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Metabolic Fate of Hydrazines and Hydrazides

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Keyphrases ☐ Hydrazines, hydrazides—metabolic fate ☐ *N*-Acetylation—hydrazino compounds detoxification ☐ C—N bond cleavage—hydrazino compounds detoxification ☐ Isonicotinic acid hydrazide—metabolism ☐ Pharmacogenetics—hydrazide metabolism

Although hydrazine was synthesized in 1887, surprisingly little has been learned of its metabolic fate until relatively recently. Pharmacological and biological effects of high level hydrazine administration have been described, such as hypoglycemia (1–3), increased ammonia levels in the blood and spinal fluid (4), decreased hepatic lipid mobilization (5, 6), blood dyscrasias, and convulsions. An extensive discussion of the pharmacology of these compounds is beyond the scope of this paper and will not be reviewed here.

Hydrazine and its methyl derivatives have received considerable interest recently due to their extensive use as high-energy fuels. The fate of hydrazine is important because some derivatives may yield hydrazine by *in vivo* hydrolysis:

$$R{-}CONHNH_2 + H_2O \rightarrow R{-}COOH + H_2NNH_2 \quad \text{(Eq. 1)}$$

The probable cleavage of hydrazine from unsymmetrically substituted hydrazides was demonstrated by the presence of hydrazine in the urine of rabbits after intraperitoneal injection of glutamylhydrazine (I)[1] (7) and after the oral administration of benzhydrazide, nicotinoyl and isonicotinoyl hydrazides, and certain ring-substituted benzhydrazides (8).

Hydrazine is extensively metabolized by few animals, and appears rapidly in the urine of animals such as the dog and rabbit. McKennis *et al.* (9) have suggested that in all animals studied, except the dog which has limited capabilities to acetylate hydrazines, hydrazine is metabolized to acetylhydrazine and so rapidly converted to the diacetyl derivative that the monoacetylhydrazine intermediate cannot be detected in the urine (9–11). On the other hand, diacetylhydrazine is excreted unchanged.

From the observation that blood ammonia levels of the dog were elevated by the administration of hydrazine, (4) whereas diacetylhydrazine had no effect (11), it would appear that some hydrazine was also degraded to ammonia *in vivo*.

Alkyl-Substituted Derivatives of Hydrazine—The methyl-substituted derivatives of hydrazine are metabolized quite rapidly. In four species studied (mouse, rat, monkey, and dog) all excreted 25–40% of the administered dose of ^{14}C-monomethylhydrazine (MMH-^{14}C) (II) in 24 hr. except the dog which excretes only one-half as much in the first 2 hr. (12). At all experimental periods reported, about 50% of the excreted activity was in the form of the unchanged compound. The activity which remained in the body was localized primarily in the liver, but significant quantities were also found in the kidney, bladder, pancreas, and blood serum.

The initial metabolic reaction involving methyl-substituted hydrazines is apparently demethylation fol-

[1] Roman numerals in parentheses refer to structural formulas in *Appendix A*.

lowed by oxidation, since large proportions of the activity from both unsymmetrical dimethylhydrazine (UDMH) (III) and monomethylhydrazine (MMH) labeled with ^{14}C were expired in 10–24 hr. as one-carbon fragments. Approximately 30% of a subacute dose of UDMH-^{14}C injected intraperitoneally into rats was recovered as respiratory $^{14}CO_2$ in the first 10 hr. The detoxication system can apparently be saturated since convulsive doses of UDMH-^{14}C resulted in significantly lower respiratory $^{14}CO_2$ (13).

The metabolism of MMH-^{14}C presented a slightly different picture. Approximately 45% of the administered dose was expired in 24 hr. and most of the remaining activity was excreted *via* the urine. Int his case only 20–25% of the dose is represented by $^{14}CO_2$, and, unlike UDMH, the remainder is $^{14}CH_4$. Within the first 3 hr. the output of $^{14}CH_4$ is tenfold that of $^{14}CO_2$, but sustained $^{14}CO_2$ production resulted in ratios of 3:4 at later experimental periods. The differences in the expiratory patterns of the two gases was interpreted by Dost *et al.* (13) to mean that $^{14}CH_4$ and $^{14}CO_2$ were produced by independent mechanisms, but the possibility of direct conversion of CH_4 to CO_2 was not completely eliminated.

Neuman and Nadeau (14) demonstrated that in a purely chemical system a dilute aqueous solution of MMH yields methane, nitrogen, and a small quantity of carbon monoxide when allowed to come in contact with a dilute solution of sodium hypochlorite. Phenylhydrazine (IV) can be degraded *in vitro* by oxyhemoglobin to benzene and molecular nitrogen in the presence of oxygen (15). Also, using a system resembling biological conditions, Eberson and Persson (16) demonstrated the cupric ion catalyzed breakdown of a model compound, β-phenylisopropylhydrazine (V), into isopropylbenzene, phenylacetone, 2-phenyl-2-propanol, and propenylbenzene. Taken collectively, these data would indicate that monoalkyl- and arylhydrazines are attacked by mild oxidizing systems, presumably through free radical formation. If in fact, free radicals are formed in the demethylation of MMH, then CH_4 would be the expected metabolic product (13).

Data would indicate that a substantial portion of the methyl carbon of 1-methyl-2-*p*-(isopropylcarbamyl)benzene hydrazide (MIH) (VI), a cytostatic methyl derivative, is also converted to methane. MIH is very rapidly degraded *in vivo* and has a serum half-life of less than 15 min. in man, dog, and rat (17). Within 8 hr. after administration, 7–10% of the dose was expired as $^{14}CH_4$ and 11–22% was expired as $^{14}CO_2$ (18). The demethylation reaction occurred primarily in the liver and could be enhanced by pretreatment with phenobarbital suggesting that hepatic microsomal enzymes were responsible (19).

