THE FATE OF DRUGS IN THE ORGANISM[1,2]

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Because of the extremely large number of investigations dealing with the metabolism of drugs, it is impossible to avoid limiting this report to a few critically chosen topics. The space available did not permit discussing (12) all drugs which depress or stimulate the CNS. Their metabolism has been reported in detail in previous volumes of this series. The drugs discussed in this article are ones which play an important role in therapy and yet have not been surveyed extensively in the last two editions of the *Annual Review of Pharmacology* (1, 2). It has not been possible to limit this survey of the literature to articles published in the years 1963 and 1964; it has often been necessary to refer to earlier publications. In the process, the less familiar German literature also has been taken into consideration.

A report dealing with the chemical reactions which take place in the organism and with the metabolites thus produced would not be complete without calling to notice the subsequent effects brought about by the biotransformations. All research involved with the metabolism of drugs should be directed toward discovering the relationships between the fate of the therapeutic agent in the organism and the observed effects it has upon animal or man. Such an attempt would facilitate the elucidation of hitherto unexplained phenomena in the behavior of certain drugs.

Enzymatic reactions in the liver.—Lipid-soluble foreign substances, such as drugs, which find their way into the body, would be doomed to remain there for days, weeks, and, in many cases, even for months were the organism not capable of converting them into more polar, less lipid-soluble substances (3, 4, 5). Such a conversion prevents them from re-entering the blood path by way of simple diffusion, through the tubular cell membranes of the kidney once they have been filtered through the glomerulus into the tubular lumen (6, 7). The speed with which the drug penetrates the barrier is dependent upon the diffusion gradient and the relationship of its solubility in lipids to that in water.

An excellent summary of the processes involved in the enzymatic conversion of drugs to more polar compounds, which almost exclusively takes place in the liver, has been published (8). A more extensive report on the transformations carried out by the drug-metabolizing liver enzymes is, therefore, unnecessary. Only a few facts which were either discussed very

[1] The survey of literature pertaining to this review was concluded in July, 1964.

[2] The following abbreviations are used: ATPase (adenosine triphosphatase); BSP (bromosulfophthalein); 6-MP (6-mercaptopurine); NADPH [nicotinamide adenine dinucleotide phosphate (reduced form)]; RNA (ribonucleic acid); and SKF 525-A (propyldiphenyl acetic acid, ester of the diethyl-aminoethyl alcohol).

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briefly in the article referenced above or which became known only in the last year will be dealt with here.

Oxidizing, reducing, hydrolyzing, and conjugating enzymes are among those responsible for the conversion of drugs to more polar compounds. Almost all of these are either tightly or loosely bound to the liver microsomes. Those loosely bound can be solubilized. From a morphological point of view, the microsomes, as carriers of the enzymes, must be regarded as artifacts. They consist of small vesicles which are fashioned out of the disintegrated membranes of the endoplasmic reticulum when liver is homogenized, or they are composed of ribosomes which are either attached to a portion of membrane or exist freely (9, 10). The ribosomes contain ribonucleic acid (RNA). The structures of the endoplasmic reticulum, which were first fully revealed under the electron microscope, fill out the largest portion of the liver cell. They form a network of connected canaliculi lined with membranes, which probably, like a sewer system, drain off the polar lipid insoluble metabolites produced on their membranes. Those membranes occupied with ribosomes, so-called rough membranes, can be clearly distinguished from those having none, known as smooth membranes. The enzymes which hydroxylate drugs occur in both types of membranes, but are more abundantly represented in the smooth than in the rough membranes (11, 12). They hydroxylate lipid-soluble drugs or foreign substances but not the intrinsic lipid-insoluble compounds of the body (13). The natural androgenic steroid hormones require NADPH and O_2 for the oxidation, and are attacked by the same or by analogous enzymes which are also located in the microsomes, and are influenced in their activity by many compounds and sex hormones similarly (14). Following incubation of testosterone or Δ^4-androsten-3,17-dione with microsomes from rat liver, various metabolites were identified. These were hydroxylated at C-atom 7, 6, 16, or 2 and in either the α or β position (14). Similar hydroxylation products were discovered after perfusion of the isolated liver of a dog with testosterone (15). 6-β-Hydroxycorticosterone was detected also in rat urine, following administration of corticosterone. The excretion of this metabolite was considerably reduced by a compound known to be an inhibitor of microsomal enzymes, so that it is quite probable that this hydroxylation also takes place with enzymes of liver microsomes (16). It is altogether possible that one or the other of these hydroxylated, strongly polar steroids has a biologic effect, e.g., in the kidney, but this has not yet been clarified.

Comparative studies of the activity of microsomal enzymes found in the liver of many vertebrate species and which are capable of oxidizing and conjugating drugs with glucuronic acid make evident the importance of these enzymes for the elimination of foreign substances from the organism (17, 18). Oxidizing microsomal enzymes are not present in fish and amphibians; they are not required by these water-dwelling species since the lipid-soluble substances ingested can diffuse through the membranes of the gills or through the skin into the surrounding aqueous medium. But the higher vertebrates,

having gone from water to land life in the process of evolution, can no longer rid themselves of lipid-soluble substances in this manner. The development, then, of specific enzymes capable of making lipid-soluble substances water soluble represents a prerequisite for the furtherance of life. Fish still have enzymes for glucuronidation, whereas the amphibians do not.

The enzymes which oxidize and conjugate drugs are also developed quite late in the ontogeny. The microsomal enzyme systems, which are responsible for oxidations and glucuronidations, first begin to form in the human and in other mammals after birth (19, 20). Because of this, newborns detoxify drugs very poorly or not at all and are, consequently, very sensitive to all drugs requiring oxidation or conjugation before being excreted through the kidneys (21, 22).

Of course, it is not conceivable that nature should have prepared, in advance, a specific microsomal enzyme for the oxidation of every existing or yet-to-be-synthesized foreign substance; this would require an endless number of such enzymes. Rather, it is much more likely that only a restricted number exist, and that each is responsible for the hydroxylation of a large group of analogues. The spatial arrangement of the molecule seems to play an important role here; as can be seen in the case of the oxidation of glutethimide, one of the optical isomers is hydroxylated on the glutarimide ring, whereas the other is hydroxylated on the ethyl substituent (23).

