BIOCHEMICAL EFFECTS OF DRUGS[1]

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Certain representative biochemical effects have been selected for this first volume of the *Annual Review of Pharmacology*. Where a biochemical effect appears pertinent to an understanding of the drug's mechanism of action, we have framed our discussion accordingly.

Drugs Influencing Amine Metabolism

Reserpine and other Rauwolfia alkaloids.—In the past several years, studies on the various biochemical effects induced by reserpine and related Rauwolfia alkaloids have stimulated the interest of biochemists as well as pharmacologists. Classical pharmacological techniques were employed initially to investigate the mechanisms of the central and peripheral pharmacologic effects induced by reserpine (depression, hypotension, hypothermia, bradycardia, etc.). Early conclusions were that reserpine acts primarily by reducing the activity of the central sympathetic regulating centers (1) or by producing a central block or inhibition of afferent nerve impulses which normally stimulate sympathetic activity (2).

The idea that reserpine might release certain body amines arose from observations that serotonin (5-hydroxytryptamine) administration to mice produces certain effects similar to those of reserpine (3, 4, 5): sedation, potentiation of hypnotics, and blockade of this action of both reserpine and serotonin by the serotonin antagonist, lysergic acid diethylamide. Shore *et al.* (5) observed that administration of reserpine to dogs caused a marked increase in the urine levels of 5-hydroxyindoleacetic acid, a metabolic product of serotonin, suggesting that serotonin had been released from its stores and undergone metabolism. This view was verified by direct analysis of tissue serotonin levels which showed that reserpine treatment lowers the serotonin content of intestine (6), brain (7, 8), platelets (9, 10), and other tissues including rat mast cells (11).

From an examination of the effect of several Rauwolfia alkaloids on brain serotonin levels, Brodie *et al.* (12) concluded that only those which elicit a sedative response affect the levels of serotonin in brain. Hess *et al.* (13) demonstrated that the depressant effects of reserpine persist long after the time that any appreciable levels of reserpine remain in the brain, whereas the pharmacologic effects parallel temporally the decline in serotonin levels.

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

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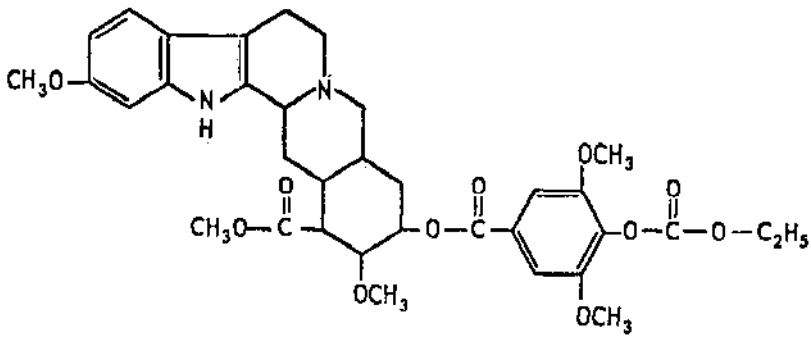
Sheppard *et al.* (14) reported the presence of trace amounts of radioactive material in the brain long after injection of reserpine-C^{14}, and interpreted their results as an indication that reserpine per se, rather than changes in brain amine levels, may be responsible for the persistent effects of a single dose of reserpine. Their results show, however, no correlation between levels of radioactive material and pharmacological effects.

Studies in man have shown that clinically effective doses of reserpine are effective in altering blood serotonin levels (15). A prolonged biochemical "lesion" in tissues was evidenced by the demonstration that after reserpine administration, platelets and brain fail to take up as much added serotonin as do normal tissues (16).

The inference that brain serotonin is important in the pharmacologic action of reserpine (17), made on the basis of the above mentioned find-



Su 3118

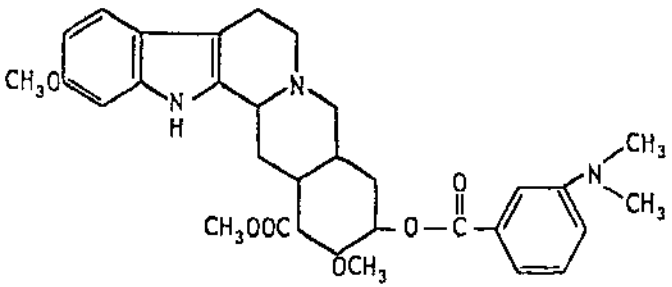
FIG. 1

ings, was challenged by the observation that reserpine also releases catecholamines from tissues including brain (18, 19, 20), heart (20, 21), ganglia (22), arteries (23), and blood (24). Furthermore, almost identical dose and temporal relationships are evident in the effects of reserpine on serotonin and norepinephrine levels in brain (25). More recently, the identification of dopamine (hydroxytyramine) in brain was followed by the observation that reserpine treatment leads to the release of this amine, too (26).

Persuasive evidence is now available that the depletion of norepinephrine from peripheral sympathetic nerve endings is largely responsible for the lowered peripheral sympathetic tone induced by reserpine. Thus it has been demonstrated that after depletion of tissue norepinephrine by reserpine or similarly acting congeners, animals no longer show the usual peripheral responses to electrical or chemical stimulation of sympathetic nerves, although the response to injected norepinephrine is unchanged or potentiated (19 to 22, 27). One semisynthetic Rauwolfia alkaloid, Su 3118 (Fig. 1), exerts an effect primarily on peripheral norepinephrine stores over a wide

dosage range, although higher doses of the drug also cause depletion of amines from the brain with a consequent central depression (28).

To ascribe the central effects of reserpine to a single amine has proved to be a more difficult problem. Arguments that the central depressant effects of reserpine are caused by depletion of brain catecholamines have been based on observations such as the ability of injected dihydroxyphenylalanine and the inability of injected 5-hydroxytryptophan to reverse the depressant effects of reserpine (29), or on attempted differential depletion of the amines by certain drugs (30). More recently, however, evidence has been accumulating that the depressant effects of reserpine are indeed more closely related to changes in brain serotonin levels than to brain catecholamine levels. For example, by the use of another semisynthetic reserpine analog, Su 5171 (Fig. 2), which alters brain norepinephrine levels longer than brain serotonin levels, it has been shown that the depressant effects of the alkaloid closely parallel changes in the level of serotonin, but not norepinephrine (31).



Su 5171

FIG. 2

Stronger evidence for the primary importance of brain serotonin in the central actions of reserpine has been obtained from experiments in which the alkaloid has been administered to cold-exposed animals (32, 33). Under this condition, reserpine lowers brain norepinephrine levels, but has little effect on brain serotonin levels and produces no sedation until the brain serotonin levels finally decline (33).

In studies on the mechanism of reserpine-induced release of serotonin in a model system of rabbit platelets *in vitro,* Carlsson *et al.* (34) found that the releasing activity of reserpine is remarkably potent, one molecule of the alkaloid effecting the release of hundreds of serotonin molecules. In spite of this, platelets are not broken by the drug (9). An analysis of the kinetics of serotonin uptake by platelets in the presence and absence of reserpine has suggested that the drug interferes with a serotonin transport mechanism which maintains serotonin levels within the platelet against a concentration gradient (35).