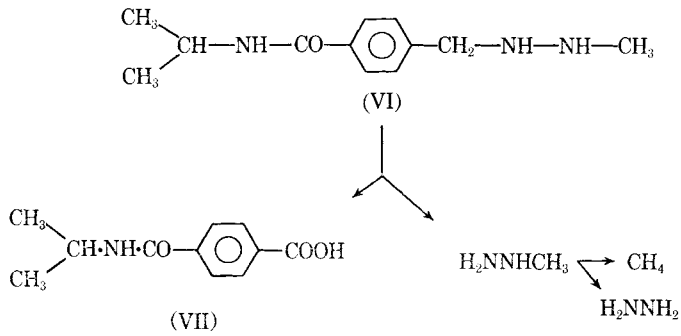
By comparison of the rates of CO_2 formation from MIH and MMH, Dost and Reed (17) suggested that MMH may be an intermediate in the metabolism of MIH based on data supporting the following scheme:

$$MIH\text{-}{}^{14}CH_3 \longrightarrow H_2NNH\text{-}{}^{14}CH_3 \longrightarrow {}^{14}CH_4 \longrightarrow {}^{14}CO_2 \quad \text{(Eq. 2)}$$

The hypothesis of Dost and Reed has been greatly strengthened by the demonstration of *N*-isopropylterephthalmic acid (VII) (18–20) as a metabolite of MIH. *N*-Isopropylterephthalamic acid is formed quite rapidly from MIH and can be demonstrated in the urine 10 min. after intravenous injection of the parent compound and accounts for 24–25% of the administered dose in 24-hr. urine collections from both man and rodents, whereas the parent compound could not be detected in urine (18, 20).

The metabolic scheme of Eq. 3 can now be expanded to include the fate of the aromatic ring.

The methyl carbons of 4-chloro-3-sulfamoylbenzoic acid 2,2-dimethylhydrazide (VIII) have also been shown to be metabolized to ethanolamine-trapable components of expired air, presumably CO_2, to the extent of 3–5% of the administered dose (21). Since traces of UDMH and MMH were identified in the urine of dogs, rats, monkeys, and man, the formation of CO_2 is probably by a route similar to that shown in Eqs. 4 and 5.



(Eq. 3)

$$H_2NNHCH_3 \rightarrow CH_4 \rightarrow CO_2 \quad \text{(Eq. 4)}$$

$$H_2NNH_2 \rightarrow CH_3CONHNHCOCH_3 \quad \text{(Eq. 5)}$$

With symmetrically substituted hydrazines, *i.e.*, hydrazo compounds, a postulated reaction is the cleavage between the two nitrogen atoms to yield two amines (22) by a reaction similar to that shown in Eq. 6.

$$R\text{—}NH\text{—}NH\text{—}R' \longrightarrow R\text{—}NH_2 + H_2N\text{—}R' \quad \text{(Eq. 6)}$$

However, little experimental evidence has been made available to support this hypothesis other than the report by Schwartz (23), who reported rats given methylamine, excreted it unchanged; but when MMH or MIH was administered, methylamine was excreted in addition to the parent compounds. Formation of methylamine from MMH and MIH, therefore, demonstrates that reductive cleavage of N—N bonds of alkyl- or arylhydrazines can occur *in vivo* although it has not yet been demonstrated with the simplest of arylhydrazines, phenylhydrazine (IV).

Phenylhydrazine is excreted slowly by rabbits, requiring approximately 4 days for 60% excretion. Small amounts of the hydrazine moiety were liberated but 17% of the parent compound was hydroxylated and conjugated by glucuronidation. Approximately 20% was excreted as hydrazones of pyruvate and α-ketoglutarate, but acetylation was not shown to occur. Two derivatives of phenylhydrazine, *o*-hydrazino-*p*-hydroxybenzoic acid (IX) and *p*-hydrazinosalicylic acid (X), have been reported to be excreted partly unchanged (22).

Isoniazid and its Derivatives—The discovery in 1952, that isoniazid (isonicotinic acid hydrazide, INH) (XI) was a highly efficacious chemotherapeutic agent for tuberculosis, stimulated extensive research on the carbonyl derivatives of hydrazine. Of the aromatic hydrazides, INH has received the most intensive metabolic investigation and, since its metabolism demonstrates many of the detoxication mechanisms of hydrazides, the metabolism of INH will be discussed at length here.

INH is quite rapidly absorbed and single oral doses of INH to dogs typically result in peak plasma levels within 30 min; it disappears at approximately 19%/hr. (24). The isopropyl derivative of INH, iproniazid (XII), also quickly reaches peak levels in the plasma, but unlike INH it persists in the plasma at measurable levels after 24 hr. (25). Distribution of INH and iproniazid parallel plasma levels as indicated by liver concentrations which reach a maximum within 30 min. after an oral dose (26).

In 24-hr. urine, 44–86% of the dose of INH could be accounted for as the parent compound plus a major metabolite, isonicotinic acid (INA) (XIII) (25). Most of the activity was in the form of INH from 0–24 hr., but from 25–48 hr. most of the activity was in the form of INA (27). Iproniazid on the other hand was excreted less rapidly in humans during the first few hours following administration. However, after 48 hr. about 65% of the dose from either drug could be found in the urine as unchanged drug. The excretion of INA, was about the same for both drugs 8 hr. after administration, but at 48 hr. the urinary output of INA from iproniazid was about 50% higher than from INH indicating a slightly longer retention of intact iproniazid in the body (28).

INH can be detoxicated by two primary routes of metabolism—hydrolysis and direct conjugation. In man, the major mode of detoxication is direct conjugation, but the extremely labile C—N bond between the aromatic carbonyl and the hydrazine moieties results in a highly important metabolic degradation route by hydrolysis (Eq. 1). In animals, such as the dog, which are apparently deficient in the ability to acetylate free amines, the hydrolysis to free acid plus hydrazine or its corresponding substituted derivative becomes a major pathway in the metabolism of INH as well as *N*-acetyl-INH (8, 27). Benzohydrazide, ring substituted derivatives of benzohydrazide, and to some extent salicyclohydrazide are also hydrolyzed (29, 30). The cleavage of the hydrazide bond would be by a hydrolase, whereas, the rapid breakdown of the resulting hydrazine to ammonia would be catalyzed by an oxidase (31, 32). These two enzymes could account for the increase in blood ammonia levels in nonacetylating animals that have been observed after both hydrazine and INH administration (4, 8).

