weight sleep more than six hours, whereas adult mice given 10 times as large a dose sleep less than an hour.

The adverse effect of drugs on newborn infants as a result of inefficient metabolic conversion is illustrated by the striking "gray-baby syndrome" in infants that may come a few days after treatment with the antibiotic chloramphenicol: abdominal distention, respiratory difficulty, cyanosis (blue skin color as a result of insufficient oxygenation of the blood) and shock. The condition is apparently the result of deficient metabolism of the antibiotic and deficient conjugation with glucuronic acid. In adults about 90 percent of the antibiotic is excreted in the urine in the form of conjugated metabolites within 24 hours after it has been orally administered; only a small amount is excreted unchanged. In comparison a 10-day-old infant in one study excreted less than 50 percent of the drug in 24 hours.

Rates of drug metabolism are very dif-



PATHWAY of a drug that is transformed in the liver is shown schematically. The drug (colored arrows) enters the liver through the large portal vein, passes into the general circulation, has its effect on the target organ and eventually returns to the liver. On each passage through the liver a fraction of the drug is converted, usually into inactive metabolites (black arrows). The metabolites may be carried by the bile into the intestines for excretion or may pass through the circulation to the kidneys, to be excreted into the urine.

ferent in different species, and the effective dosage varies accordingly. The anti-inflammatory agent phenylbutazone is metabolized slowly in man; its half-life in the plasma averages about three days. In the horse, the dog, the rabbit, the rat and the guinea pig, however, the drug is metabolized much more quickly and the half-life ranges from three to six hours. (Knowledge of the rapid rate of metabolism of phenylbutazone in the horse is of practical importance because the drug is administered to treat arthritic conditions in racehorses.) A dose of hexobarbital (adjusted for the body weight of the animal) that makes mice sleep for an average of 12 minutes puts rabbits to sleep for 49 minutes, rats for 90 minutes and dogs for 315 minutes. When the enzymatic oxidation of hexobarbital by microsomes from the liver of these animals was measured, the fastest oxidation was carried out, as expected, by microsomes from the mouse; the rates of oxidation by microsomes from the rabbit, the rat and the dog were proportionately lower. Differences in the rates and patterns of drug metabolism in the various animals seem to explain most of the species differences in effect, but there may also be differ-

ences in the distribution of the drug, the response of the target tissues and excretion.

Even among human patients there are marked individual variations in the metabolism of drugs that are handled primarily by microsomal enzymes. The variability causes some patients to metabolize a drug so quickly that therapeutically effective blood and tissue levels are difficult to achieve and others to metabolize the drug so slowly that they suffer toxic effects. It can therefore be difficult for the physician to predict just what dosage of some drugs will provide a safe and therapeutic effect in an individual patient. Very large individual differences have been noted in the metabolism of the coumarin-derivative anticoagulant drug Dicumarol; the half-life can vary from seven to 74 hours. That makes it hard to predict how much of the drug will provide the desired anticoagulant effect in a patient. Marked variations have also been observed in the metabolism in man of phenylbutazone and of diphenylhydantoin, a drug that is administered to control epileptic seizures.

Genetic factors may play an impor-

tant role in the metabolism of drugs, as Elliot S. Vesell of the Pennsylvania State University College of Medicine has shown. The large individual differences in Dicumarol half-life persist to some extent when fraternal twins are compared but almost disappear in identical twins, and similar results have been reported for other drugs studied in twins. The antituberculosis drug isoniazid has been the subject of a number of investigations covering different populations. Isoniazid is metabolized in man primarily by an acetylation reaction catalyzed by the enzyme *N*-acetyl transferase. Soon after the drug was introduced it became apparent that individuals vary in their ability to acetylate it. The distribution of the acetylation rates for a group of subjects tends to be bimodal, that is, plotting the rate against the number of patients exhibiting each rate yields a curve with two peaks. Apparently there are two classes of individuals: those who acetylate isoniazid rapidly and excrete the drug primarily as acetylisoniazid and those who acetylate and excrete the drug more slowly and excrete more of it in an unchanged form. The frequency of rapid inactivators is about 90 percent in a population of Eskimos or of Japanese, whereas among both whites and blacks in North America there are about as many slow inactivators as there are rapid ones.