Tetrabenazine and other benzoquinolizines.—Another series of compounds that markedly lower brain serotonin and norepinephrine levels is the benzoquinolizine series, of which tetrabenazine has been the most widely investigated. Pletscher and co-workers (36) found that this drug and its active congeners release brain serotonin and norepinephrine and produce central depression similar to that induced by reserpine. Unlike the Rauwolfia alkaloids, however, the benzoquinolizines are relatively short acting in their actions on brain amines and in producing sedation. The short duration of action of these compounds suggests that they may act "reversibly," rather than "irreversibly" as does reserpine.

Tetrabenazine seems to have little effect on peripheral amine stores (37), although a recent report holds that heart norepinephrine levels are lowered by the drug (38). Consistent with the relative absence of peripheral norepinephrine release is the observation that, unlike reserpine, tetrabenazine has little or no effect on blood pressure (39).

In spite of their differences, tetrabenazine and reserpine appear to act in the brain at the same receptor sites, since it has been demonstrated by Quinn *et al.* (37) that treatment of animals with tetrabenazine just prior to administration of reserpine markedly lessens the duration of action of reserpine. The results of this experiment provide further evidence for the "irreversible" nature of reserpine action, because tetrabenazine is of itself short-acting, but remains in the brain for a time sufficient to inhibit the action of reserpine at its receptor sites during the even briefer physical presence of reserpine in the brain.

Effect of reserpine on histamine levels.—In addition to affecting serotonin and catecholamines in tissues, reserpine lowers histamine levels in rabbit blood (40), but not in other rabbit tissues (41). The alkaloid does not lower histamine levels in rat skin (mast cells), although serotonin is released from this tissue (11). The effect on histamine levels in rabbit blood, like the effects on other amines, seems to be confined to sedative Rauwolfia alkaloids since an almost inactive one, methyl reserpate, causes little lowering of rabbit-blood histamine levels (41). Inasmuch as Burkhalter *et al.* (41) found that infusion or injection of serotonin or its precursor causes a lowering of blood histamine levels, there is the possibility that the serotonin released by reserpine in the blood may, in turn, be responsible for the histamine release from rabbit platelets. Surprisingly, neither reserpine nor serotonin effects the release of histamine from rabbit platelets *in vitro,* although serotonin is readily released by reserpine in the same system.

Guanethidine.—The saying, "It never rains but it pours," seems to apply to amine-releasing agents, and a new hypotensive drug, guanethidine, must be added to the growing list. Investigation into the mode of action of this drug has revealed that it causes peripheral effects that, as with the Rauwolfia alkaloids, might be described as "chemical sympathectomy," since the action of the drug renders the sympathetic system unresponsive to

stimuli, although the action of injected norepinephrine is not inhibited [Maxwell *et al.* (42)]. Suggestions that the drug might alter the state of the adrenergic transmitter have been borne out by the observation of Cass *et al.* (43) that guanethidine causes a marked lowering of norepinephrine levels in various peripheral organs, but not in brain or adrenal glands. The rate of norepinephrine depletion of tissues by guanethidine is considerably slower than that observed following reserpine administration, suggesting a difference in the mode of action. It seems possible that guanethidine might act by blocking the synthesis of norepinephrine rather than by a true release process.

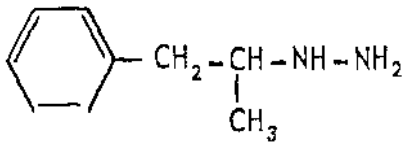
Bretylium, a hypotensive drug which causes a pharmacologic effect similar to that of guanethidine, does not appear to act by depletion of norepinephrine stores, but possibly by blockade of nerve impulses along the postganglionic sympathetic fiber, an action aptly described by Boura & Green (44) as "adrenergic neurone blockade."

α-Methyl-dihydroxyphenylalanine.—Experiments *in vivo* with α-methyl-dihydroxyphenylalanine, an inhibitor of the enzyme which decarboxylates dihydroxyphenylalanine (45, 46) and 5-hydroxytryptophan (47), showed that the drug causes a lowering of serotonin levels in brain and intestine (48) and of norepinephrine levels in brain and peripheral organs (49). In preliminary studies, Oates *et al.* (50) observed hypotension and sedation in hypertensive patients given α-methyl-dihydroxyphenylalanine.

Monoamine oxidase inhibitors.—The close association of interests between the clinical investigator and the basic researcher has seldom been more evident than in studies of the actions of monoamine oxidase inhibitors. Much of the impetus for the clinical trial of these drugs arose from laboratory studies, although many of the discrete pharmacologic effects are seen only in the clinic.

Observations in 1952 that tubercular patients undergoing therapy with iproniazid showed signs of central stimulation (51) led to the abandoning of the drug as a tuberculostatic agent in favor of its congener, isoniazid. In that same year, Zeller *et al.* (52) reported that iproniazid inhibits the activity of monoamine oxidase. The various observations remained unrelated until reports appeared indicating that administration of reserpine to animals pretreated with iproniazid does not cause the usual depressant effect of the Rauwolfia alkaloid, but instead elicits a marked degree of central stimulation (53, 54, 55). Measurement of the serotonin and norepinephrine content of various tissues, including brain, showed that pretreatment with the monoamine oxidase inhibitor also prevents the usual serotonin- and norepinephrine-lowering effects of reserpine (53, 54, 56).

These observations and the recollection of the central excitation seen in tubercular patients on iproniazid therapy prompted a reinvestigation of the clinical usefulness of iproniazid, but this time as a central stimulant to combat mental depression (57). The resulting favorable clinical results and the assumption that the antidepressant effects were associated with the mono-



JB 516

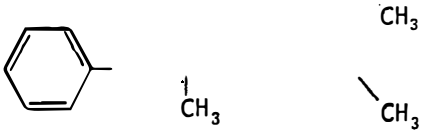
FIG. 3

amine oxidase blocking action of iproniazid led to the rapid synthesis and clinical trial of a number of other monoamine oxidase inhibitors.[3]

Attempts to elucidate in animals the mechanisms involved in the central stimulant effect seen in patients have led to observations that pretreatment with monoamine oxidase inhibitors not only reverses reserpine-induced depression and prevents the decrease in the levels of brain catecholamines and serotonin, but that the inhibitors cause a marked increase in the concentrations of these amines in brain (54, 60 to 64). Thus, a single large dose of iproniazid, or JB 516 (Fig. 3), causes a two- to threefold increase in serotonin and norepinephrine levels in rabbit brain. There exists a species difference with regard to this effect; rabbits, rats, mice, and monkeys show an elevation in both serotonin and norepinephrine brain levels after treatment with monoamine oxidase inhibitors, whereas dogs and cats show an elevation only in serotonin levels (63). Pretreatment of dogs with a monoamine oxidase inhibitor, however, does prevent the usual lowering of brain norepinephrine levels by reserpine (65).[4]

[3] Doubt has been expressed that the clinical antidepressant action of monoamine oxidase inhibitors is related to blockade of the enzyme; instead, it is thought that it is attributable to some other action of the drugs. However, the primary criterion for selection of the drugs for further investigation has been their ability to block monoamine oxidase *in vitro* and *in vivo*. One report (58) holds that 1-benzyl-2-methyl-5-methoxytryptamine (BAS), though a sedative agent in man, inhibits monoamine oxidase, a conclusion based on indirect evidence. Direct measurement of the ability of BAS to block brain monoamine oxidase *in vivo* or *in vitro*, however, has demonstrated that this agent has little or no monoamine oxidase inhibitory activity (59).