INA may be excreted as the free acid or it may be conjugated with natural products before excretion. In the rabbit, conjugation with glucuronic acid occurred to a limited degree (33), whereas, in other mammals such as man and rat, INA was excreted primarily as the glycine conjugate—isonicotinoyl-glycine (INA-G) (XIV) (34–37). The simplest aromatic hydrazide, benzohydrazide; was also hydrolyzed, conjugated with glycine, and excreted as hippuric acid (XV) by the rabbit (30). The general reaction for glycine conjugation may be visualized by the following equation:

$$\text{aryl—COOH} + \text{H}_2\text{N—CH}_2\text{—COOH} \longrightarrow \text{aryl—CO—NH—CH}_2\text{—CCOH} \quad \text{(Eq. 7)}$$

In addition to conjugation of hydrazine and hydrazides with acetate, numerous excretion studies in animals and man have revealed that a secondary route of metabolism of hydrazides is their condensation with endogenous carbonyls, such as α-ketoglutarate (α-KGA) and pyruvate, to form hydrazones. The presence of compounds resembling hydrazones in the urine of rats, humans, and monkeys receiving INH orally, was reported (38, 39). The two primary hydrazones were identified by paper chromatography as pyruvic acid isonicotinoylhydrazone (INH-PA, XVI) and α-ketoglutaric acid isonicotinoylhydrazone (INH-KA, XVII) (35, 37). The discovery of hydrazones of INH with pyruvate and α-ketoglutarate has thus implicated the possibility of their production from any substituted hydrazide which would yield free hydrazide during its metabolic transformations. The formation of hydrazones of INH with pyruvate exceeds that with α-KGA by two to three times.

The isonicotinoyl hydrazones which have been reported qualitatively in urine have also been reported to possess *in vitro* antimicrobial activity (40, 41). Since these activities are equivalent to INH on a molar basis it appears that these highly labile derivatives are not active *per se* but liberate INH quantitatively during the prolonged periods of incubation employed during *in vitro* tests for antimicrobial activity. Schmidt and Hughes (42) using INH-PA and INH-KA and Nielsch and Giefer (43) employing INH-KA have suggested that orally administered hydrazones of INH are promptly converted to INH in the gastrointestinal tract since the patterns of urinary metabolites found after hydrazone administration were essentially identical to those observed following oral administration of INH. Highly sensitive radiotracer techniques have not disclosed the presence of these hydrazones in the blood of rats receiving ^{14}C-labeled INH (44), but at convulsant doses of INH to rabbits INH-PA could be detected in the blood (45). Increases in the amounts of total isoniazid containing derivatives in the urine of dogs after intravenous administration of α-keto acid hydrazones suggested that these derivatives were quite stable since approximately 50% of the administered doses was excreted unchanged (46).

The pattern of urinary metabolites following the administration of glucose hydrazones of INH sug-

gested an even greater *in vivo* stability of this derivative since nearly 80% was excreted as the unchanged drug (46).

The lowered excretions of isonicotinic acid derivatives following the administration of INH hydrazones compared to INH injection suggested the *in vivo* conversion of the hydrazone derivatives to INH may be prerequisite to the formation of isonicotinic acid from INH hydrazones.

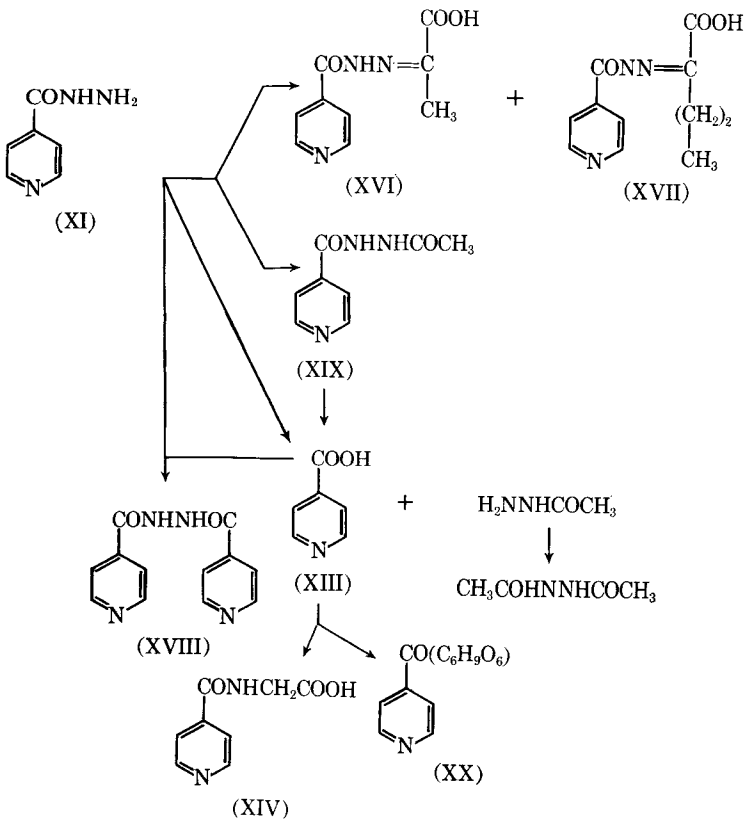
The formation of amides from hydrazides has not been demonstrated (27, 32). Likewise the *N'*-methylation, which has been shown to be an important metabolic route of nicotinic acid, has not been demonstrated with INH (27). A somewhat unusual product, which represented only 1–4% of the total excretion, has been isolated from the urine of mice and guinea pigs given ^{14}C-INH intraperitoneally. This compound was identified as DiINH (XVIII) formed apparently from the condensation of INH with INA and is believed to be formed nonenzymatically in the urine since DiINH has been found as an end product of *in vitro* reactions in the presence of hemin and phosphate or tris buffers (47, 48).

The distribution of ^{14}C activity among metabolites resulting from INH administration is exemplified by the results found in normal mice where 26% of the dose was excreted as unchanged drug and 55% as INA. The remaining 19% of the activity was found primarily as the acetyl-INH (XIX) and secondarily as the INH hydrazones and INA derivatives. Very slight amounts of INH are oxidized completely to CO_2. If one considers that the average 20-g. mouse expires 0.05 mole CO_2/24 hr., then less than 1% of the injected dose was expired through the lungs by normal mice (26, 49). The complete metabolism of isonicotinic hydrazide may be summarized in Scheme I.

Quantitatively, acetylation of INH to N^1-acetyl-N^2-isonicotinyl-hydrazine (XIX, AcINH) is one of the most important metabolic alterations of the drug in primates and results in complete loss of antimycobacterial activity. AcINH appeared as the major metabolite of INH in both man and monkey. Of the total metabolite excreted AcINH represented 91% in the former case and 50–100% in the latter (40, 50–52).

When the metabolite AcINH was administered to adult male humans or rabbits, most of the dose was excreted as 1,2-diacetylhydrazine in 48 hr. In contrast, the dog excreted 48% of the dose as monoacetylhydrazine within 35 hrs., but no INH was excreted (53, 54). The observation in dogs and rabbits that INA but not INH is excreted following administration of AcINH fully supports the suggestion that acetylhydrazine is an intermediate in INH metabloism and is consistent with the report by Wenzel (55) that AcINH was cleaved in serum to INA but not INH.