A clear relation has now been established between this variation in acetylation rate and the incidence of isoniazid toxicity. Several studies have shown that

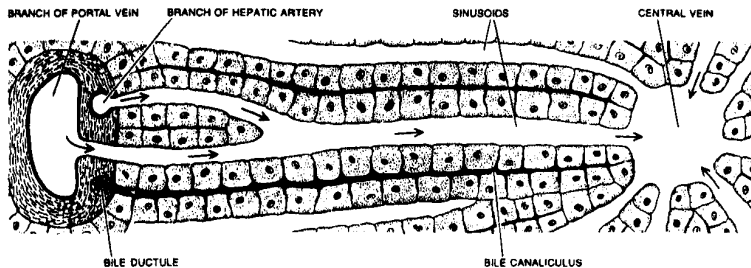
the slow inactivators are more susceptible to a toxic effect of isoniazid: a disorder of the peripheral nerves caused by a specific vitamin B₆ deficiency resulting from an interaction of the drug and the vitamin.

The first indication that microsomal enzymes were special kinds of complexes was developed some 20 years ago by Gerald C. Mueller and James A. Miller at the University of Wisconsin. They discovered that compounds known as aminazo dyes could be oxidized (specifically, *N*-demethylated) by liver microsomes and that the oxidation required molecular oxygen (O₂) and the coenzyme NADPH (reduced nicotinamide-adenine dinucleotide phosphate). Soon afterward Bernard B. Brodie's group at the National Heart and Lung Institute showed that there was a similar requirement for the oxidative metabolism of a number of drugs. In 1957 Howard S. Mason of the University of Oregon Medical School proposed that such oxidations are catalyzed by a class of "mixed-function oxidases": enzyme complexes that require oxygen and NADPH, are nonspecific (they catalyze the oxidation of different kinds of compounds) and catalyze the consumption of a molecule of oxygen for each molecule of the drug or other substrate, with one atom of oxygen appearing in the metabolized substrate and the other atom usually combining with two hydrogen atoms to form water.

The key enzyme of these oxidases is cytochrome P-450. A cytochrome is a

complex of protein and heme, the iron-containing ring structure that is the oxygen-binding component of hemoglobin. Like hemoglobin, the various cytochromes serve to bind oxygen, which they deliver to their substrates in such processes as cell respiration. Cytochrome P-450 gets its designation from the fact that in the reduced form it binds carbon monoxide and then absorbs light most intensely at a wavelength of 450 nanometers. The amplitude of the absorbance peak is the basis of quantitative studies of the enzyme. In the mixed-function oxidases cytochrome P-450 serves as the terminal oxidase: it accepts electrons passed along by several intermediates, binds oxygen and then delivers the oxygen to oxidize its substrate and (usually) produce water (see illustration on page 30). In addition to NADPH the system includes the enzyme cytochrome P-450 reductase. Another heme protein, cytochrome b₅, is present in liver microsomes and may participate in drug oxidations, but its precise function is not clear.

Nor is it known just how the various components of the mixed-function oxidase complex are arrayed in the tubular membranes of the endoplasmic reticulum. It appears that the reductase and cytochrome b₅ are on the outside of the membranes, with cytochrome P-450 in the deeper layers. The enzyme glucuronyl transferase, which catalyzes the most important conjugation reaction, is also present within the membranes. The drug or other fat-soluble compound to be transformed is presumably bound and



LIVER LOBULE, the functional unit of liver tissue, is defined by branches of the portal vein, the hepatic artery, the bile duct and lymphatic vessels, which run together through the tissue, outlining the lobules. (The lymphatics are not shown here.) The venous blood, with its nutrients and other ingested substances, and the oxygenated arterial blood enter the fine sinusoids and perfuse the