[4] The ability of monoamine oxidase inhibitors to reverse the depressant effects of reserpine and to prevent the usual rapid decrease in the brain content of catecholamines and serotonin following reserpine administration was originally interpreted as evidence that the amines were released by reserpine from their binding sites, but that since they could not be acted upon by the inactivated monoamine oxidase, they remained in the brain in a free form (53). This view has been challenged by suggestions that the monoamine oxidase inhibitors not only block the enzyme, but prevent release of the amines as well. Recently, however, Spector *et al.* (66) have demonstrated that monoamine oxidase inhibitors do not prevent the reserpine-induced release of platelet serotonin or the release of brain serotonin and catecholamines, but appear, instead, to stabilize the liberated amines.



JB 807

FIG. 4

After a single large dose of iproniazid in rabbits, no obvious gross pharmacologic signs can be detected in the animals in spite of the elevated brain amine levels, but prolonged daily injections of the drug provoke a degree of excitation and peripheral sympathomimetic signs together with a further elevation of the brain amine levels (63, 64).

Attempts to associate these pharmacologic signs with one or the other of the brain amines have not yielded clear-cut answers. There is no obvious relationship between the excitatory effects and the degree of elevation of brain serotonin levels. A better association exists in the case of brain norepinephrine levels. Spector *et al.* (63) observed that with several monoamine oxidase inhibitors, excitation in rabbits did not occur until brain norepinephrine levels reached a critical value. Upon discontinuance of the drug, the excitation ceased when the brain norepinephrine levels decreased, even though the serotonin levels were still elevated. The picture is by no means clear, however, as one monoamine oxidase inhibitor, JB 807 (Fig. 4), closely related to JB 516, causes a marked increase in the level of both brain amines, but does not produce detectable excitatory effects in rabbits (64).

It has not been possible to produce these pharmacologic effects by administration of monoamine oxidase inhibitors to dogs or cats (63). In these species, as pointed out before, although a marked rise in brain serotonin levels follows treatment with the drugs, no rise in brain norepinephrine levels occurs. These results suggest again a possible association between central stimulation and elevated brain norepinephrine levels.

Metabolism of catecholamines.—The influence of monoamine oxidase inhibitors on the metabolism of norepinephrine in the brain has led to the hypothesis that the major pathway of catecholamine metabolism in brain is via oxidative deamination by monoamine oxidase (67). This conclusion might seem at variance with studies on the fate of catecholamines injected into the bloodstream. In the latter experiments, Axelrod *et al.* (68) have shown that the major metabolic route of administered catecholamines involves initially an ether formation by 0-methylation of the 3-hydroxyl group. It seems clear, however, that both monoamine oxidase and catechol-0-methyl transferase are of utmost importance, each in its own domain. Monoamine oxidase is probably responsible for metabolism of catecholamines within tissues and for regulation of the tissue stores of these

amines, whereas 0-methyl transferase appears to be mainly responsible for the "detoxication" of catecholamines which find their way into the bloodstream. For example, it has been demonstrated that administration of monoamine oxidase inhibitors does not potentiate the cardiovascular effects of injected epinephrine (69), in contrast to pyrogallol, an inhibitor of the methylating enzyme (70) which potentiates the effects of injected epinephrine (71). However, pyrogallol, even in very large doses, does not elevate catecholamine levels in brain (72), nor does it block the norepinephrine-depleting effect of reserpine even though the activity of the brain-methylating enzyme is blocked by parenteral administration of pyrogallol. Similar results have been obtained from studies on peripheral tissues. Thus, pyrogallol lowers the rate of metabolism of circulating injected norepinephrine but not the rate of metabolism of the injected norepinephrine taken up by heart tissue, whereas monoamine oxidase inhibitors have no effect on the circulating norepinephrine levels but lower the rate of disappearance of norepinephrine taken up by the heart (72).

Association of brain amine changes with convulsive threshold.—Changes in brain amine levels may have a role in the effects of certain drugs on the threshold of electroshock convulsions. A marked facilatory effect of reserpine on experimentally induced convulsions was noted by Chen *et al.* (73) in 1954 before it was known that the drug releases brain amines. More recently, Prockop *et al.* (74) showed that centrally acting monoamine oxidase inhibitors exert a marked anticonvulsant action in rats subjected to electroshock. The onset of the anticonvulsant effect following injection of iproniazid is delayed until the brain amine levels have increased, and the effect persists until the amine levels fall toward normal values. The peak anticonvulsant effect of the more rapidly acting monoamine oxidase inhibitor, JB 516, occurs much sooner than that following iproniazid injection. Thus, there appears to be a marked correlation between brain amine levels and the convulsive threshold. In these experiments, diphenylhydantoin was found to completely block convulsions without causing a concomitant rise in brain amine levels. Thus, the anticonvulsant effect of diphenylhydantoin does not appear to be associated with an alteration of brain amine levels despite the report that very high doses of this drug cause a rise in brain serotonin levels (75).

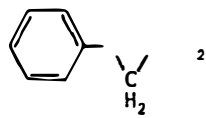
That the facilatory effects of reserpine, on the one hand, and the anticonvulsant effects of the monoamine oxidase inhibitors, on the other, are not caused by the presence of the drugs per se was shown in experiments where monoamine oxidase inhibitors were given to animals previously treated with reserpine (low brain amine levels) or where reserpine was given to animals pretreated with monoamine oxidase inhibitors (high brain amine levels). Again, those animals with low amine levels showed facilitation of convulsions, whereas those animals with high brain amine levels showed a resistance to electrical stimuli (74). Lessin & Parkes (76) have noted that reserpine or tetrabenazine also lowers the survival time of mice

treated with pentylenetetrazol and that pretreatment of the animals with iproniazid inhibits this effect.

Anticonvulsant effects of monoamine oxidase inhibitors also have been observed in man. Grisoni *et al.* (77) observed that iproniazid exerts a normalizing action on the EEG pattern of epileptic patients. More recently, Carter (78) found that isocarboxazid, a potent monoamine oxidase inhibitor, reduced the frequency of grand mal seizures in a high percentage of a group of epileptic patients.