Hughes (50) has stated that acetylation of isoniazid is indeed a significant reaction both toxicologically and chemotherapeutically. Variations in completeness of acetylation by different subjects and species may well account for the differences observed in the toxicity of INH. Moreover, if the dog is unable to acetylate INH, this circumstance may well account for the fact that INH is more toxic for this species than for man or monkey. This hypothesis is supported by the fact that when



Scheme I

INH was administered to dogs in equimolar amounts to the tolerated level of AcINH, convulsions resulted (53).

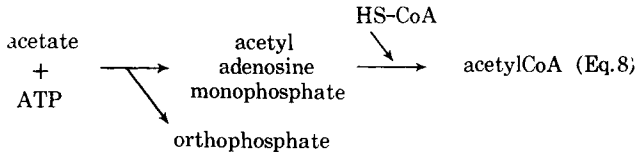
The metabolism of hydrazine, acetylhydrazines, and INH to 1,2-diacetylhydrazine provides an example of detoxication mechanisms in a literal sense. Monoacetylhydrazines appear consistently to have toxic properties (7, 29, 30) which may approach that of hydrazine (6), whereas 1,2-diacetylhydrazine produces fewer or diminished toxic reactions. The LD_{50} for acetylhydrazine given intraperitoneally to mice was found to be 153 ± 3.4 mg./kg. (58) and the intravenous LD_{50} was 175 mg./kg. (57) and more than 3000 mg./kg. for 1,2-diacetylhydrazine. The maximum acceptable daily dose of INH in mice is 64 mg./kg. (58) and the corresponding value for AcINH, the primary metabolite of INH in man, monkey, and rat (35, 50) is 1000 mg./kg., again indicating a reduction in toxicity following acetylation.

Pharmacogenetics in Hydrazide Metabolism—For many years, researchers worked under the assumption that the enzymes responsible for the deactivation of foreign compounds were uniform. However, as soon as one hereditary variant enzyme system was demonstrated for pseudocholinesterase (silent gene) (59), a number of further examples of hereditary variation of drug metabolism became evident, and the variation among individuals and among species in their response to drugs began to fit into a logical pattern. Shortly after the introduction of INH as a chemotherapeutic treatment for tuberculosis, individual differences in the conversion of the drug to inactive metabolites became evident (60). Subsequent studies showed these differences to be reproducible, stable, individual manifestation of INH metabolism whether the drug determination was made in the urine or plasma (7), and regardless of administration route (61). Broader investigations of large populations of tuberculosis patients, healthy subjects, specific families, and identical and fraternal twins have clearly established that the inactivation capacities are under genetic control (62, 63) and are due to the inherent capacities of subjects to acetylate the drug to the metabolite AcINH (36, 64, 65).

When not confounded by impaired intestinal absorption, the separation of two INH inactivator phenotypes in human populations can be clearly differentiated into a bimodal distribution. The terms rapid and slow inactivators have been used to designate the individual capabilities in degrading INH and the phenomenon becomes readily apparent even on a small number of subjects (36).

The trait of slow acetylation of INH has been shown in man to be an autosomal recessive trait, and remarkably good agreement between observed and predicted results in each category of rapid or slow inactivator status was demonstrated by Evans *et al.* (66) in confirming the recessive trait hypothesis.

A direct correlation between an individual's ability to acetylate INH and his ability to *N*-acetylate sulfamethazine (a sulfonamide), hydralazine (XXI) and phenelzine (XXII) has been demonstrated (64, 67). Evans and White demonstrated that the biochemical basis for the human polymorphism for isoniazid resides in the enzymatic step occurring in the liver which tranfers acetyl groups from the acetylCoA to the receptor drug molecule (64). The formation of acetylCoA from acetate and ATP has been shown to be two enzymatic steps (68):



When the acetate, ATP and CoA were replaced in human liver homogenates by acetylCoA, the bimodal pattern of sulfamethazine acetylation became more distinct (64). Since the transfer of the acetyl group from acetylCoA to the receptor drug is thought to be a single enzymatic step (69), it was concluded that the observed polymorphism resides in this step.

Either the existence of two hepatic acetyltransferases with different reaction rates or a difference in enzyme concentration could account for the differences observed between acetylator phenotypes. The similarities of a number of properties (Michaelis constants, heat inactivation, and substrate specificities) of semipurified enzymes obtained from rapid or slow inactivators would suggest that the amount of enzyme, rather than specific activity of the enzyme, is responsible for the differences (70).

Although humans readily acetylate sulfanilamide *in vivo* (71), human liver homogenates are unable to acetylate the drug (62). In the same system, the acetylation of *p*-aminobenzoic acid (PABA) by acetylCoA did not occur, even though the acetylation of PABA by human red cell homogenates has been shown (72) and acetylated PABA was observed in human abdominal veins (73). The acetylation of PABA as well as that of *p*-aminosalicylic acid (PAS) by human red cell homogenates was found to be unimodal (72) and the serum fall-off of PAS concentration following intravenous injection was found to occur at the same rate in both rapid and slow inactivators of INH (74). Therefore, it seems probable that while INH and related compounds are inactivated by acetylation in the liver, the drugs which are acetylated by monomorphic mechanisms are acetylated by extrahepatic systems.

To clarify the question of which reaction, acetylation or hydrazone formation, is the primary mode of deactivation of INH and which reaction determines inactivator status in human subjects, Peters *et al.* (36) studied the deactivation of INH in a group of healthy volunteers. After separation into inactivator phenotypes, the amounts of each type of metabolite of INH were determined. Peters *et al.* (36) found the amounts of INH hydrazones (INH-PA and INH-KA) seemed to parallel approximately the amounts of INH excreted, suggesting the extent of hydrazone formation was dependent upon the amounts of unchanged INH available for conjugation. AcINH and derivatives of INA, on the other hand, were excreted in amounts inversely proportional to the amount of INH present, thereby indicating that when AcINH was formed as a metabolite it was probably excreted in this form without undergoing further metabolism.

When the same group of volunteers was given an equimolar dose of AcINH, only small amounts of the hydrazone derivatives were excreted which would confirm

the hypothesis that hydrazone formation is dependent upon the presence of INH and is not a metabolic end-product of AcINH.