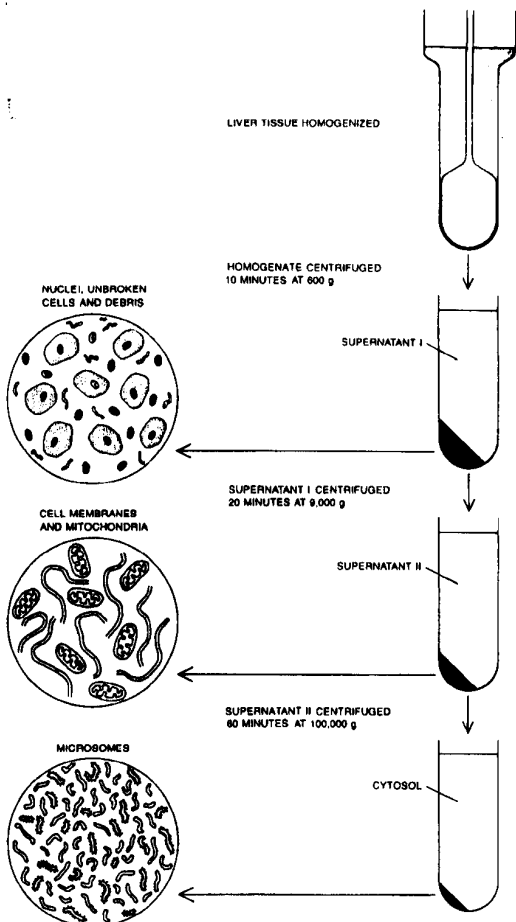
liver cells, which carry out the metabolic functions discussed in the text. The blood drains into a central vein in each lobule and thence to sublobular and hepatic veins. The liver cells also secrete bile, which is collected by bile canaliculi that feed into bile ductules and eventually into the main bile duct. This two-dimensional view of part of a lobule is highly simplified and diagrammatic.

metabolized by the mixed-function oxidase system that contains cytochrome P-450 and then is often converted into a highly water-soluble compound by conjugation with glucuronic acid; the transformed product passes into the lumen, or central channel, of the tubular membranes and is excreted from the cell into the bile or the bloodstream.

The cytochrome P-450 mixed-function oxidase system has come to be recognized as having a central role in the body's defense against chemical agents, whether they are normal body constituents or are introduced from the environment. It is now clear that the system is responsible for the detoxification of many of the potentially harmful environmental pollutants. The system is highly inducible, that is, its activity can be greatly increased by exposure to a wide variety of environmental agents and drugs that act as substrates for the system. Such chemicals stimulate the synthesis of cytochrome P-450 and other components of the complex. More than 200 steroid hormones, drugs, insecticides, carcinogens and other foreign chemicals are now known to stimulate drug metabolism in experimental animals, and many of them have been shown to do the same thing in man. On the other hand, some substances (such as lead and other heavy metals) inhibit the mixed-function oxidase system, although they are fewer in number and less diverse than the inducers. The P-450 system is also the site of much competitive interaction among drugs and other chemicals that are undergoing transformation.

The consequences of this inducibility, inhibition and competition have important implications in drug therapy. Patients are often given several drugs at the same time. Certain combinations can have unpredictable and often undesirable effects if one drug inhibits or stimulates the metabolism of another or competes with it. For example, phenylbutazone, the coumarin anticoagulants and chloramphenicol compete with the metabolic inactivation of tolbutamide, a drug given to reduce the blood-sugar level in diabetics; the competition can lead to excessive tolbutamide activity and thus to serious hypoglycemia, or low blood sugar.

Phenobarbital is a prime example of a drug that has a different effect: enzyme induction. When rats are treated with phenobarbital, there can be a three- to fourfold increase in the microsomal content of cytochrome P-450 and a two-