There has been no lack of attempts to bring the enzymes, considered to be mixed function oxidases (24), into solution in order to study their properties. It was possible to separate one or more enzymes from the lipid-containing microsomal membranes by means of a phospholipase from snake venom. The enzyme preparation was capable of oxidizing aniline to *p*-aminophenol, and its activity was not essentially reduced (25, 26). An acetanilid hydroxylase was solubilized from pig liver, by means of pancreatic lipase and, afterwards, purified 20-fold (27). Yet these attempts still make only a limited contribution to the final explanation of the reaction mechanism, since the enzyme was forced out of its natural relation with the lipids through the action of the lipase. Apparently these enzymes represent a special, structurally bound type. Before a substrate reacts with such an enzyme, it must probably be accumulated in lipid micelles which exist in close contact with the enzyme. Consequently, it is extremely difficult to evaluate any kinetic data based on the reaction rates of this enzyme system with various substrates. In spite of all this, one can still conclude, from the existing data, that there are a certain number of different oxidases in the microsomal membranes which differ from one another in their characteristic properties (27–30).

Enzymes which split esters and acid amides are also found in the endoplasmic reticulum. They can be more easily solubilized. It was, therefore, possible to prepare an enzyme from pig liver microsomes which has only a small degree of substrate specificity (31). It hydrolyzes acid amides such as acetanilid and other anilid derivatives, as well as esters, the latter with a

much higher turnover number. Consequently, a great number of drugs containing acid amide or ester bonds can be split by this enzyme. On the other hand, it is not capable of hydrolyzing ester- or acid amide bonds of intrinsic substances. In this respect, it resembles the above mentioned hydroxylases which possess a similar unspecificity. This enzyme has been purified. It is homogenous in electrophoresis, ultracentrifugation, and column chromatography. Its molecular weight is about 175,000 (31a). An enzyme with very similar properties was isolated and highly purified from rat liver microsomes (31b). It was demonstrated that this enzyme is a pure tissue-specific antigen. Traces of this antigen, however, could be detected in the kidney (31c).

Another attempt was made, using a different method, to obtain a homogeneous protein from lyophilized microsomes of rabbit liver which could hydrolyze both acid amides and esters. Only the component which hydrolyzed the acid amides could, here, be considered as homogenous (32). In this type of experiment, it is difficult to decide whether an enzyme can change its characteristics as a result of minor structural changes incurred during its preparation and, further, whether such changes might enable it to react with other closely related substrates.

Until recently, the relationships between the cytochromes, which are found in the liver microsomes (and which have properties not always corresponding to those of the cytochromes in the mitochondria), and the processes involved in the hydroxylation of foreign substances were not known. Cytochrome-b_5, which is present in the microsomes, is a component of the NADPH-cytochrome-*c*-reductase system, and, therefore, cannot be considered to have a part in the electron transport from NADPH to the hypothetical hydroxylase (33, 34, 35).

In addition to cytochrome-b_5, there is another microsomal cytochrome which reacts with carbon monoxide following reduction. Only this CO derivative is detectable since it possesses an exceptionally wide absorption band for a cytochrome at 450 mμ, without exhibiting the corresponding absorption in the visible region. This cytochrome can be solubilized by means of snake venom lipase. In the process, though, its properties are altered, inasmuch as the absorption peak of the reduced CO-cytochrome shifts from 450 to 420 mμ (35, 36, 37).

In the light of the observation that the C_{21}-hydroxylation of 17-hydroxyprogesterone, as carried out by the microsomes of bovine adrenals, was inhibited by CO and that this inhibition was nullified upon exposure to sunlight (38), it could be demonstrated, using the ingenious method by which Warburg discovered cytochrome oxidase 40 yr ago, that a microsomal cytochrome of the adrenal cortex is involved in the hydroxylation of progesterone at position C_{21}. The region of the light spectrum found to be effective in abolishing the inhibiting effect of CO upon C_{21}-hydroxylation was determined to be between 400 and 500 mμ. The maximum or peak of this photochemical activating spectrum was found to be at 450 mμ (38). This corresponded to the spectrum of the CO-cytochrome which had already been de-

scribed in the liver microsomes. Thus, these experiments reveal the first evidence for the existence of a cytochrome which is involved in the oxygen activation of the hydroxylation reaction. This CO-cytochrome appears to have the same function in the hydroxylation of foreign substances in the liver (39).

Important problems connected with drug metabolism have been discussed in recent symposia. A short list of these problems, with the appropriate references, follows for the benefit of the interested reader: (*a*) individual species and variability in drug metabolism as cause of drug toxicity (40, 41, 42); (*b*) drug metabolism in the impaired liver and in the premature and newborn animal and its implication for drug toxicity (43, 44); (*c*) the influence of starvation and factors in the diet on drug metabolism (45, 46, 47); and (*d*) relationship of drug metabolism to methemoglobin formation (48).

Much has been written in the past few years concerning the inhibiting effect on the breakdown of drugs brought about by certain compounds (49). Among the best known of these inhibitors are the diethylaminoethyl alcohol esters of propyldiphenyl acetic acid (SKF 525-A) and of phenyldiallyl acetic acid (CFT 1201) (50, 51). However, these inhibiting compounds have not taken on any practical importance, in terms of therapy, because of their toxicity and inconsistency of effect, even though, theoretically, they might be used to prolong the effects of a drug in the organism.

There is still much discussion as to just how this inhibition of the esterases and hydroxylases which is involved in drug breakdown can best be explained (49, 57–60). The inhibiting substances themselves are N-dealkylated and hydrolyzed by microsomal enzymes just as are other foreign substances. They are thus capable of competitively inhibiting the turnover of other substances, inasmuch as they too compete for binding on the hydroxylating or hydrolyzing enzymes. However, such a mode of action by no means explains all of the observations.

Less well known is the fact, already many times confirmed, that the above mentioned inhibitors, as well as others which are capable of inhibiting the hydroxylation and hydrolysis of drugs, can also bring about an increase in the activity of the same enzymes after a 12 hr or longer inhibition period. There results, then, as can be seen by *in vitro* or *in vivo* experiments, a diphasic course in the oxidation rate of the drugs. The activity of the enzyme rises even further after repeated application of the inhibiting compound (51a, 52–56).

This type of activation of microsomal enzymes can also be observed after pretreatment of animals and human beings with numerous lipid-soluble drugs (54, 63). The hydroxylation of androgens (14) as well as that of many drugs is thereby accelerated (61, 62). There seems to be no change in the activity of androgens (14). However, the enhanced breakdown of drugs has great practical significance. If the drug is metabolized to less effective or ineffective compounds, it will exert a shorter and sometimes weaker effect.

It would extend too far to list all of the substances capable of stimulating

microsomal enzymes involved in oxidation processes. The same holds for a listing of all those substances which exhibit an accelerated turnover, *in vitro* as well as *in vivo*, as a result of the respective enzymes having been activated. Various reviews provide information here (63–67).