The rapid inactivators formed and excreted INA-derivatives equally as fast from AcINH as from INH, but the slow inactivators could excrete INA-derivatives in quantities equimolar to that of AcINH only when AcINH was administered. Therefore, since the differences in output of AcINH and INA-derivatives could not be accounted for by differences in abilities to excrete AcINH or form INA-derivatives, it was concluded that the primary alteration determining inactivator status must lie in the acetylation of INH.

Although it was concluded that the rate-limiting reaction in the formation of INA-derivatives was the acetylation of INH, only insignificant amounts of acetylhydrazine were found. Peters *et al.* (36) did not speculate on the fate of the hydrazine moiety, but its conversion to ammonia is probable (4, 12).

The INH inactivator status seems to make little difference for antituberculosis therapy, but without supplemental pyridoxine, slow inactivators more frequently exhibit toxic side effects (peripheral neuropathies) to INH treatment (75). Enhancement of INH toxicity has also been reported in pyridoxine deficient rats (44), as well as pyridoxine antagonism in bacterial cultures. Furthermore, only three metabolites were found in the urine of pyridoxine deficient rats, whereas, four metabolites were demonstrated in the rats given adequate levels of pyridoxine (44).

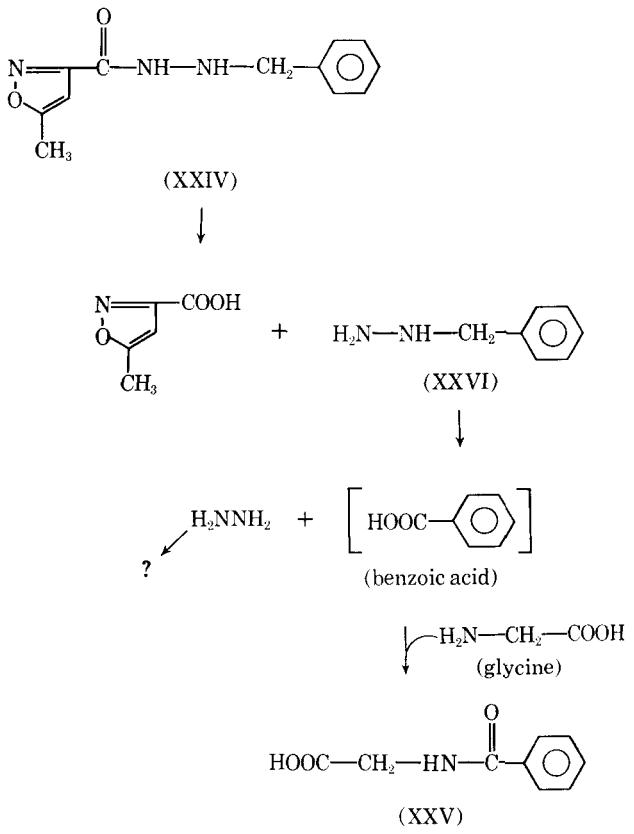
The administration of INH to both humans and dogs causes an increase in excretion of pyridoxine (76, 77) which was thought to be the result of combining of the hydrazide with the coenzyme and the subsequent excretion of the complex (76). Although no direct evidence for a hydrazone between INH and pyridoxine has been reported (44), such a complex has been postulated *in vivo* between γ-glutamylhydrazide (I) and pyridoxal phosphate (XXIII). This hypothesis was based on the fact that equimolar doses of γ-glutamylhydrazide plus pyridoxal phosphate or pyridoxal phosphate-γ-glutamylhydrazone had identical effects in relation to the production of convulsions, the decrease of brain level of α-aminobutyric acid, and the pyridoxal reversible inhibition of glutamic acid decarboxylase activity (78).

INH administration also causes a reversible inhibition of pyridoxine dependent enzyme systems. INH induced inhibition of L-glutamic acid decarboxylase is reversed by pyridoxine administration both *in vivo* and *in vitro*. However, the *in vitro* inhibition of glutamic-aspartic transaminase activity by hydrazides may be explained by the substrate complexing with hydrazide and α-ketoglutaric acid (79).

By graphically measuring the biological half-life of INH in various species, Peters (51) found that the rat was the most rapid deactivator of INH of the species tested, and the rhesus monkey appeared to be about one-half as active as the rat in disposing of the drug. The dog, on the other hand, reflected its inability to acetylate INH by a comparatively slow half-life. Man showed a wide range of biological half-lives reflecting genetic polymorphism. It is interesting to note that monkeys do not exhibit the genetic polymorphism of acetylation capabilities as does man. Peters *et al.* (80) found that in three species of monkeys tested (rhesus, cynomoglus, and mangabeys) no clear-cut polymorphism existed nor was there a sex or weight correlation with deactivation of INH. The total excretion of all metabolites was the same in all groups; however, the excretion of the INH varied as much as four times. It was concluded that the major differences in INH metabolism between man and all monkeys were quantitative and not qualitative.

Miscellaneous Substituted Hydrazides and Hydrazines—Isocarboxazide (XXIV) is a potent monoamine oxdase inhibitor which has been used for the treatment of various depressions and in management of angina pectoris. When rats were administered ^{14}C-isocarboxazide intraperitoneally, 75% of the administered activity appeared in the urine within the first 24 hrs. (81). Approximately 64% of the urinary activity was found to be due to labeled hippurate (XXV).

Structurally, isocarboxazide is both a substituted hydrazine and a hydrazide. *In vitro* studies (82) have indicated that rat liver homogenates rapidly metabolized isocarboxazide under either aerobic or anaerobic conditions. The major product of enzymatic breakdown of isocarboxazide was shown to be benzylhydrazine (XXVI), indicating the hydrazide bond was metabolized first, and yielding 5-methylisoxazolylcarboxylic acid (XXVII) as the other product. These data were interpreted as reflecting the hydrolytic cleavage of isocarboxazide followed by cleavage of the benzyl moiety and further oxidation to benzoate which then can conjugate with glycine to form hippurate as shown in Scheme II.