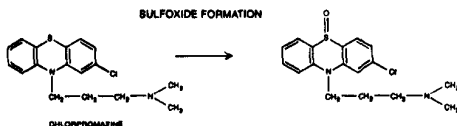
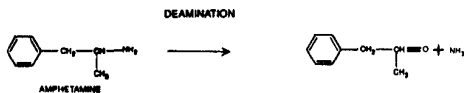
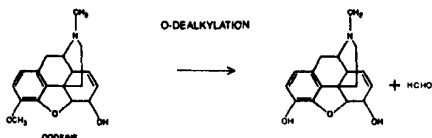
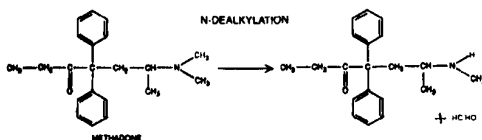
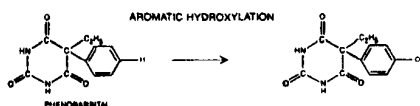
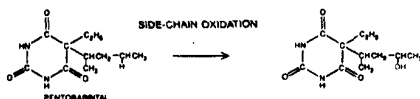
LIVER MICROSOMES, the membrane structures that contain most of the liver enzymes engaged in detoxification, are isolated by spinning homogenized liver tissue at successively higher speeds, which produce successively higher gravity (g) forces, in a centrifuge. Homogenized liver tissue is centrifuged for 10 minutes at 600 g; a pellet of dense material, primarily whole cells, cell debris and cell nuclei, collects at the bottom of the tube. The supernatant, or liquid portion, is centrifuged more strongly, isolating less dense structures such as mitochondria and pieces of membrane. When supernatant II is spun at very high g forces in an ultracentrifuge, the microsomes are separated from the cytosol, or cell fluid.

fold increase in the reductase, and drugs such as methadone and ethylmorphine are metabolized three or four times as fast. In accordance with these experimental observations chronic administration of phenobarbital to patients decreases the effects of many drugs

by hastening their inactivation. Sedative doses of the drug reduce the concentration in the blood plasma of phenylbutazone, the analgesic antipyrine and the coumarin anticoagulants; it also decreases their pharmacological actions. Anticoagulant therapy is particularly

sensitive to dosage. A patient who is satisfactorily maintained on a given coumarin-drug dosage while he is being given phenobarbital as a sedative may hemorrhage when the phenobarbital is withdrawn because the P-450 system is no longer being induced and the anticoagu-

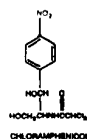
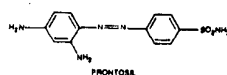
ADMINISTERED DRUGS OXIDATION



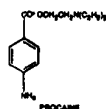
BIOTRANSFORMATIONS carried out in the liver include oxidation, reduction, hydrolysis and conjugation, examples of which are given here. The molecular sites of each reaction are indicated in

TRANSFORMED PRODUCTS

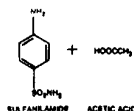
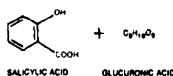
ADMINISTERED DRUGS REDUCTION



HYDROLYSIS



CONJUGATION



color. In a few cases transformation converts an inactive form of a drug (such as Prontosil) into an active form (sulfanilamide), but most of the reactions lead to inactivation. Conjugation with a nat-

Int is therefore being inactivated more slowly.

Alcohol is converted into acetaldehyde largely in the liver, perhaps to some extent by the mixed-function oxidase system; heavy drinkers are found to have an increased concentration of

cytochrome P-450. The habitual consumption of alcohol therefore stimulates the metabolism of a wide variety of drugs. This helps to explain why heavy drinkers are less affected than other people by barbiturates and other sedatives—when they are sober. A single very large dose of alcohol taken together with another drug, on the other hand, inhibits the drug's metabolism, presumably by competing with the drug for the appropriate enzymes. This effect, in addition to the depressant effect of alcohol on the central nervous system, helps to explain the enhanced sensitivity to barbiturates and other sedatives of a person who has been drinking heavily. The synergistic actions of alcohol and sedatives in the brain can cause death.

The inducibility of microsomal enzymes by drugs suggests a form of therapy for certain conditions in which normal body constituents ordinarily metabolized by such enzymes are present in excessive amounts. Long-term administration of phenobarbital, for example, can lower the concentration of bilirubin, the pigment that produces jaundice, in the blood of patients with chronic obstruction of bile flow in the liver. The excessive bilirubin levels that are normally observed in infants after birth can also be markedly reduced if the mother is given a small dose of phenobarbital for a number of days before delivery. Presumably the drug crosses the placenta and stimulates the conjugating enzyme system that is ordinarily slow to develop in the fetus and the newborn infant.