Increase in enzyme activity could be the result of the production of a larger amount of the enzyme, itself, or it could possibly be due to the formation of an enzyme-activating or enzyme-stimulating substance. Highly suggestive for the theory of enzyme induction was the observation that ethionine, a protein synthesis-inhibiting compound, could prevent this increase in enzyme activity without interfering with the normal activity or normal rate of drug oxidation (62). Even though this observation suggested an increased synthesis of oxidizing microsomal enzymes, it could not be considered an actual proof, since ethionine might also interfere with other biochemical processes in the cell.

Direct proof is given, though, by the fact that the amount of spectrophotometrically determined CO-cytochrome increases three- to sevenfold during the induction period, following an injection of phenobarbital, a well known inducing drug. The time course of this typical enzyme induction corresponds very closely to the rise and fall of the oxidation rates of two barbiturates (67). Only after repeated application of phenobarbital, or other drugs which are lipid-soluble, can one also ascertain a twofold increase in the amount of cytochrome-b_5. At the same time, one observes a severalfold increase in the activity of microsomal esterases and reductases, whereas certain enzymatic reactions which have nothing to do with the oxidative breakdown of foreign substances are accelerated either only slightly or not at all (67a). An augmentation in the amount of smooth membranes of the endoplasmic reticulum of the liver cell, as observed with the electron microscope, is closely associated with such an increase in enzyme activity. Morphological changes in the other cell structures were not observed (68). Chemical determinations of the lipid, protein, and RNA content of the various cell particles and microsome fragments confirmed the morphologic findings; that is, that the absolute amount of smooth membranes increased about twofold (67a).

These results are in accord with others which showed that pretreatment of rats with phenobarbital stimulated the incorporation of labeled amino acids into microsomal proteins of cell-free liver preparations (68a). The influence that such a conspicuous increase in certain structures in the liver cell could have upon other functional processes has not yet been investigated.

Cardiac glycosides.—The metabolism of the cardioactive glycosides was last handled briefly in the *Annual Review of Pharmacology* in 1961 (69). In the past few years, only a summary presentation has been published (70). It was known from earlier experiments (71, 72) that the sugar components of cardiac glycosides can be split off in the animal organism. Yet, the question remained whether the inherent enzymes of the organism, itself, catalyzed this hydrolysis, or whether the cleavage is carried out by enzymes of the intestinal flora. Experiments on rats have shown that the glucose group from

genuine cardiac glycosides is principally removed by action of enzymes from the intestinal bacteria. Animal enzymes appear unable to bring about such a cleavage under physiologic conditions. On the other hand, desoxy sugars cannot be split off by intestinal bacteria. This reaction, instead, is catalyzed by as-yet-unknown enzymes in the liver. The enzymatic activity in other organs, as determined by incubation of tissue slices, is relatively unimportant in respect to glycoside metabolism. When compared with liver slices assigned 100 percent, the activity for the small intestine and adrenals was found to be 20 percent, for the kidneys 5 percent, and for the heart and diaphragm the activity never reached 1 percent (73). These results are in agreement with others showing that digitoxin cannot be metabolized in the heart tissue of guinea pig (74). All previous conceptions of glycoside metabolism in the heart were accordingly refuted.

The very slow rate of cleavage is dependent upon the position of the sugar component in the sugar chain; as a rule, the rate decreases as the polarity of the molecule increases. The deoxy-sugar residue which is linked directly to the genin is the one most rapidly removed (75).

The mono-, bis- and tridigitoxosides of digitoxigenin and of digoxigenin, of which 6 percent of the total consisted of digitoxin, 20 percent digoxin, and 62 percent digoxigenin-bis-digitoxoside, could be demonstrated in the urine and feces of rats after intravenous injection of digitoxin (72). These findings are in agreement with *in vitro* experiments. They also confirm the earlier observed 12β-hydroxylation of digitoxin to digoxin (76).

Neither the hydroxylation nor the glycoside cleavage can be considered a detoxification process since the monodigitoxoside of digitoxin possesses the same effect as the tridigitoxoside (77). However, the duration of effect of the monodigitoxoside is considerably shorter, and this conforms with the above mentioned accelerated transformation.

The genins, on the other hand, are transformed essentially faster than glycosides in rat liver slices. The hydroxyl group at position 3 is interconverted from the β to the α position, whereby 3-dehydrogenin is found as an intermediate product (78). The enzyme involved is probably the same which facilitates similar reactions in steroid hormones. The increased polarity of the molecule due to the additional hydroxyl group on the genin reduces the rate of transformation, so that digoxigenin (12β-hydroxydigitoxigenin) and gitoxigenin (16β-hydroxydigitoxigenin) undergo epimerization more slowly on C_3. A reduction of the double bond in the lactone ring and an interconversion from the 17α-H to 17β-H configuration were not found to take place. Digitoxigenin can be hydroxylated at position 12 *in vivo* as well as *in vitro* precisely as can digitoxin and its mono- and bis-digitoxoside derivatives (75). Beyond that, metabolites with higher polarity but still soluble in chloroform are produced *in vitro* from digitoxigenin as well as from other genins. They are probably the product of further hydroxylations on the steroid nucleus (78). The genins and their hydroxylation products are difficult to recover *in vivo* since they are quickly transformed to highly polar metabolites that

are not soluble in chloroform (72). Following incubation of digitoxigenin with liver slices from different species, six water-soluble metabolites can be isolated using paper chromatography. Three of these are probably identical with 3-digitoxigenin sulfate, the appropriate epi-isomers, and 3-epidigitoxigenin glucosiduronate (79).