Cardiovascular effects of monoamine oxidase inhibitors.—In addition to the clinically observed antidepressant effects, monoamine oxidase inhibitors have been found to lower standing blood pressure in man (79) and to alleviate the pain associated with angina pectoris (80).

The mechanism of the cardiovascular actions is unclear, especially since the drugs do not appear to cause clear-cut effects in laboratory animals. Several possible pharmacologic mechanisms, including coronary artery dilation, ganglionic blockade, and an inhibition of catecholamine release, have



SKF 385

FIG. 5

been postulated to explain the clinical cardiovascular effects [see review by Zbinden *et al.* (81)]. An elevation in catecholamine levels in peripheral tissues occurs in the heart (82, 83) and autonomic ganglia (84). Elevated blood serotonin levels have been reported in patients and laboratory animals treated with monoamine oxidase inhibitors (85, 86). Presumably all of the extra blood serotonin is localized in the platelets, as in the case with the normal serotonin content of blood.

Other actions and types of monoamine oxidase inhibitors.—Of course, monoamine oxidase inhibitors do not necessarily affect only monoamine oxidase. Indeed, monoamine oxidase inhibitors possessing the hydrazine moiety also inhibit diamine oxidase (87, 88). The action on diamine oxidase appears to be attributable to the hydrazine moiety since aminoguanidine and isoniazid do not block monoamine oxidase but do block diamine oxidase, whereas certain potent nonhydrazine monoamine oxidase inhibitors, such as SKF 385 (Fig. 5), have no action on diamine oxidase (88). Inhibition of diamine oxidase does not appear to be implicated in the hypotensive action of monoamine oxidase inhibitors since SKF 385 also causes postural hypotension (89).

Udenfriend *et al.* (90) have pointed out that monoamine oxidase inhibitors fall biochemically into two categories: irreversible and reversible. The

inhibitors in clinical use, such as iproniazid, irreversibly inhibit the enzyme since inhibition *in vivo* persists long after the drug has disappeared from the body (91, 92), and the inhibitory action *in vitro* cannot be removed by dialysis. Harmaline, a reversible inhibitor, blocks monoamine oxidase only while present in sufficient concentration in the tissues, and its action *in vitro* can be removed by dialysis (90). It appears that the reversible inhibitors occupy the same sites that are altered by the irreversible inhibitors since pretreatment of animals with harmaline blocks the prolonged effect of subsequently administered iproniazid (66, 93).

It should perhaps be pointed out that although the major investigative labors have gone into a study of the effects of monoamine oxidase inhibitors on serotonin and the catecholamines, other amines are affected by these drugs. It has been shown that after administration of these inhibitors to human subjects, several amines, including tryptamine, phenylethylamine, tyramine, and its *o*- and *m*-isomers, appear in the urine (94 ,95). It is possible that one or more of these amines or some as yet undiscovered amine might be important in the action of monoamine oxidase inhibitors.

Relationship Between Vitamin B_6, γ-Aminobutyric Acid, and Seizure-Producing Drugs

During the past 10 years γ-aminobutyric acid has become of considerable importance in pharmacology. The compound appears to be formed and to occur only in the central nervous system. Several functions have been attributed to γ-aminobutyric acid in the brain: (*a*) The compound may act as a neurohumoral agent. (*b*) It may participate in ammonia detoxification via the formation of γ-guanidinobutyric acid. (*c*) γ-Aminobutyric acid may be an important substrate in oxidative metabolism via the following pathway: α-keto-glutaric acid → glutamic acid → γ-aminobutyric acid → succinic semialdehyde → succinic acid. Recent reviews have appeared on the metabolism and postulated roles for γ-aminobutyric acid in brain (96 to 100).

Several lines of investigation have indicated a relationship between vitamin B_6, γ-aminobutyric acid, and seizure-producing drugs. It is known that a dietary deficiency of vitamin B_6 (pyridoxine) in man and animals facilitates seizures. Furthermore, vitamin B_6 antagonists, either structural analogues (deoxypyridoxine, methoxypyridoxine) or carbonyl reagents (isonicotinylhydrazide, semicarbazide, and thiosemicarbazide), are potent convulsants. A possible explanation for the seizures produced by these compounds has come from the observation that vitamin B_6 in the form of pyridoxal phosphate serves as a coenzyme necessary for certain transaminase and decarboxylase reactions involved in the metabolism of γ-aminobutyric acid. Glutamic acid decarboxylase required for the conversion of glutamic acid to γ-aminobutyric acid is found only in brain and is very sensitive to a deficiency of pyridoxal phosphate or to interference with this coenzyme.

Killam & Bain (101) showed that carbonyl reagents markedly inhibit the activity of this enzyme and that the decreased activity is correlated with decreased levels of γ-aminobutyrate in brain.

McCormick & Snell (102) have proposed a mechanism for the action of carbonyl reagents on brain metabolism other than through their direct effect on enzymes involved in amino-acid metabolism. They have isolated pyridoxal phosphokinase from human cerebral cortex and purified the enzyme 200-fold. Both α-methylphenethylhydrazine and isonicotinylhydrazide were found to be extremely effective inhibitors of this kinase. The actual inhibitors, however, are the corresponding hydrazones formed between these hydrazines and pyridoxal. The oxime, hydrazone, and semicarbazone of pyridoxal are all potent inhibitors with affinities for the kinase well over 100 times that of pyridoxal. In view of the low concentration at which the carbonyl reagents inhibit pyridoxal phosphokinase, McCormick & Snell (102) suggested that the seizure-producing effects of these compounds result in part from their capacity to lower or to eliminate production of pyridoxal phosphate. Thus, the carbonyl reagents were postulated to lower brain γ-aminobutyrate levels as a consequence of inhibition of the pyridoxal phosphokinase rather than from inhibition of the glutamic decarboxylase.

Baxter & Roberts (103) reported that the vitamin B_6-dependent γ-aminobutyric acid-α-keto-glutaric acid transaminase of beef brain which catalyzes the conversion of γ-aminobutyric acid to succinic semialdehyde is markedly inhibited by hydroxylamine. They showed that administration of hydroxylamine results in increased γ-aminobutyrate levels in rat brain (104). Eidelberg *et al.* (105) found that administration of hydroxylamine to cats elevates brain levels of γ-aminobutyrate and reduces the duration of electrically induced seizures. The possibility was suggested that the increased γ-aminobutyrate levels may result from a more potent inhibitory effect of hydroxylamine on the γ-aminobutyric acid-α-keto-glutaric acid transaminase than on the glutamic acid decarboxylase. Rindi & Ferrari (106) reported that the seizure-producing compound toxopyrimidine (2-methyl-4-amino-5-hydroxymethyl pyrimidine) lowers the level of γ-aminobutyric acid in rat brain presumably by inhibition of glutamic decarboxylase. Nishizawa *et al.* (107) administered toxopyrimidine and hydroxylamine to mice and measured the activity of both brain glutamic-aspartic transaminase and glutamic decarboxylase and found that when convulsions are induced by toxopyrimidine administration, both enzyme systems are inhibited. When sufficient hydroxylamine is given to cause convulsions, the decarboxylase is inhibited, but the transaminase is not affected unless lethal doses of hydroxylamine are administered. They suggested that the convulsions are related to inhibition of glutamic decarboxylase.