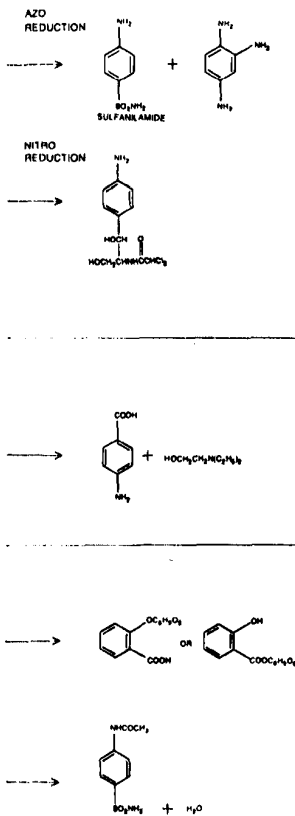
Halogenated hydrocarbon insecticides such as DDT are potent stimulators of drug and steroid sex-hormone metabolism in mammals and in birds; the breakdown of sex hormones explains in part the devastating effects of DDT on reproduction in some bird populations. The minimum exposure to DDT that will stimulate the metabolism of phenobarbital and decrease its hypnotic action in experimental animals is one that results in concentrations of from 10 to 15 micrograms of DDT per gram of fat; that is a level commonly found in human fat tissues. Among the other insecticides that induce microsomal enzymes in experimental animals are chlordane, aldrin and dieldrin. It is interesting that piperonyl butoxide, a synergist that was added to insecticides to inhibit the enzymatic defenses insects had developed against DDT and its chemical relatives, also inhibits the activity of the microsomal enzymes in the mammalian liver.

The polychlorinated biphenyls (PCB's)

constitute another class of environmental pollutants that have been shown to induce microsomal enzymes. The PCB's are lubricants, heat-exchange fluids, insulators, plasticizers for paints and plastic compounds and a major component of the lens-immersion oil used in microscopy. Whereas some of the consumer-product applications have recently been curtailed, the immersion oils are still handled daily by many laboratory workers. PCB's have been found in the tissues of numerous bird and fish species and in human fat and milk, although the route of entry into the human body has not been accurately determined. In recent experiments we have been able to show that the application of pure PCB's or of microscope immersion oil to the skin of experimental animals in very small amounts (one microliter) causes a marked increase in mixed-function oxidase activity and reduces the pharmacological effect of xoxazolamine, a muscle relaxant, and of hexobarbital in the live animal. These findings suggest that trivial skin exposure to chemicals can have significant and perhaps harmful biological effects in man.

A number of chemicals to which human beings are regularly exposed have been identified as chemical carcinogens, that is, they cause cancers when they are applied to the skin of experimental animals or otherwise administered to them. Clearly a factor that inhibits or stimulates the metabolism of such compounds may affect the development of human cancers. Benzopyrene, benzantracene and similar polycyclic aromatic hydrocarbons are among the most ubiquitous carcinogens; they are present in tobacco smoke, in polluted city air and in charcoal-broiled and smoked foods. The polycyclic hydrocarbons are metabolized by a mixed-function oxidase enzyme that in this case is called aryl hydrocarbon hydroxylase because of the particular oxidation it catalyzes. Like other microsomal oxidations, this one requires NADPH and molecular oxygen, but the terminal oxidase of the system induced by the polycyclic hydrocarbons is somewhat different from the oxidase induced by drugs. The catalytic properties of the cytochrome are changed, as are its spectral properties: the absorbance maximum of the complex of carbon monoxide with the reduced cytochrome is at 448 nanometers rather than at 450, and so the enzyme is designated cytochrome P-448. Apart from the polycyclic hydrocarbons, only one other class of compounds has so far been noted to induce the formation of cytochrome P-448: we have found that the PCB's induce

TRANSFORMED PRODUCTS



uril substance such as glucuronic acid often follows metabolism of drugs and facilitates excretion. Not all the by-products are shown.

some of the newly identified cytochrome along with cytochrome P-450. This suggests the possibility, for which there is developing evidence in animals, that the PCB's too may have carcinogenic properties.