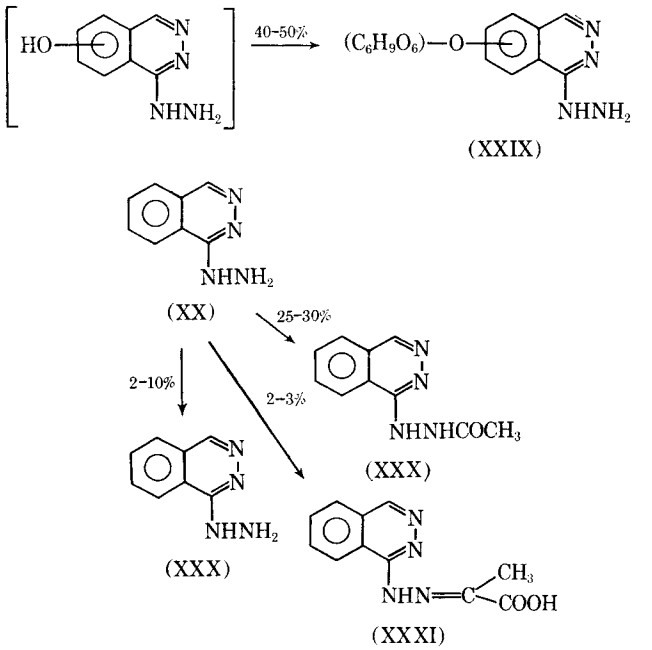
Scheme II

The presence of a monosubstituted hydrazine side-chain of the antidepressant drug phenelzine (XXII) suggested that like hydrazine and INH, phenelzine might be metabolized by acetylation. Evans *et al.* (67) have shown acetylation of phenelzine to be clearly polymorphic for INH. Among 47 patients with various depressions treated with phenelzine, two groups of acetylators were found. Differences in clinical response to the drug were not apparent, but the slow acetylators had many more side effects.

Hydralazine (XXI), a hypotensive drug which is the hydrazine derivative of phthalazine is quickly and extensively metabolized. Approximately 75% of the dose appears in the first 24-hr. urine collection with less than 2% of the dose as unchanged drug (83–86). The major metabolite of hydralazine isolated from the urine of rats and rabbits was the glucuronide conjugate implicating ring hydroxylation as the primary metabolic reaction. *N*-acetylhydralazine was present in considerable quantities and small but significant amounts of the pyruvic acid hydrazone were also found. No ethereal sulfates were detected (85).

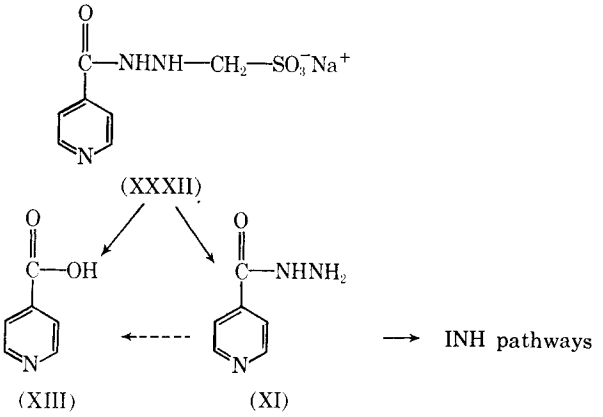
The *N*-acetylation of hydralazine occurs to a significant extent but appeared to be less important than hydroxylation in the deactivation since nearly 50% of the drug is converted to the glucuronide conjugate. *N*-acetylation of hydralazine occurs in pigeon liver homogenates and *in vivo* in guinea pigs (87, 88) and man but not in the dog.

Due to the instability of hydralazine at pH 8, it was suspected that if urine pH values were very high dimerization to di-phthalazinyl-hydrazine (XXVIII) would occur since no free hydrazine was evolved. However, since the pH of the urine collected remained very close to neutrality such a degradation product would not be expected in this case. Schematically the metabolic fate of hydralazine is as follows:



Scheme III

A comparative study in rats between INH and its structural analog isonicotinoylhydrazinomethanesulfonic acid (INHSM, XXXII) has indicated that both were excreted at approximately the same rate following i.p. injection, but when administered orally there were considerable differences in absorption rates. Whereas 66% of the administered INH was absorbed from the gut in 30 min., 25% of the administered INHSM still remained in the gut at the end of 6 hr. (88). INA plus an unknown metabolite were reported to be metabolic by-products of INHSM, but INA formation was somewhat less rapid from INHSM than from INH, implying that INHSM was slightly more stable *in vivo* than INH (88). Some INH was also reported to be formed from INHSM as shown in Scheme IV.



Scheme IV

Benzohydrazide (XXXIII) is a relatively toxic substance and when administered orally to rabbits approximately 20% of the dose appears in the urine as hydrazino nitrogen (6). A larger proportion (50–60%) is hydrolyzed to benzoic acid and excreted as hippuric acid and free hydrazine.

The *p*-chloro- and *p*-methyl- derivatives of benzohydrazide were also excreted largely as the corresponding glycine conjugates—*p*-chloro-hippuric and *p*-toluric acid, respectively. On the other hand, little hydrolysis of *p*-hydroxybenzohydrazide occurred. The latter was metabolized mainly by conjugation with glucuronic acid (29).

Salicylohydrazide (XXXIV), a compound with tuberculostatic properties is excreted mostly as the glucuronic acid conjugate by the rabbit. Less than 30% of the dose was hydrolyzed to salicylic acid and this was excreted largely as salicyluric acid (XXXV). Small amounts of gentisic acid (XXXVI) are also excreted. The acetyl derivative, *N*-acetylsalicylohydrazide (XXXVII) was also largely excreted as the glucuronide (29).

CONCLUDING REMARKS

A variety of substituted hydrazines and exotic hydrazides have come upon the research and medical scene since the introduction of hydrazine and INH, *e.g.*, isocarboxazid, nialamide, pivazid, mebamazine, N^1-benzyl-N^2-(1,4-benzodioxane-2-yl-methyl)hydrazine, N^1-picolinoyl-N^2-benzylhydrazine, pheniprazine, *etc.* In most cases, the metabolism of these compounds has not been reported, and research efforts have been concentrated on their pharmacological properties.

Of primary interest has been the monoamine oxidase inhibition potential of these compounds, but even with this interest few generalizations can be made even now. For example, seemingly random synthesis of compounds of various combinations of aromatic and heterocyclic ring systems with hydrazines has only led to the conclusion that replacement of the aromatic ring with the heterocyclic system may or may not increase monoamine oxidase inhibitive activity. However, with the wide spectrum of potential clinical properties of hydrazines and hydrazides, such as tuberculostatic, antidepressive, antihypertensive, treatment of angina pectoris, and polycythemia vera, it is obvious that more research on the metabolism of these compounds will be forthcoming.