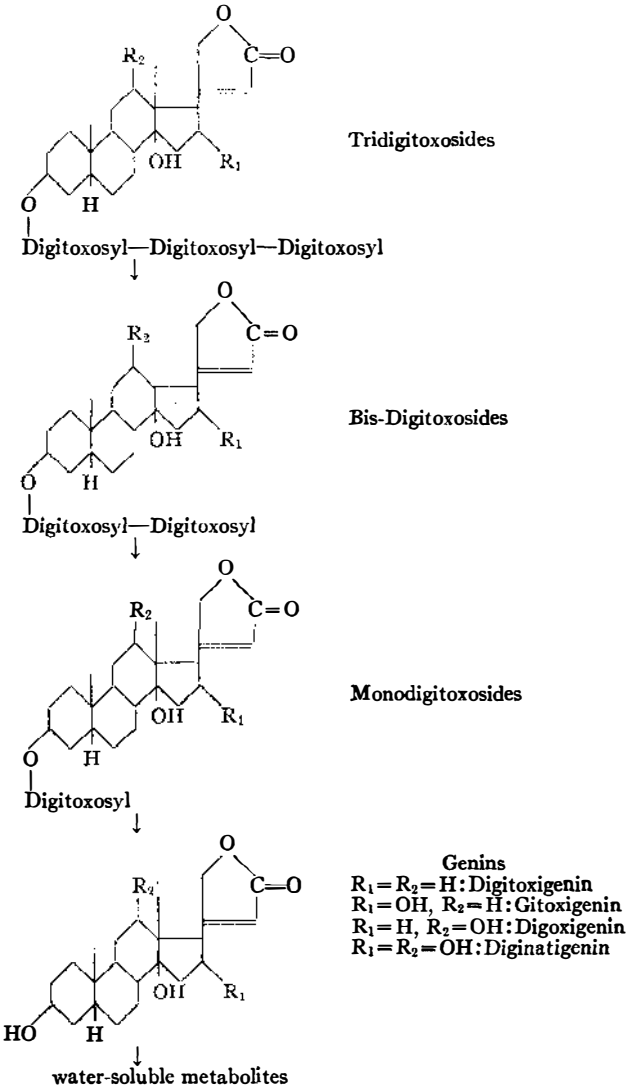

FIG. 1. Stepwise degradation of tridigitoxosides by enzymes in the organism.

The cumulative effect of the cardiac glycosides varies quite markedly among the different species. The previously often-discussed unresolved question of whether this is based upon an accumulation of substance or of effect has been, to a large extent, answered by experiments dealing with the breakdown rate of digitoxin in the liver of different species. The results speak clearly for an accumulation of substance (80). Digitoxin is best suited for such studies, since it is not excreted through the kidneys of mammals in an amount worth considering. This is due to its high lipid-solubility. Only by frogs and toads is digitoxin excreted unaltered. Here, it is eliminated by way of the skin and, possibly, also through the kidneys. The measurement of the amount of digitoxin transformed by liver slices to ineffective metabolites has now made it evident that the accumulation of digitoxin is clearly dependent upon its rate of detoxification (80).

Toads, frogs, and humans cannot break down digitoxin, whereas a transformation was demonstrated by pigeons, cats, rats, guinea pigs, rabbits, and dogs. The rate of detoxification in these animals increases in the order in which they are here listed, just as the tendency towards accumulation decreases. The rapid detoxification of digitoxin in dogs concurs with the observation that 70 percent of the radioactivity from tritium-labeled digitoxin was excreted by this species through the bile and urine within 9 hr and that it was exclusively in the form of water-soluble metabolites (81). The differences in the rate of metabolism of the glycosides among the species might possibly be traced back to the different rates by which the three terminal digitoxose residues are split off. Another possibility comes to mind as a consequence of the experiments on dogs cited above. Here, two of the water-soluble substances isolated from bile and urine contained one part digitoxigenin for every three parts digitoxose. This observation would speak for the dog's ability to conjugate the intact glycoside also. Accordingly, the possibility cannot be excluded that the conjugate, once excreted in the bile, is then hydrolyzed in the intestines and again reabsorbed in the form of digitoxin. However, on the other hand, no relationship between the breakdown rate in the liver of the species investigated and the acute toxicity produced by the digitoxin administered could be established. Studies made on the toad (82), an animal which is exceptionally resistant to digitoxin, showed that the insensitivity of this species can be explained neither on the basis of a rapid excretion or detoxification nor as a result of an inadequate uptake on the part of the heart muscle. Probably the differences in digitalis sensitivity, among the different species, depend upon the varying affinity of the digitalis molecule for a certain receptor. Such a receptor could be the Na^+, K^+-activated membrane ATPase of the heart muscle (70, 83).

Glycosides which contain strophanthidin as genin and are characterized by the aldehyde group on C_{19} are much more rapidly eliminated than are those of digitoxigenin and digoxigenin. Their excretion is accelerated by their high polarity. Upon intravenous application of ouabain labeled with tritium, the human organism eliminates 57 percent of the radioactivity

through the urine and 5 percent through the bile within 24 hr. The aldehyde group of strophanthidin is reduced by liver slices and liver homogenates from the rat (85). One fourth the amount of the enzymic activity detected in the liver is found in the kidneys. Only traces can be found in the lungs, and the other organs are completely free. A detoxification process is not involved here; but the metabolite produced can be eliminated more quickly on account of its higher degree of polarity.

Further metabolites are formed when cymarin (strophanthidin-β-d-cymaroside) is incubated. They arise as a result of demethylation of the cymarose-moiety or splitting off of the cymarose (85).

Cardiac glycosides having a higher degree of polarity than digitoxin suffer a considerable loss of effect when taken orally. A possible hydrolysis affected by gastric and intestinal juices plays a minor role here (86). Enteral absorption quotas for the various cardioactive glycosides, determined chemically for the first time, agree approximately with the values obtained from biologic tests (87). The absorption quota approaches 100 percent for digitoxin but decreases with the increasing polarity of the other cardiac glycosides. Of strophanthidin glycosides, cymarin, K-strophanthoside and convallatoxin were more absorbed than was expected on the basis of biologic tests (87). A portion of each of these polar glycosides undergoes biliary excretion even during the absorption period. For convallatoxin and convallatoxol, this portion amounts to 60 percent of the total amount absorbed (88).

The glycoside moiety circulating in the enterohepatic circulation is deprived of any effect upon the heart. The enteral absorption quota can be enhanced by esterification of one or more of the hydroxyl groups, since the polarity of the compound is thereby reduced (89). Acetylation is the most advantageous, because the acetyl group is easily removed, enzymatically, in the organism. The cleavage begins in the intestinal mucosa. The highest enzymatic activity was found in the liver microsomes (90). Penta-acetyl-gitoxin is absorbed equally as well as digitoxin. At the same time, it is eliminated as rapidly as gitoxin following cleavage of its acetyl groups. An excessive accumulation is thereby avoided. This glycoside appears to possess a somewhat wider therapeutic index because of its lower degree of extracardiac toxicity. It has consequently already been tested clinically (91).

The earlier prevailing notion that the heart possesses a high affinity for glycosides and takes up more than other organs per unit of weight was refuted some time ago by experiments on humans with C^{14}-labeled digitoxin (92). The highest concentrations of digitoxin were found in the excretory organs such as the kidney, gall bladder, and intestines. These experiments were confirmed by studies on rats, whereby digitoxin was administered and paper chromatographic separation was applied (93). It should be pointed out, though, that the rats received the very high dose of 5 mg per kg, in order to make possible the identification of the digitoxin in the various organs. The highest concentration is exhibited in the adrenals, whereas the lowest is found in the brain. The concentration in the heart was never

twice as high as that in the blood. From these experiments, it could also be concluded that there is a substantial enterohepatic circulation of digitoxin.