The level of γ-aminobutyric acid in brain depends, presumably, on the relative rates of its formation and utilization by two vitamin-B_6-requiring enzymes, glutamic acid decarboxylase and γ-aminobutyric acid-α-ketoglu-

tarate transaminase. Consequently, drugs that inhibit the decarboxylase decrease the level of γ-aminobutyric acid, whereas those that selectively inhibit the transaminase elevate the level of this compound. Roberts & Eidelberg (96) have pointed out that a causal relationship between changes in seizure susceptibility with changes in γ-aminobutyric acid levels in brain still has not been established.

Interaction of Nicotinamide with Drugs

Kaplan *et al.* (108) reported that the amount of diphosphopyridine nucleotide in mouse liver increases markedly upon the injection of large doses of nicotinamide. Burton *et al.* (109) subsequently demonstrated that the administration of reserpine or chlorpromazine prior to the injection of nicotinamide results in elevated levels of diphosphopyridine nucleotide in liver for a prolonged period of time. Burton *et al.* (110) recently showed a correlation between the ability of reserpine and reserpine derivatives and of chlorpromazine and phenothiazine derivatives to tranquilize animals and their ability to maintain elevated diphosphopyridine nucleotide levels in liver. They noted that sedative drugs such as phenobarbital and ethanol are not able to maintain these high nucleotide levels. In another study, Burton *et al.* (111) demonstrated that nicotinamide acts in mice in conjunction with either reserpine or chlorpromazine to reduce spontaneous activity and to increase the duration of pentobarbital anesthesia. The biochemical mechanisms involved in the effect of reserpine and chlorpromazine in prolonging the increased diphosphopyridine nucleotide levels remain to be established.

Stimulatory Effect of Drugs on Ascorbic Acid Synthesis

Various drugs possessing completely unrelated chemical and pharmacological properties have been shown by Longenecker *et al.* (112), Baumann *et al.* (113), and Burns *et al.* (114) to increase markedly the urinary excretion of ascorbic acid in rats. These drugs include the hypnotics, chlorobutanol (Chloretone) and barbital; the analgesics, aminopyrine and antipyrine; the muscle relaxants, orphenadrine and meprobamate; the antirheumatics, phenylbutazone and oxyphenbutazone; the uricosuric agent, sulfinpyrazone; the antihistaminics, diphenhydramine and chlorcyclizine; the anticoagulant, bishydroxycoumarin (Dicumarol); and the carcinogenic hydrocarbons, 3-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene. Tracer studies by Horowitz & King (115) and Burns and coworkers (116, 117) showed that this increased excretion results from an accelerated L-ascorbic acid synthesis from D-glucose through the intermediate formation of D-glucuronic acid, L-gulonic acid and L-gulonolactone.

The striking effect of a single 10 mg. dose of 3-methylcholanthrene on the urinary excretion of ascorbic acid is shown in Figure 6 (114). By six days after administration of the hydrocarbon, the rate of ascorbic acid excretion was 50 to 75 times greater than the control value, and, in fact, during the 19-day period of the experiment about 140 mg. of the vitamin

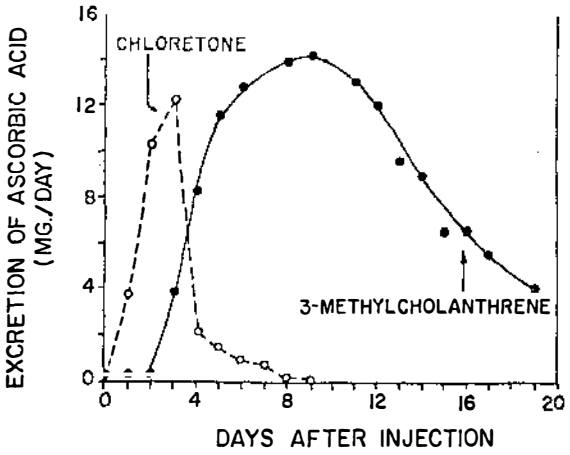


FIG. 6. Stimulation of L-ascorbic acid excretion by 3-methylcholanthrene and chlorobutanol (Chloretone).

was recovered in the urine. For comparison, the effect of a single 40-mg. dose of chlorobutanol is also shown. It will be noted that chlorobutanol exerts an immediate effect on ascorbic acid excretion which reaches a peak by the third day and falls to a low value by the fifth day. With 3-methylcholanthrene, no effect was observed for two days, but after this period the excretion values remained markedly elevated for at least 18 days. No explanation can be offered at present for the unusually prolonged effect of 3-methylcholanthrene on ascorbic acid synthesis.

Various drugs have been reported (112, 114) to exert little or no effect on ascorbic acid excretion in rats: urethane, acetanilide, phenacetin, *p*-aminophenol, phenol, sulfanilamide, codeine, quinine, salicylates, borneol, α-naphthol, phenolphthalein, ethyl biscoumacetate (Tromexan), reserpine, chlorpromazine, chlorzoxazone, carbon tetrachloride, sodium bromide, nicotinamide, and keto-oxyphenbutazone. Interestingly enough, Minesita *et al.* (118) observed that the administration to rats of the alkaloid lycorine inhibited ascorbic acid synthesis and decreased ascorbic acid content of various organs, producing a condition resembling scurvy. Other studies by Conney *et al.* (119) showed that lycorine administration to rats antagonized the stimulatory effect of chlorobutanol on ascorbic acid synthesis.

Available evidence indicates that the drug-induced synthesis of ascorbic acid results from increased metabolism of glucose through the glucuronic acid pathway (Fig. 7). The existence in animals of this route of glucose metabolism has been revealed largely by studies on the biosynthesis of L-ascorbic acid and L-xylulose. The reactions of this pathway have been reviewed recently by Touster (120), Burns (121), Strominger (122), and Burns & Conney (123). According to this scheme, D-glucose is oxidized to D-glucuronic acid, which undergoes reduction to L-gulonic acid; the latter

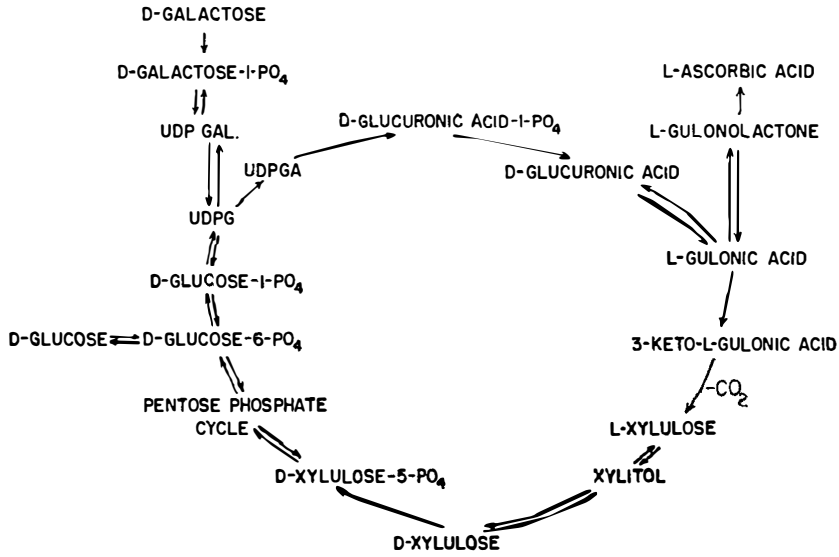


FIG. 7. The glucuronic acid pathway of glucose metabolism.