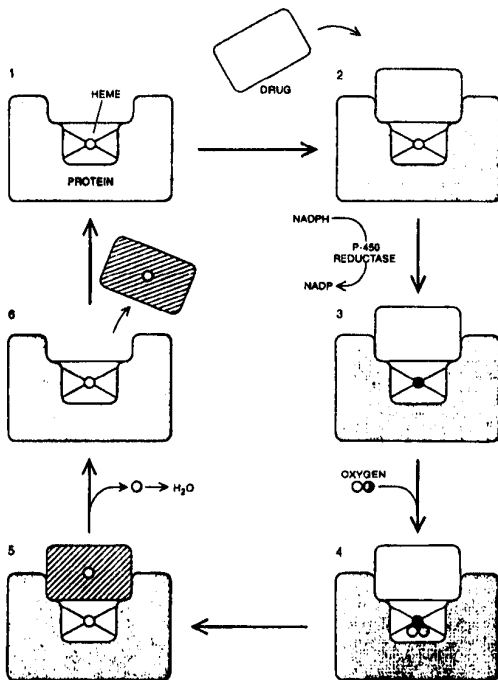
The aryl hydrocarbon hydroxylase system has been the subject of intensive investigation as a possible link in the causation of some cancers. The reason is that rather than detoxifying its polycyclic-hydrocarbon substrates it seems to make some of them more toxic: interme-

diates of polycyclic-hydrocarbon metabolism such as apoxides are more active in the malignant transformation of tissue-cultured cells than the parent products are. Moreover, the enzyme system for carcinogen metabolism is available at many sites in the body that are exposed to polycyclic hydrocarbons and is induced not only in the liver but also in the gastrointestinal tract, the kidneys, the skin and the lungs. Cigarette smoking markedly induces aryl hydrocarbon hydroxylase activity even in the human

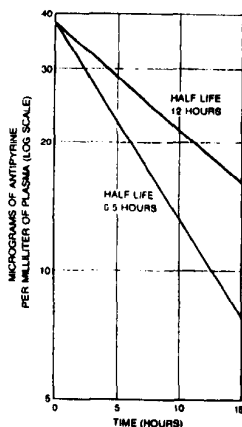
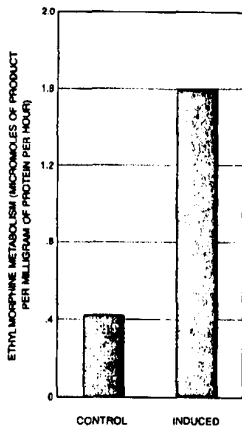
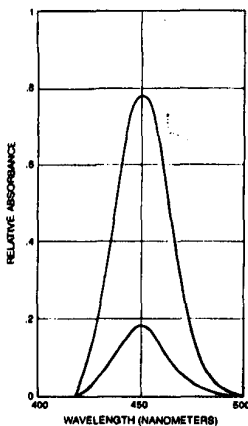
placenta, as Allan H. Conney, Richard M. Welch and their colleagues showed at the Wellcome Research Laboratories. Little or no such enzyme activity was found in placenta from nonsmokers.

We have investigated the cytochrome P-448 system in human skin and have found that there is marked variability in the hydroxylase activity of different skin samples and that incubation of skin in tissue culture with the polycyclic hydrocarbon benzanthrone induces more enzyme activity. A skin biopsy is easy to do, so that assaying skin aryl hydroxylase in the presence or absence of polycyclic hydrocarbons may provide a convenient test of individual differences in the capacity to metabolize certain environmental carcinogens. The possibility that induction of aryl hydrocarbon hydroxylase is of considerable significance in chemical carcinogenesis is suggested by the results of some recent experiments performed by Gottfried Kallermann and his associates at the University of Texas. They found that the inducibility of such hydroxylase activity in lymphocytes is significantly greater in cigarette smokers who have lung cancer than it is in healthy nonsmokers.