Anticancer drugs.—An extensive discussion of the latest findings, concerning the metabolism of cytotoxically active substances, would not fit the space allotted to this survey since alkylation reactions with the intrinsic substances of the body would have to be considered; these have already been discussed in summary elsewhere (94–97). The present state of our knowledge concerning the metabolism of certain cytotoxic agents, to which belong busulfan (myleran), ThioTEPA (N,N′,N″-triethylene thiophosphoramide), and the halogenated purines, has also been reviwed recently (98). Further knowledge which is essentially new has not been gained since then. Interesting details, concerning metabolism of cyclophosphamide (cytoxan, endoxan) (see Figure 2) have been made known, which have not yet been compiled in a review.

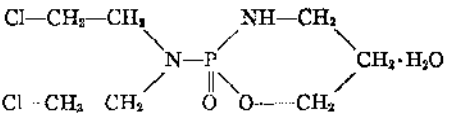

FIG. 2. Structural formula of cyclophosphamide.

Cyclophosphamide is one of those few substances which are not detoxified by a conversion process in the liver. On the contrary, it is first made active in the liver. The chemical reactivity of this compound, when estimated on the basis of the number of chloride ions removed in an aqueous solution, is very small, when compared with that of other nitrogen-mustard compounds (99). Because of this, no effect *in vitro* on cultures of cells derived from human tumors could be detected (100), even though these tumors responded *in vivo* to cyclophosphamide therapy. However, blood serum or liver extract from rats, which had previously been treated with cyclophosphamide, possessed an inhibiting action (100). Cyclophosphamide, when mixed with homogenates derived from various normal and neoplastic tissue from the mouse and then incubated *in vitro*, only became active when the homogenate was from liver tissue. These observations were confirmed and also extended (99).

The alkylating agent arises when cyclophosphamide is incubated with liver slices. Its quantity can be measured sufficiently accurately by means of a cytostatic test. A similar converting activity can be detected in kidney and lung slices, but to a lesser extent. Other organs and a whole series of tumors which were incorporated in the experiments possessed no activating effect. The responsible enzyme itself could only be demonstrated in the microsomal fraction of the liver. The reaction is dependent upon NADPH and O_2. The production of the effective substance is inhibited by SKF 525-A (99). It is not yet known at which position of the molecule the oxidation takes place, but it is known that the ring is thereby opened. This follows

from the fact that $^{14}CO_2$ was found in the expired air of mice which had received cyclophosphamide labeled on the carbon atom adjacent to the ester bond in the ring (101). The substance produced by the microsomal oxidation following injection of cyclophosphamide could be detected in the blood serum and in all of the organs; it is not accumulated in tumor tissue. Its half-life time in serum at 37° C was only 15 to 30 min. The rate of production in the liver, on the other hand, is initially greater, but then it takes on the same order of magnitude so that the cytotoxic activity in the serum of the rat remains the same from approximately 15 min to 2 hr. The absolute level of activity is dependent upon the dose.

To this time, the alkylating agent has not yet been definitely identified. It possibly is identical with bis-(β-chlorethyl)-amine or its derivative, N-β-chlorethylaziridine (102, 103). The latter arises spontaneously from the former, by the release of chloride. Against such an assumption are observations in which differences were ascertained between the mode of action of bis-(β-chlorethyl)-amine and the alkylating agent originating from cyclophosphamide (104).

The concentration of the effective agent in the rat is increased three- to fourfold by pretreatment with phenobarbital (105). This supports the likelihood that the microsomal oxidation of cyclophosphamide to the active substance is accelerated by enzyme induction (67a), and this could open up new therapeutic possibilities.

On the other hand, an enzyme inhibitor can enhance the cytotoxic effect of the purine derivatives substituted at the 6 position, such as 6-mercaptopurine, 6-methylpurine, 6-propylthiopurine, and 6-chloropurine. All of these substances can be convered by a xanthinoxidase to uric acid derivatives. When a xanthinoxidase inhibitor, such as 4-hydroxypyrazolo-(3,4*d*)-pyrimidine (HPP), is administered simultaneously with one of the above mentioned purine derivatives, the cytotoxic potency of that drug is increased manyfold (106). Specifically, when the inhibitor is administered with 6-mercaptopurine (6-MP), the excretion of 6-MP in the urine by the mouse and by the human is increased fourfold, whereas 6-thiouric acid is then found only in very small amounts (106).

Sulfonamides.—The metabolism of the sulfonamides was discussed in the previous edition of this *Review* (1). Nevertheless, certain details concerning sulfonamide behavior in the organism will be dealt with here, because a knowledge of these processes can shed light on the fate of other chemotherapeutic agents.

The binding of sulfonamides to the plasma proteins and the lipid solubility of these substances have been more intensely investigated than for any other class of drugs. A comparison of the physicochemical data acquired and the behavior of these substances in the organism allows for many interesting conclusions. For this reason, data taken from the very latest studies have been compiled in Table I. Elimination generally decreases with increasing lipid solubility and the ensuing increase in back diffusion in the renal tubules.

Since only the nondissociated portion is capable of permeating the tubu-

TABLE I
SULFONAMIDES

	1[a]		2[b]	3[c]	4[d]	5[e]			
	CONC. IN PLASMA OF NOT PROTEIN BOUND S.A. AT A LEVEL OF 10 MG %		SOLUBILITY IN CHLOROFORM	H.L.T. OF S.A. IN PLASMA	pKα	EXCRETION IN URINE (PER CENT)			REF.
						FREE	ACET.	GLUC.	
	Mouse mg % (α)	Man mg % (α)		Hr					
Sulfisoxazole	6.9	1.5	3.8	6	5.0	70	23	7	(1)
Sulfathiazole	—	2.5	6.8	4	7.25	68	31	1	(1)
Sulfadiazine	9.7	5.5	11.9	17	6.5	92	8	0	(111)
S. Methoxydiazine	5.0	3.0	51	37	7.2	46	16	38	(111)
Sulfaphenazole	2.0	0.07	54	11	6.1	7*	5	88	(112)
S. Methoxypyridine	5.7	1.3	69	35	7.2	24*	49	26	(112)
S. Dimethoxine	3.8	0.35	74	41	6.3	7	15	78	(113)
						21*	16	64	(112)
						9	21	70	(114)

[a] Re: the binding to the plasma proteins (107, 108). Here the freely dissolved portion which is found at 37° C and at a total concentration of 10 mg percent is given in mg percent (α). The values listed were compared with those from another experiment in which a different method was used to determine the degree of protein binding, and they were found to be substantially in agreement (109). Since the mouse is mainly used for testing *in vivo* the chemotherapeutic effect of the sulfonamides, a comparison is made possible by the listing of the α values for mice serum in the first column and the values for human serum in the second column (107).