acid then serves as the precursor of either L-ascorbic acid or L-xylulose. Administration of barbital or chlorobutanol to rats stimulates the conversion of D-glucose-1-C^{14} or D-galactose-1-C^{14} to labeled D-glucuronic, L-gulonic, and L-ascorbic acids (114, 124, 125). Administration of barbital to guinea pigs, a species which cannot make ascorbic acid, leads to the increased urinary excretion of D-glucuronic acid (124). Martin *et al.* (126) observed no detectable conversion of D-galactose-1-C^{14} to ascorbic acid in guinea pigs treated with 3-methylcholanthrene. It should be noted that the guinea pig, as well as man and monkey, lacks the ability to convert L-gulonolactone to L-ascorbic acid (121).

Further evidence for the effect of drugs on the glucuronic acid pathway came from earlier studies by Enkelwitz & Lasker (127). They showed that aminopyrine and antipyrine, which stimulate the synthesis of ascorbic acid in the rat, also markedly elevate the urinary excretion of L-xylulose in human subjects with essential pentosuria. This observation can be explained in terms of the scheme in Figure 7, since administration of these drugs would be expected to increase the formation of L-xylulose from D-glucose. The pentosuric subject is not able to metabolize L-xylulose (120), and thus the pentose would be excreted in the urine.

The mechanism by which drugs stimulate the formation of ascorbic acid is not known. The possibility that a renal mechanism is involved has been ruled out since chlorobutanol induces the synthesis of the vitamin in nephrectomized rats (114). The available evidence (111, 114, 121) indicates that drugs such as chlorobutanol and barbital increase ascorbic acid bio-

synthesis by stimulating the formation of free D-glucuronic acid. Increased synthesis of labeled free D-glucuronic acid from D-galactose-1-C^{14} has been observed by Evans *et al.* (125) in liver homogenates of rats pretreated with chlorobutanol, but the specific enzymatic steps involved have not yet been defined.

The formation of a glucuronide by the drug is not required since barbital, one of the most potent drugs stimulating the synthesis of ascorbic acid, is recovered unchanged in the urine (124). It should be noted that borneol and aniline, potent in forming glucuronides, have no effect on the synthesis of ascorbic acid or its precursor, L-gulonic acid. Aniline was shown by Smith & Williams (128) and by Axelrod *et al.* (129) to cause appreciable excretion of free D-glucuronic acid resulting from the breakdown of a labile aniline N-glucuronide.

Ganguli *et al.* (130) reported that the administration to rats of adenosine triphosphate, malic acid, or thyroxine suppresses the chlorobutanol-stimulated synthesis of ascorbic acid. They also showed the administration of chlorobutanol elevates the inorganic phosphorus level in liver while the levels of several hexose and triose phosphates are diminished. Straumfjord & West (131) reported that the stimulation of ascorbic acid formation by chlorobutanol is not impaired in the alloxan-diabetic rat. The relevance of these findings to the effect of drugs on ascorbic acid synthesis is not clear.

Many reports have appeared in the literature showing that a variety of drugs depress the level of ascorbic acid in the adrenal glands. However, there appears to be no obvious relationship between this particular drug effect and the effect of drugs on ascorbic acid synthesis. Administration of barbital or chlorobutanol to adrenalectomized rats produces about the same increase in ascorbic acid excretion as that observed in normal rats (124). Possible hormonal control over this phenomenon is suggested from results showing that the effect of chlorobutanol and barbital on ascorbic acid excretion is less in hypophysectomized rats than in normal rats (124).

Whatever mechanism is proposed for the stimulatory effect of drugs on ascorbic acid synthesis must take into consideration that the amount of glucose metabolized through the glucuronic acid pathway is greater than the amount of drug administered; in fact the amounts of free D-glucuronic, L-gulonic, and L-ascorbic acids excreted in urine of drug-treated rats are minimum values for the total amount of these acids formed since they are in turn extensively metabolized (116, 117, 132).

The enhanced formation of ascorbic acid through the glucuronic acid pathway may represent an adaptive response of the body to foreign compounds. Reports have recently appeared (133) indicating that the administration of D-glucuronolactone reduces the toxicity of various foreign compounds in animals by an unknown mechanism. It is of considerable interest that those drugs which are potent in stimulating the synthesis of ascorbic acid, such as phenobarbital, barbital, aminopyrine, and the previously men-

tioned carcinogenic hydrocarbons, have been shown by Conney & Burns (134) and Conney *et al.* (135) to increase the activity of liver microsomal enzymes which metabolize various foreign compounds. This observation suggests a relationship between the stimulatory effect of drugs on ascorbic acid synthesis and on their ability to increase the activity of drug metabolizing enzymes in liver microsomes.

Ascorbic acid deficiency may also influence the pharmacological effect of drugs. For example, ascorbic acid-deficient guinea pigs are more sensitive to the action of theophylline (136), ether (137), pentobarbital (138), procaine (139), and zoxazolamine (119). It is possible that ascorbic acid may exert this effect, at least in part, by influencing drug metabolism. Udenfriend *et al.* (140), Brodie *et al.* (141), and Dalgliesch (142) have shown that a model system consisting of ascorbic acid, ferrous ion, ethylenediaminetetraacetic acid, and oxygen can catalyze the hydroxylation of acetanilide, antipyrine, aniline, anthranilic acid, and kynurenine to yield products identical with those formed in the body. Axelrod *et al.* (136) further reported that the rate of hydroxylation of several of these compounds is markedly reduced in guinea pigs depleted of ascorbic acid and that on repletion of the animals with ascorbic acid the rate of hydroxylation is restored to normal. Although these results suggest a role for the vitamin in the metabolism of drugs, attempts to define such a function at the enzyme level have not been successful. Conney *et al.* (119) recently reviewed the various metabolic interactions between ascorbic acid and drugs.