It is clear that the liver's mixed-function oxidase system is implicated in a number of processes affecting human health. The ability of a drug or other foreign substance to stimulate the metabolism of another drug by the system may explain some of the adverse drug reactions observed in clinical practice. Drug interactions have been well documented in anticoagulant therapy; more research is required to explore other drug interactions, since most patients are given several drugs at the same time. The ability of environmental pollutants to modify drug action is now under active investigation. It is clear that insecticides, for example, stimulate drug-metabolizing enzymes and that heavy-metal substances such as lead and methyl mercury inhibit the enzymes; the clinical significance of these effects may be appreciable in populations that are exposed occupationally to such agents. Finally, recently acquired evidence seems to indicate that the induction of aryl hydrocarbon hydroxylase in human tissues by polycyclic hydrocarbons may play a significant role in chemical carcinogenesis. Research on this biological action of carcinogens may provide important leads toward predicting the susceptibility of individuals to certain kinds of chemically induced cancer.

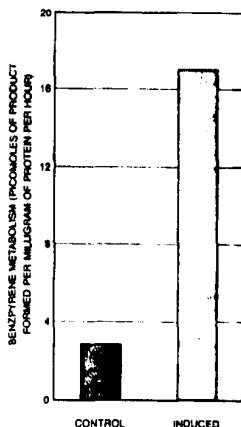
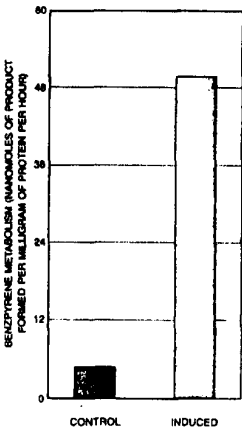
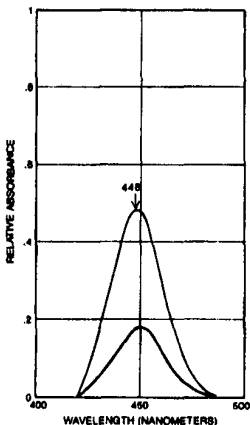


OXIDATION OF A DRUG by the enzyme cytochrome P-450 is visualized here as a sequential process. The enzyme (1) is a complex of a protein and the oxygen-binding compound heme, which contains an iron atom that is initially in the ferric (Fe^{+++}) form (open circle). The cytochrome binds the drug (2). Then (3) the enzyme cytochrome P-450 reductase, utilizing the coenzyme NADPH, reduces the iron of the heme to the ferrous (Fe^{++}) form (black dot), in which it can bind a molecule of oxygen (4). It supplies one atom of oxygen to oxidize the drug and one generally to form water, in the process reverting to its oxidized form (5). The drug, oxidized and in most cases inactive, is thereupon released (6).



PHENOBARBITAL induces cytochrome P-450 and thus stimulates the metabolism of drugs oxidized by that enzyme. The curves (left) show the light-absorbance spectrum, with a peak at 450 nanometers, characteristic of reduced cytochrome P-450 complexed with carbon monoxide, the usual means of identifying and quantifying the enzyme. In a rat treated with phenobarbital the cytochrome P-450 content of microsomes (colored curve) is substantially higher

than that of microsomes from an untreated rat (black curve). More of the test drug ethylmorphine is therefore metabolized (center) by liver tissue from a phenobarbital-treated rat (colored bar) than by the same amount of tissue from an untreated rat (gray). Similarly, phenobarbital speeds up metabolism of the drug antipyrine in man (right): antipyrine level in plasma falls faster after phenobarbital treatment (colored curve) than ordinarily (black).



CARCINOGENS induce a different species of cytochrome, cytochrome P-448. The curves (left) compare the absorbance of microsomes from the liver of a rat treated with the carcinogen methylcholanthrene (color) with the absorbance of control microsomes (black); the induced cytochrome has a peak at 448 nanometers.

Methylcholanthrene enhances metabolism of another carcinogen, benzo(a)pyrene (center): the microsomes of treated rats produce more metabolite (color) than control microsomes (gray) do. Similarly, treating human skin in tissue culture with the carcinogen benzo(a)anthracene stimulates skin-cell metabolism of benzo(a)pyrene (right).