[b] Re: the solubility in chloroform (109). Here the portion which goes over from the aqueous phase (isotonic phosphate buffer solution with pH 7.4) to the chloroform phase is expressed in per cent. The volume ratio of aqueous to chloroform phase was 1:1.

[c] Re: the half-life-time in plasma, as a measure of the elimination rate (110).

[d] Re: the dissociation constant (109). Here the pH value listed is the one found when one half of the compound is present in the dissociated state (pK).

[e] Re: the percentile distribution of the excreted product found in the urine. The fraction of unaltered sulfonamide which is marked here with * contains acid-soluble glucuronide, so that the actual portion of unaltered substance is, in reality, considerably less, whereas the glucuronide fraction is correspondingly larger.

lar cells, the dissociation constant in the renal tubules is of great importance in determinating the extent of backdiffusion. But since the pK value fluctuates, with one exception, between 6.1 and 7.25, it can hardly be evoked to explain the substantial differences in elimination rate found in Table I. Moreover, sulfonamides as weak acids can be secreted by the renal tubules, but this factor appears to play a minor role in determining the rate of elimination of the listed compounds.

Considering the extremely strong protein binding and high lipid solubility of several sulfonamides, the clearance of these substances should be very small, and their elimination should take an even longer period than actually found. They are converted, however, to highly polar and easily excretable glucuronides (115).

Whereas an increased affinity of a drug for the proteins of the blood plasma reduces clearance through the kidneys, it does not interfere with glu-

curonidation. The latter even increases with advancing lipid solubility, so that it becomes a facilitating factor in the elimination of lipid-soluble sulfonamides.

The glucuronides formed are conjugated on the N_4, the N_1 or the N position of heterocyclic substituents. Two different glucuronides of sulfa-dimethoxine have been isolated from human urine and identified. N_4-glucuronide, which is acid-labile, represented 11 percent, while the acid-stable N_1-glucuronide 89 percent of the total glucuronide fraction isolated (113, 114). Both conjugates were also found in the urine of rats and of dogs (See Figure 3).

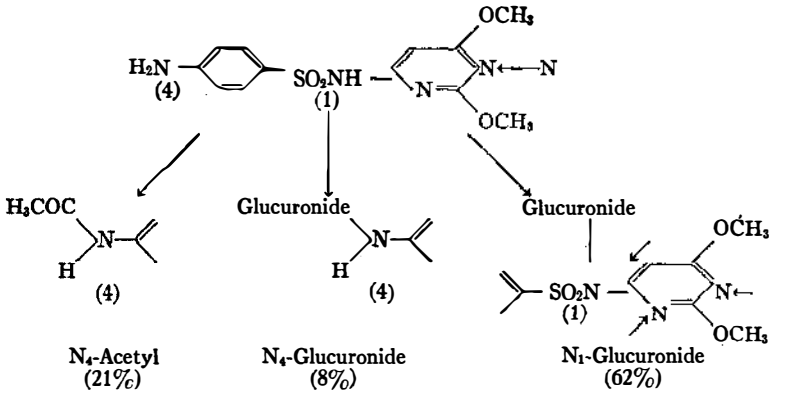

FIG. 3. Sulfadimethoxine metabolism. (In parenthesis are listed the percentile portion of the metabolites found in human urine.)

The excretion of sulfadimethoxine-glucuronide, by way of the bile and the urine, rose as much as threefold when the rats were pretreated with phenobarbital. This result permits the conclusion that phenobarbital provokes an increased production of the N-glucuronyltransferase, just as it induces accelerated synthesis of microsomal enzymes of the liver (116, 117).

Two different glucuronides are also detected in the urine following application of sulfamethoxydiazine. The smaller portion was identified as the N_4-conjugate, whereas the larger portion, judging from its observed characteristics (118), is thought to be an N_1-glucuronide. A metabolite of sulfisomazole, discovered in the urine, likewise exhibited the same characteristics as the synthetically produced N_1-glucuronide (112). Sulfaphenazole, on the other hand, does not form a N_1-glucuronide (112), but rather an acid-stable glucuronide. The latter metabolite is conjugated either on the nitrogen atom of the heterocyclic ring or on the phenyl residue following hydroxylation, by means of which its notably rapid elimination can be explained. The brief 11-hr half life of this sulfonamide, which is so highly lipid-soluble and exhibits the most extensive degree of protein binding, would not be conceivable were the drug not virtually completely metabolized.

The extent to which the sulfonamides are bound to the proteins of the plasma of mice is markedly less than it is in human blood plasma. This is very important, as has been pointed out before (119). An 11 times higher level of free sulfadimethoxine and a 30 times higher concentration of free sulfaphenazole are present in the mouse plasma as compared with the human when both have the same concentration of total sulfonamide. The greater affinity shown by the other sulfonamides for the proteins of human plasma is not as obvious. But the notable differences, here, between mice and humans should be considered when evaluating the various sulfonamides, since their chemotherapeutic effectiveness is dependent upon the concentration of unbound sulfonamide in the blood plasma (119–122).

Effectiveness of a new sulfonamide tested on mice does not permit the conclusion that it possesses a similar activity in men.

Equipotent doses of 5 sulfonamides tested on mice varied between 1 and 4.5. However, the mean ultrafiltrate concentration during the first 39 hours was identical in 4 of 5 sulfonamides, although the course of the concentration curve in plasma and ultrafiltrate differed widely (123). The chemotherapeutic effect of sulfadimethoxine could not be completely accounted for on the basis of this criterion. Its concentration in the ultrafiltrate achieved about 30 percent when sulfadimethoxine was equally active (123).

Comparing the level of sulfonamides in plasma and in tissue fluid, it was found that the concentration of the unbound substance was the same, although the concentration of the protein-bound part was always lower in the tissue fluid than in the plasma (108).

The physicochemical conditions underlying the binding of the various sulfonamides were investigated on a large number of modified sulfonamides. On the basis of these studies, we can conclude that the protein binding increases with an increasing dissociation constant. In spite of this, neutral molecules can be more strongly bound if the appropriate substituents are present, since ionic and hydrophobic bonds exist side by side. These same regularities also hold true for other classes of substances, for example, the antibiotics (124).