The number of well-founded conclusions which can be made is in direct proportion to the paucity of systematic information available. However, a few speculative remarks can be made.

There appears to be only two mechanisms of detoxication which are characteristic of hydrazino compounds. Of the two mechanisms *N*-acetylation of the parent compound is the most significant from a toxicological approach. This reaction probably becomes less important as the toxicity of the parent compound decreases and alternate pathways become available. The second mechanism observed *in vivo* is the release of the hydrazino group through cleavage of the C—N bond.

The availability of a free hydrazine group appears to be related not only to toxicity, but also to the extent of metabolism. As pointed out earlier, the monoacetylhydrazines exhibit toxicities approximately that of hydrazine, yet, 1,2-diacetylhydrazine exhibits an LD_{50} several thousand times higher (6, 57, 58). Correspondingly, 1,2-diacetylhydrazine is excreted unchanged in the urine whereas, no urinary monoacetylhydrazine could be detected (13). A similar phenomenon is seen with INH in which the maximum tolerated daily dose in mice is 64 mg./kg. (58) and the corresponding value for AcINH, a metabolite of INH, in man, monkey, and rat is 1000 mg./kg. (35, 50). INH is primarily converted to AcINH which constitutes 91% of the total metabolites of INH excreted by man. Again the reduction in toxicity is shown following acetylation of the hydrazine group.

Deacetylation is not a common mechanism in the metabolism of hydrazino compounds. Instead, the carbonyl to nitrogen bond formed as a result of acetylation of a hydrazide compound appears to have greater *in vivo* stability than the bond between an aryl carbonyl carbon and the hydrazino nitrogen. The greater lability of the aryl carbonyl to nitrogen bond was demonstrated by the fact that following the administration of AcINH to rabbits, monoacetylhydrazine, and INA were observed in the urine but no free INH could be detected (54). In man, INA and diacetylhydrazine are the end-products of AcINH (53).

The toxicities of many hydrazino compounds are probably related to their release of hydrazine. Benzohydrazine is frequently lethal to rabbits at oral doses of 100 mg./kg. and is 60% hydrolyzed to hydrazine (6). On the other hand, the oral dose required to produce convulsions in 50% of the rabbits tested with salicylohydrazide was 600 mg./kg. *N*-Acetylsalicylohydrazide is well tolerated even at 800 mg./kg. (30). The main difference in the metabolism of these compounds appears to be the availability of alternate pathways of metabolism. With salicylohydrazide, the alternate to hydrolysis is glucuronidation which occurs in 50–60% of the dose. Some of the hydrazino group is lost since some salicyluric acid was recovered. With *N*-acetylsalicylic-hydrazide, the only active pathway is glucuronidation. *p*-Chloro and *p*-methylbenzohydrazide are nearly as toxic as the parent compound, benzohydrazide. Both are almost completely hydrolyzed. The *p*-hydroxyl derivative which exhibits very low toxicity is conjugated through the hydroxyl group.

Apparently the hydrazide bond exhibits a greater lability *in vivo* than does the alkylhydrazine bond. Phenelzine (XXI), which is 2-phenylethylhydrazine, is *N*-acetylated and excreted (67), but benzohydrazide (XXIX) is extensively hydrolyzed (6). A unique example of the relative stabilities of the hydrazide bond to the hydrazine bond is the metabolism of isocarboxazide (XXIII). Isocarboxazide which contains both types of bonds, is hydrolyzed first at the hydrazide bond (82).

These observations would suggest toxicity is related to the *in vivo* release of hydrazine. However, as pointed out by McIsaac and Williams (30) 20% hydrolysis of a 600-mg. dose of salicylohydrazide would yield as much hydrazine as complete hydrolysis of a 100-mg. dose of benzohydrazide. Therefore, if hydrazine is the toxic agent then it must necessarily be released from benzohydrazide much more rapidly than from analogs which have lower toxicities.

SUMMARY

The metabolism of hydrazine and its alkyl derivatives has been discussed as well as the metabolic fate of hydrazides. There appeared to be only two mechanisms of detoxication which were characteristic of hydrazino compounds. Of the two mechanisms *N*-acetylation of the parent compound was found to be the most significant in the toxicological approach. This reaction probably becomes less important as the toxicity of the parent compound decreases and alternate pathways become available. The second mechanism discussed is the release of the hydrazino group through cleavage of the C—N bond. The latter reaction appeared to be related not only to the toxicity but also to the extent of metabolism.

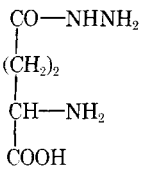
Deacetylation appeared not to be a common mechanism of the metabolism of hydrazino compounds. Instead, the carbonyl to nitrogen bond formed as a result of acetylation of the hydrazide compound appeared to have greater *in vivo* stability than the bond between the aryl carbonyl carbon and the hydrazino nitrogen bond. On the other hand the hydrazide bond itself has been shown to exhibit greater lability *in vivo* than the acetylhydrazide bond.

The toxicity of many hydrazino compounds has been related to their release of hydrazine. These compounds frequently showed much higher toxicity than the corresponding *N*-acetyl derivatives.

The pharmacogenetics of hydrazide metabolism also has been discussed with special reference to the individual and species variation observed.

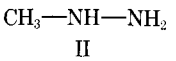
APPENDIX A

Key to Structural Formulas



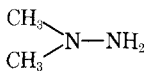
I

l-γ-glutamylhydrazide



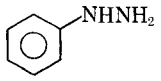
II

monomethylhydrazine



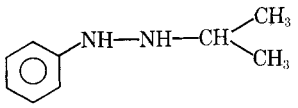
III

unsymmetrical dimethylhydrazine (1,1-dimethylhydrazine) (UDMH)



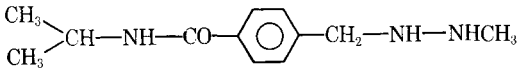
IV

phenylhydrazine



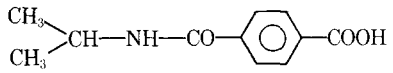
V

β-phenylisopropylhydrazine



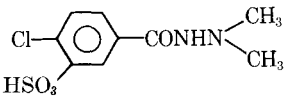
VI

1-methyl-2-*p*-(isopropylcarbamyl)benzene hydrazide (MIH) (Natulan)