Stimulatory Effect of Drugs on Drug-Metabolizing Enzymes

Many drugs are metabolized by enzymes in liver microsomes which require reduced triphosphopyridine nucleotide and oxygen. These enzyme systems have been reviewed recently by Brodie *et al.* (143) and by Axelrod (144). Several laboratories reported that the administration of various foreign compounds markedly increases the activity of these drug-metabolizing enzymes in liver microsomes. Conney *et al.* (145, 146) observed that pretreatment of rats with polycyclic hydrocarbons such as 3,4-benzpyrene, 3-methylcholanthrene, and 1,2,5,6-dibenzanthracene greatly increases the activity of enzyme systems in liver microsomes which N-demethylate 3-methyl-4-monomethylaminoazobenzene, hydroxylate 3,4-benzpyrene, and reduce the azo linkage of 4-dimethylaminoazobenzene. Subsequent studies by Conney and co-workers (134, 135, 147, 148) showed that pretreatment of rats with a variety of compounds such as phenobarbital, barbital, aminopyrine, phenylbutazone, orphenadrine, chlorcyclizine, and 3,4-benzpyrene causes a marked increase in the activity of the enzymes in liver microsomes which metabolize hexobarbital, pentobarbital, aminopyrine, phenylbutazone, 3-methyl-4-monomethylaminoazobenzene, 3,4-benzpyrene, and zoxazolamine. The ability of phenobarbital to stimulate the activity of liver microsomes

to oxidize hexobarbital and to N-demethylate monomethylaminopyrine was observed by Remmer (149, 150) in rats, mice, and rabbits. Inscoe & Axelrod (151) reported that the activity of liver microsomes to form *o*-aminophenol glucuronide is increased by administration of 3,4-benzpyrene and 3-methylcholanthrene. Cramer *et al.* (152) showed that the administration of 3-methylcholanthrene causes a marked increase in the ability of liver microsomes to hydroxylate the carcinogen 2-acetylaminofluorene.

Administration of certain drugs can actually enhance the ability of liver microsomes to metabolize the same or a closely related compound (135). Thus, the administration of phenylbutazone, aminopyrine, 3,4-benzpyrene, or phenobarbital will increase, respectively, the ability of rat liver microsomes to metabolize phenylbutazone, aminopyrine, 3,4-benzpyrene, or hexobarbital.

Pretreatment of rats with certain drugs can appreciably influence the pharmacological activity of other drugs as shown by the following examples:

(*a*) Pretreatment of rats with drugs that stimulate the zoxazolamine-metabolizing enzyme system shortens the duration of action of zoxazolamine (135, 153). Since this drug is hydroxylated to a pharmacologically inactive metabolite (154), any increase in the activity of the zoxazolamine-metabolizing enzyme system in liver would be expected to shorten the duration of a paralytic dose of zoxazolamine. Indeed, this was found to be the case. Pretreatment of rats with phenylbutazone, aminopyrine, barbital, orphenedrine, phenobarbital, or 3,4-benzpyrene, all of which increase the activity of the zoxazolamine-metabolizing system in the liver microsomes, causes a concomitant decrease in the duration of zoxazolamine paralysis in the intact animal. For instance, the duration of zoxazolamine paralysis in control rats averages 730 minutes, in phenobarbital-treated rats 102 minutes, and in 3,4-benzpyrene-treated rats only 17 minutes.

(*b*) Pretreatment of rats with phenobarbital, phenylbutazone, chlorcyclizine, or orphenadrine noticeably increases the activity of the liver microsomes to metabolize hexobarbital, and this effect is paralleled by a shortened duration of action of hexobarbital (135). For example, control rats given hexobarbital sleep for an average of 216 minutes, while phenobarbital pretreated rats given hexobarbital sleep for an average of 11 minutes. The ability of phenobarbital to stimulate markedly the metabolism of hexobarbital has led Remmer (149, 150) and Conney and co-workers (135) to suggest that tolerance to barbiturates may result, at least in part, from an accelerated rate of metabolism of the barbiturate to pharmacologically inactive metabolites. Although pretreatment of rats with chlorcyclizine, orphenadrine, or phenobarbital shortens the duration of action of hexobarbital by accelerating its metabolism, pretreatment of rats with these drugs does not shorten the duration of action of barbital, a drug which is not metabolized in the rat (148). It should be noted that the stimulatory effect of drugs

upon drug metabolism furnishes an explanation for the recent findings of Thompson *et al.* (155) that rats fed on a diet containing chlorcyclizine develop resistance to the hypnotic action of pentobarbital.

(*c*) Murphy & DuBois (156) reported that the administration of 3-methylcholanthrene, 3,4-benzpyrene, or 1,2,5,6-dibenzanthracene increases markedly the activity of the enzyme system in liver microsomes which catalyzes the conversion of the dimethoxy ester of benzotriazine dithiophosphoric acid (guthion, BDB) and ethyl *p*-nitrophenylthiobenzenephosphonate (EPN) to their corresponding oxygen analogues which are active anticholinesterase agents. In contrast to the above cited examples where the hydrocarbons increase the conversion of drugs to inactive metabolites, the hydrocarbons in this case increase the conversion of the organic thiophosphates to active metabolites. For example, the inhibition of cholinesterase caused by the intraperitoneal administration of 2 mg./kg. of guthion was increased by approximately 50 per cent 48 hours after the injection of 3-methylcholanthrene.

Several lines of evidence (135, 145, 146) suggest that compounds such as 3,4-benzpyrene and phenobarbital increase the activities of drug-metabolizing enzymes by inducing enzyme synthesis. Administration to rats of the amino acid antagonist, DL-ethionine, completely prevents the phenobarbital- or hydrocarbon-induced increases in the activity of the azo dye demethylase or zoxazolamine metabolizing system, whereas the administration of DL-methionine nullifies the inhibitory action of DL-ethionine. Administration of phenobarbital, 3-methylcholanthrene, and 3,4-benzpyrene also stimulates liver growth. Livers from drug-treated rats are usually 20 to 30 per cent heavier than the livers from controls, and the amount of microsomal or total liver protein is increased proportionately.

Administration of drugs which stimulate the drug-metabolizing enzymes in liver microsomes can also exert other biochemical responses in animals. Pretreatment of rats with chlorcyclizine or phenobarbital markedly stimulates the activity of the liver-microsome system that oxidizes reduced triphosphopyridine nucleotide (148). Methylcholanthrene-treated rats were reported by Glenn *et al.* (157) to have increased hepatic activity for the metabolism of hydrocortisone. Jondorf *et al.* (158) found that liver tryptophan peroxidase activity was markedly increased and that serum alkaline phosphatase was substantially decreased following administration of 3-methylcholanthrene. Conney & Burns (134) and Burns *et al.* (114) reported that those drugs which stimulate the activity of drug-metabolizing enzymes in rats also stimulate ascorbic acid synthesis.

Effect of Drugs on Cholesterol Metabolism

During the past several years considerable efforts have been made to find compounds that inhibit cholesterol synthesis. These studies have been motivated by the hypothesis that hypercholesterolemia is one of the predis-

posing factors in the genesis of atherosclerosis. One such compound is triparanol (MER-29) which has been introduced recently into clinical practice. Blohm & McKenzie (159) were the first to report on the striking inhibition of cholesterol synthesis brought about by the administration of triparanol to rats. They found that the drug reduces the incorporation of acetate-1-C^{14} into cholesterol of liver and intestine of intact rats. In a subsequent study, Blohm *et al.* (160) showed that triparanol decreases the levels of cholesterol in the following tissues and fluids of the rat: blood plasma, erythrocytes, liver, adrenal, lung, aorta, skeletal muscle, and bile. In a recent conference triparanol was reported (161) to lower plasma cholesterol in a majority of patients.