Anabolic androgens and gestagens.—Along with the modified glucocorticoid steroids, the derivatives of testosterone and nortestosterone are being used to a great extent, both therapeutically and on the basis of a social indication. These compounds have an androgenic, anabolic, gestagenic, and, through their metabolites, an estrogenic effect. A marked disparity exists between the level of our knowledge of their fate in the organism and the extent to which they are being used in practice. It is, therefore, important that the little which is known about their metabolism be dealt with thoroughly.

The close chemical relation between the anabolic androgens and the gestagens is illustrated in Figure 4.

Certain effects can be suppressed and others intensified by modifying the molecule. The fate of the individual compounds in the organism is thereby also influenced.

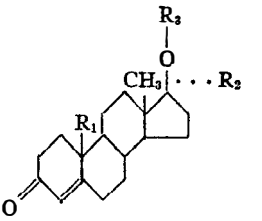

FIG. 4. Chemical relationships between anabolic androgens and gestagens.

1. $R_1=CH_3$ $R_2=CH_3$ $R_3=H$
   17α-Methyltestosterone (androgenic)
2. $R_1=H$ $R_2=H$ $R_3=COCH_2CH_2-$ 
   19-Nortestosterone-17α-phenyl-propionate (anabolic)
3. $R_1=H$ $R_2=C_2H^5$ $R_3=H$
   17α-Ethyl-19-nortestosterone [norethandrolone] (anabolic)
4. $R_1=H$ $R_2=C\equiv CH$ $R_3=H$
   (*a*) 17α-Ethinyl-19-nortestosterone (gestagenic)
   (*b*) Norethynodrel (gestagenic)
   (double bond at $\Delta^{5(10)}$ instead of at Δ^4)

Methenolone (1-methyl-Δ^1-androsten-17β-ol-3-one) is administered in the acetate or enanthate form. (See Figure 5 for formula.) Its anabolic effect seems to be stronger than the androgenic; this is made evident in tests where the weight increase of the levator ani muscle is compared with that of the seminal vesicles of castrated male rats (125–127).

Methenolone acetate is quickly and completely absorbed, as noted in experiments on rats. The maximum level in the blood plasma of human subjects is reached after 3.5 hr (128, 129). The acetyl group is removed by hydrolysis, either in the intestinal mucosa or in the liver (128, 129). The radioactivity from tritium-labeled moieties of this compound is relatively slow to be excreted. Forty to 70 percent of the applied amount could be recovered in the excretions of the human, from which one third to one half was in the feces (128, 129); this is compared with two thirds found in feces of the rat. The half life of the radioactivity amounted to approximately two days for both the rat and the human, a value which is quite incongruous

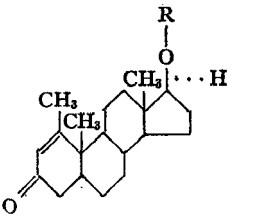

FIG. 5. Structural formula of methenolone.

R = 17-Acetate or enanthate
1-Methyl-Δ^1-androsten-17β-ole-3-one methenolone (anabolic)

with the rapid rate in which methenolone is metabolized. The extent to which it is conjugated is very noteworthy: only 2 percent of the applied dose is excreted as more highly polar metabolites in nonconjugated form. Unaltered methenolone, itself, was not detected in the excretory products. However, 3 percent of the applied dose was eliminated as sulfate or glucuronide of unchanged methenolone (129). Metabolites predominated in the conjugate fraction.

In the blood plasma, too, after 3.5 hr, only 2 percent of the radioactivity was found in nonconjugated forms. These must have been metabolites of unaltered methenolone; methenolone itself was no longer evident. The exceptionally high concentration of glucuronides and sulfates in the plasma is very unusual, since such conjugates are known to have a high rate of clearance due to their high polarity. No doubt, the enterohepatic circulation of these substances plays a certain role in the maintenance of their high concentration in the blood plasma, as could be observed in experiments on rats and dogs, but this cannot completely account for the phenomenon (128, 129). The extremely fast turnover of methenolone raises numerous questions. It is unlikely that the anabolic effect is mediated by the conjugate. The question as to the active substance in the organism must still remain open. It could be that extremely small concentrations of methenolone are sufficient to bring about the effect.

Only very limited amounts of ketosteroids such as 1-methyl-Δ^1-androsten-3,17-dione could be found among the human metabolites of methenolone (130). And these would in no way suffice to maintain an increase in the ketosteroid excretion during a period of treatment with methenolone (131, 132). 1α-methylandrosterone was likewise not detected in the urine (130). The meager formation of ketosteroids following administeration of methenolone is very noteworthy when one considers that 75 percent of an applied dose of testosterone is excreted as 17-ketosteroids (130). Even though methenolone, like testosterone, does not have an alkyl substituent at C_{17}, it apparently takes a completely different metabolic pathway. The observations cited were generally confirmed by *in vitro* experiments.

An explanation for the unusual behavior of methenolone has since been given. A 17β-hydroxysteroid dehydrogenase which can convert testosterone to a ketosteroid was found in the liver of guinea pigs (133). The activity of this enzyme is inhibited when a methyl group is attached to ring A or B, or when the double bond is displaced from Δ^4 to Δ^1 (134). The absence of a double bond in ring A likewise leaves the enzyme ineffective. 17α-Alkylation also inhibits the conversion to ketosteroids; and, consequently, 17α-methyltestosterone, in contrast to testosterone, is not oxidized on C_{17} in the presence of liver homogenates from guinea pigs (134). It is also interesting that the Δ^1 double bond is not reduced by liver slices (132).

Even though studies *in vitro* make it evident that methenolone takes a different metabolic pathway than does testosterone, the studies do not explain the extremely fast turnover of methenolone in the organism. It is pos-

sible that the conjugates found in the urine which have a steroid portion more polar than methenolone are actually hydroxylated compounds.

Much attention has been given to the question of whether or not testosterone or testosterone derivatives can be made aromatic, thereby possibly taking on estrogenic characteristics which might produce desirable or undesirable side effects. Women beyond the menopause, given 17α-ethinyl-19-nortestosterone and 19-nortestosterone-phenyl propionate, excrete 0.05 and 0.18 percent of the applied dose respectively in the form of estrogens (135). Somewhat smaller amounts, 0.02 to 0.07 percent, were found in the urine of men subjects following administration of testosterone, 19-nortestosterone, and $\Delta^{1,4}$-androstandien-3,17-dione, as well as various testosterone esters (136). Estran, estriol, and also 17β-estradiol were estabilished as being excretory products. The amounts of estrogens actually produced in the organism must be essentially higher than this, since normally only 16 percent of the estradiol is recovered in the urine. Given a therapeutic dose of 25 mg of 19-nortesterone, approximately 0.25 mg might be produced by the organism. However, an even higher amount of 17α-ethinylnortestosterone is transformed to ethinylestradiol (137, 138). It is likely that this metabolite does not exhibit its full effect because it is metabolized in the liver as soon as it is formed, but far too little attention has been given to the problem of whether the continual production of such large amounts of estrogenic substances over months and years of treatment with gestagens might not exercise a damaging effect. One should keep in mind that estrogens could be carcinogenic under certain conditions and when a certain disposition is already present.