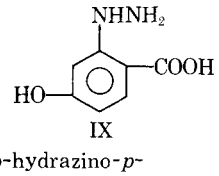
VII

N-isopropylterephthalamic acid



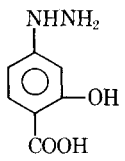
VIII

4-chloro-3-sulfamoylbenzoic acid 2,2-dimethylhydrazide



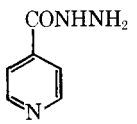
IX

o-hydrazino-*p*-hydroxybenzoic acid



X

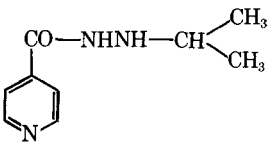
p-hydrazinosalicylic acid



XI

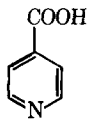
isoniazid

isonicotinic acid hydrazide (Nydrazide)



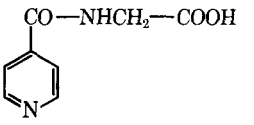
XII

iproniazid (Marsilid)



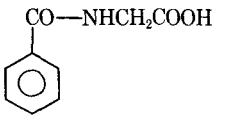
XIII

isonicotinic acid



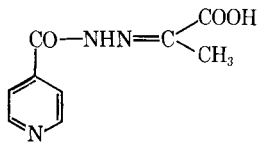
XIV

isonicotinylglycine



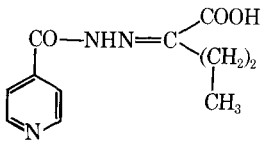
XV

hippuric acid (benzoylglycine)



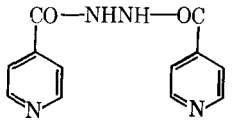
XVI

pyruvic acid isonicotinylhydrazone



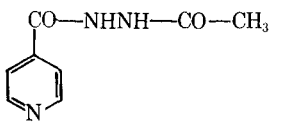
XVII

α-ketoglutaric acid isonicotinylhydrazone



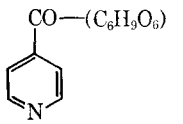
XVIII

di-isonicotinylhydrazide



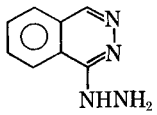
XIX

N^1-acetyl-N^2-isonicotinyl hydrazide (AcINH)



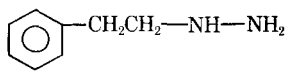
XX

isonicotinylglucuronide



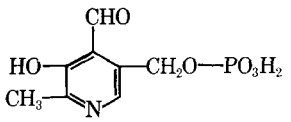
XXI

hydralazine (1-hydrazinophthalazine) (Apresoline)



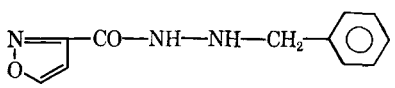
XXII

phenelzine (Nardil)



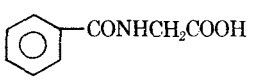
XXIII

pyridoxal phosphate



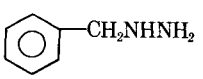
XXIV

isocarboxazide (Marplan)



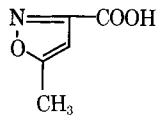
XXV

hippuric acid

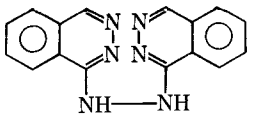


XXVI

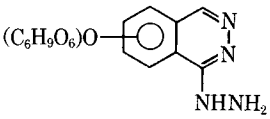
benzylhydrazine



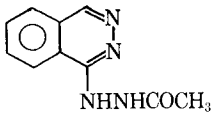
XXVII
5-methylisoxazolyl carboxylic acid



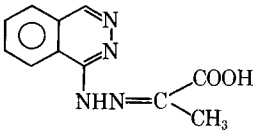
XXVIII
di-phthalazinylhydrazine



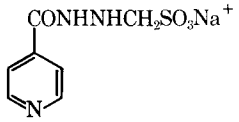
XXIX
hydralazine glucuronide



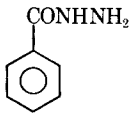
XXX
acetyl hydralazine



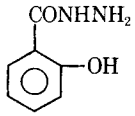
XXXI
pyruvic acid hydralazine hydrazone



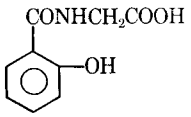
XXXII
isonicotinoylhydrazinomethane sulfonic acid (INHSM)



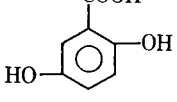
XXXIII
benzohydrazide



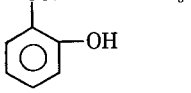
XXXIV
salicylohydrazide



XXXV
salicyluric acid



XXXVI
gentisic acid



XXXVII
N-acetylsalicylohydrazide

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[2] Abstract.

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RESEARCH ARTICLES

Hydrophile-Lipophile Balance and Cloud Points of Nonionic Surfactants

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Abstract □ The comparison of the cloud points of 165 nonionic surfactants was based on their calculated hydrophile-lipophile balance (HLB) values. The surfactants were classified according to structure and width of molecular weight distribution. Increasing length of the polyoxyethylene moiety increased the HLB and cloud points. At constant HLB, the following features were found to lower the cloud point: decreasing molecular weight, broader molecular weight distribution (probably due to the presence of fractions of such low degree of polyoxyethylation that they were insoluble in water at all temperatures), branching or greater symmetry of the surfactant molecule, the introduction of olefinic unsaturation, replacement of the terminal hydroxyl by a methoxyl group, and replacement of an ether by an ester bond. The equations for calculating the HLB, which had been derived from emulsification experiments with only a limited number of surfactants, contain the weight-percentage of polyoxyethylene as the sole variable characterizing the surfactant. Therefore, the calculated HLB is not affected by the surfactant characteristics listed above, which largely govern the values of cloud point, CMC, and interfacial tension. Additional HLB measurements are needed to determine whether the equations used to calculate HLB fully describe the emulsifying characteristics of all nonionic surfactants, *i.e.*, whether all experimental HLB values are really independent of the structure of the surfactant molecules.

Keyphrases □ Surfactants, nonionic—HLB, cloud points □ Polyoxyethylene moiety, surfactants—HLB, cloud points □ HLB determination—surfactants □ Cloud point determination, significance—surfactants

"Probably the most important single parameter affecting the type and stability of an emulsion is the puzzling hydrophile-lipophile balance number (HLB)... One is left with the conviction that the HLB has a rational interpretation and with a sense of frustration in not being yet able to show its origin conclusively" (1). One such attempt, to find a universal relation between the HLB values and critical micelle concentra-