Avigan *et al.* (162) have presented evidence for the mechanism of action of triparanol. They showed that the major block in cholesterol synthesis caused by the drug is at the last step—the conversion of 24-dehydrocholesterol (desmosterol) to cholesterol. The accumulation of significant amounts of the precursor sterol not only in liver but also in the serum of drug-treated animals and patients has been reported by Steinberg & Avigan (163) and by Blohm *et al.* (164).

Other compounds have also been studied for their ability to lower cholesterol levels: phenylethyl acetate (163, 165), Δ^4-cholestenone (163), sodium dextro-thyroxine (166), 3,5-diiodothyroacetic acid and 3,5-diiodothyroformic acid (167), and benzmalacene (168). These compounds appear to have modes of action different from that of triparanol, and their clinical value remains to be established.

Effect of Drugs on Uric Acid Biosynthesis and Excretion

Gout is a disease characterized by overproduction of uric acid leading to deposits of this substance in various tissues, especially in the joints. Drugs such as probenecid, certain phenylbutazone analogues, and zoxazolamine which increase the urinary excretion of uric acid are of considerable value in the treatment of gout. Although it is fairly well established that these drugs act by blocking the reabsorption of uric acid through the renal tubular cells, the structural features required in a drug for this activity are still not clear. Burns *et al.* (169) have reported a relationship between the chemical structure of phenylbutazone and its uricosuric activity. They found that modifications in phenylbutazone which result in increased acidity of the molecule are associated with enhanced uricosuric activity, whereas changes which decrease the acidity of the drug are accompanied by loss of this activity. The potent uricosuric activity of the highly acidic phenylbutazone analogues (pK_a 2.0 to 3.0) indicates that these drugs act in the ionic form to block the reabsorption of uric acid by the renal tubular cells, thus furnishing a possible clue to the still obscure nature of the transport mechanisms involved. The demonstration that uricosuric activity in the phenylbutazone series is related to the pK_a has proved of considerable aid

in searching for new uricosuric drugs. One such drug, sulfinpyrazone, has been found to be useful clinically as a potent uricosuric agent in the treatment of gout, and others are now under study (170).

Another approach to the treatment of gout is the use of drugs which would suppress the synthesis of uric acid. Grayzel *et al.* (171) have reported recently that 6-diazo-5-oxo-L-norleucine inhibits significantly the synthesis of uric acid in gouty patients. This drug, a structural analogue of glutamine, presumably prevents the synthesis of inosinic acid by blocking the utilization of glutamine in the enzymatic conversion of α-N-formylglycinamide ribotide to formylglycinamidine ribotide. However, the high incidence of side effects following administration of the compound prevents its practical use in the treatment of gout. Studies with this drug, nevertheless, represent a new approach to gout therapy; perhaps, safer pharmacological agents of this type will be forthcoming. Interestingly enough, Seegmiller *et al.* (172) have recently reported that 2-ethylamino-1,3,4-thiadiazole actually stimulates the synthesis of uric acid. It is possible that further studies with this compound will be of value in determining the factors which normally operate to control purine biosynthesis in man.

Primaquine-Induced Hemolytic Anemia

Administration of the antimalarial drug, primaquine, and certain other 8-aminoquinoline derivatives induces an acute hemolytic anemia in some individuals (173, 174). The incidence of this primaquine sensitivity is much greater in Negroes than in Caucasians, and the condition is apparently genetically determined (175). A similar hemolytic crisis can be induced by ingestion of fava beans, naphthalene, acetanilide, phenylhydrazine, and nitrofurantoin.

Studies by Carson *et al.* (176) indicate that primaquine-induced hemolytic anemia results from a biochemical defect which is characterized by markedly reduced activity of glucose-6-phosphate dehydrogenase in red blood cells. This enzyme requires triphosphopyridine nucleotide as a co-factor and catalyzes the first step in the metabolism of glucose through the hexose monophosphate shunt. An alteration in glutathione metabolism also occurs in the sensitized erythrocytes; this presumably results from the impaired reduction of triphosphopyridine nucleotide caused by the defect in glucose-6-phosphate dehydrogenase. Certain of the reactions of erythrocyte metabolism which are affected, either directly or indirectly, by the biochemical abnormalities in red cells of sensitive individuals are illustrated in Figure 8. Alterations in glutathione metabolism and deficiencies in glucose-6-phosphate dehydrogenase have also been reported (174) in erythrocytes of subjects sensitive to the fava bean and naphthalene.

Primaquine-sensitive hemolytic anemia represents an excellent example of a drug toxicity that can be attributed to a defect in an enzyme system.

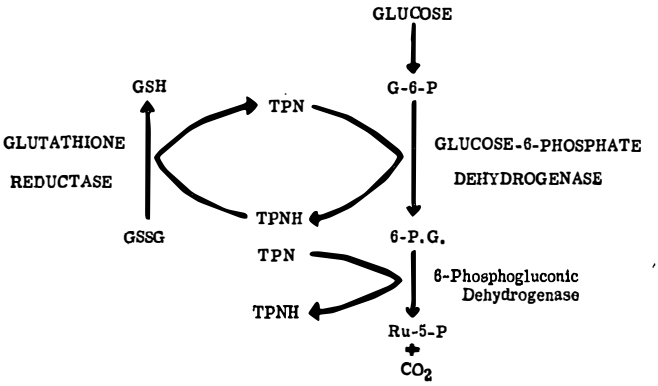


FIG. 8. Schematic representation of certain reactions of erythrocyte metabolism which are affected by the defect present in red cells of subjects with primaquine-sensitive hemolytic anemia. Abbreviations employed: G-6-P, glucose-6-phosphate; 6-P.G., 6-phosphogluconic acid; Ru-5-P, ribulose-5-phosphate; TPN, triphosphopyridine nucleotide; TPNH, reduced triophosphopyridine nucleotide; GSSH, oxidized glutathione; GSH, reduced glutathione. This scheme is taken from the review of Marks & Gross (174).

Furthermore, the importance of genetic control in a drug sensitivity is well illustrated by studies on this condition. The possibility that other drug sensitivities may also result from genetically transmitted enzyme deficiencies might be fruitful to explore.

CONCLUDING REMARKS

As evidenced by even the limited number of topics reviewed in this chapter, studies of the biochemical effects induced by drugs can contribute greatly to a better understanding of the possible mechanisms of action of the drugs. More important, perhaps, is the insight that a knowledge of drug effects can give us into normal metabolic processes. This extra dividend is perhaps the most significant factor in making the relatively new field of biochemical pharmacology increasingly important.

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