In contrast, the application of methenolone does not result in an increase in the excretion of estrogenic substances (131, 139). An explanation for this can be taken from the results of *in vitro* experiments. Microsomes from the human placenta bring about the aromatization, in the presence of NADPH and O_2, of 20 percent of 19-nortesterone and 25 percent of $\Delta^{1,4}$-androstadien-3,17-dione, as compared to 100 percent aromatization of testosterone. Formaldehyde is thereby set free in stoichiometric proportion to the amount of estradiol formed. This is evidence that the angular C_{19}-methyl group is split off after oxidation, and estradiol is thus formed following intramolecular rearrangement of the first ring (140). However, the oxidizing microsomal enzymes of the placenta are not capable of bringing about a similar transformation of 1-methyl-Δ^1-androsten-17β-ol-3-one, i.e., methenolone (134, 141). The 1α-methyl grouping can not be held responsible for the inhibition of the aromatization, since the incubation of 1α-methyltestosterone results in the formation of 1α-methylestradiol. The actual prerequisite for the completion of this reaction is a Δ^4 double bond (134). (See Figure 6.)

Less is known concerning the fate of the gestagens norethynodrel and norethindrone (norethisterone). They do not cause an increase in the excretion of ketosteroids (142). They are also predominantly eliminated as conjugates. Following the application of norethinodrel and norethindrone labeled

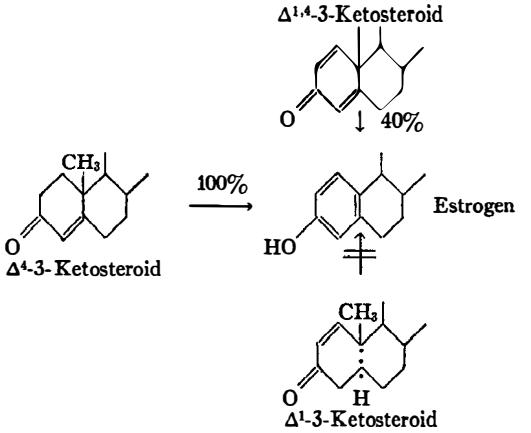

FIG. 6. Δ^4-Double bond as prerequisite for aromatization.

with tritium, 30 percent of the radioactivity of the former and 50 to 70 percent of the latter were excreted within 24 hr. Only 1 to 5 percent represented unconjugated compounds. Eight of the conjugates isolated from urine after application of norethynodrel were investigated in more detail. 17-Ketosteroids were not detected among them. Probably they are steroid alcohols. All but one possessed an intact ethinyl side chain (143) which is evidence for the resistance of the ethinyl group to enzymatic attack. Metabolites which were reduced in the 3α or 3β position and were hydroxylated at C_{10} were found among the conjugates. No conjugate of the unaltered substance was found after application of norethynodrel (143). But, following the administration of norethindrone, minute amounts of the conjugated, unaltered compound were detected in the urine (138).

Both of these gestagens also undergo a fate in the organism which is contrary to that of testosterone and nortestosterone. In spite of the structural differences between them and the anabolic androgen methenolone, there exist conspicuous similarities in their metabolism. Since the normal path of androgen degradation is not open to these substances, they are converted to highly polar steroids which are then excreted in the conjugated form. These are very likely hydroxylated steroids; in analogy, it can be assumed that they are hydroxylated by the microsomal enzymes of the liver. Such substances do not appear in significant amounts during the physiologic degradation of the natural intrinsic hormones.

It may be that the high metabolic rate of anabolic androgens in the liver and their rapid excretion in the bile canaliculi are related to the abnormal retention of BSP (sulfobromophthalein-sodium) which has been observed many times following application of these steroids (144, 145). The impairment of dye excretion can have, in severe cases, its clinical counterpart in the form of cholestatic jaundice (144).

A remarkable correlation exists between the metabolism of steroids and liver function disturbances. All anabolic androgens with an alkyl-group on C 17, as well as methenolone, may alter the human liver physiology. The retention of dye excretion can have, in severe cases, its clinical counterpart in the form of cholestatic jaundice (146). Increased serum transaminase levels are also reported (147–149). However, no alteration of the liver physiology occurs during treatment with testosterone, nortestosterone, or the phenyl-propionic-acid-ester of nortestosterone (see Fig. 4, No. 3) (144). Both types of steroids differ in their metabolism considerably, as mentioned above. Only those steroids which are degraded to ketosteroids in the same manner as the physiological hormone testosterone do not impair the liver function.

Gestagens with an ethinyl side chain, which are also not metabolized to ketosteroids, can produce the same reversible impairment. The incidence, however, is much smaller than after treatment with anabolic steroids and seems to depend rather on the lower administered dose than on minor differences in the molecular structure (147), since the daily dose for treatment of patients with anabolic steroids amounts to 40 mg whereas the dose of gestagens averages 2 to 5 mg. A dose-dependent alteration of liver function tests has also been observed when patients were treated with methenolone (149). Unexplained is the strange toxic effect of gestagens combined with an estrogenic component on the liver of women 60 to 80 years old (150). The considerable elevation of serum transaminase levels in all patients studied could be confirmed (151, 156). The reason of the age-dependent toxic effect of these preparations remains to be elucidated.

However, significant alterations of liver function tests have not been observed in women taking "contraceptive pills" before the menopause (152–154). Nevertheless, three cases of cholestatic jaundice have been reported in the Scandinavian literature (155). Recently, a severe acute cholestatic jaundice, confirmed histologically with biopsy material, was observed after a typical treatment of a younger woman during three periods with three different preparations of ovulatory drugs (156).

The state of the discussion, about the toxic effects of several not intrinsic steroids, was extensively reported, since the immense propagation of the "pill" is in considerable contrast to the meager knowledge we have about it.

The apparent relationship between the fate of these types of steroids and their toxic effect on the liver, which is dose and age dependant, seems to be an impressive argument for the necessity and usefulness of detailed studies about metabolism of drugs, especially those which are extensively used.

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