both yielded high in alcohol and ether extractions in comparison with the other two varieties of low toxicity to fish, and low active potency to dogs.

The varieties of hemp listed in Table II would hardly be considered pure genetically. It would appear likely that varieties of hemp of even lower potency upon dogs and with less toxicity to fish might be obtained by further selection and imbreeding the Kentucky and Manchurian varieties.

CONCLUSIONS

Acetone extracts of leaves of different varieties of hemp produce different degrees of toxicity in goldfish. The region of growth and the age of the hemp plants affect the degree of the extract toxicity upon goldfish. Fractions of purified resins of different potency acted upon dogs in a somewhat similar manner as they did on fish. Of four varieties used for both fish and dog tests, the degree of toxicity upon fish and potency to dogs was in the same relationship. It is believed that goldfish testing of hemp resins may facilitate hemp breeding.

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Aliphatic Amines I—A Review*

By Melvin F. W. Dunker† and Walter H. Hartung

An extensive survey of the literature on the simple aliphatic amines has been made in order to determine whether it is possible to make a correlation between structure and biological properties. A vast amount of material has been published, by far the most of it pertaining to chemical and physical properties and to methods of syntheses. The references relating to the physiological effects of the simple aliphatic amines, while numerous, represent only a small part. Since the publication of Trendelenburg's comprehensive review in 1923 (1), two partial reviews have appeared, namely, Hartung's tabulation of toxicity and pressor effects (2) and Tainter's discussion of pressor and autonomic nervous system effects (3). Many of the original reports in the literature are difficult or impossible to correlate because of differences in species, in the state of the experimental animal, dosage, technique, mode of administration and the like.

In view of the increasing commercial importance and availability of many simple aliphatic amines, it seems desirable to be better informed on their physiological properties. Furthermore it would seem presumptuous to attempt to correlate the effect of chemical structure of complex compounds with physiological action, a field in which much of the current research is being done, when the simple bases have been inadequately studied or neglected.

The one property on which good comparative data are available is the effect of amines on the blood pressure on intravenous injection. A few generalizations may be repeated here. The lower members, up to the butylamines according to Barger and Dale (4), or to isopropylamine according to Tainter, are inactive or give no constant response on blood pressure in experimental animals. As the chain is increased beyond this point, the pressor activity steadily increases to a maximum in *n*-hexylamine and then steadily decreases as the chain is lengthened to 13 carbon atoms. From propyl to hexyl, it has been shown that the normal radical is more active than the corresponding iso-chain, although Tainter (3) reports the isopropylamine to have pressor activity while the normal propylamine is inactive. Except for the sec-butylamine which was found to be indifferent (3) and the tertiary amylamine for which pressor activity is claimed (5) no other saturated branched chain amines have apparently been investigated. The primary amines seem to be somewhat more active than the secondary and tertiary amines (1).

The amines as a class appear to stimulate the tone and movement of the isolated intestinal and uterine tissues, the effect increasing somewhat with increasing length of the chain up to C_6 (6). Isoamylamine has an effect which parallels that of adrenalin on smooth muscle (7) although it is much less active, that is to say, it produces an inhibition of the intestine, contraction of the rabbit uterus in all states, an inhibition of the non-pregnant cat uterus and

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stimulation of the pregnant cat uterus. On the other hand, isohexylamine is credited with a stimulation of the isolated intestine (6). As before, the iso-form seems to be less active than the corresponding compound and the primary more active than the secondary or tertiary amines.

All of the amines studied are reported to have an effect on the central nervous system which is quite analogous to that of ammonia. There may be preliminary excitation, followed in large doses by narcosis and paralysis (1).

The amines when administered are not excreted as such, but are metabolized in the body to other products, frequently with the destruction of the aliphatic radical, the amino group forming ammonia or urea (1, 8, 9, 10).

In general there seems to be little or no effect on the various secretions although isoamylamine weakly stimulates lachrymal and salivary secretion in cats (7) and salivary secretion in rabbits (1), while trimethylamine is said to stimulate both salivary and pancreatic secretions (11).

Many amines in small doses produce no effect on the body temperature, but according to Trendelenburg (1) trimethylamine is supposed to produce a fall in body temperature in rabbits while the isomeric *n*-propylamine causes a rise in body temperature in rabbits (5).

The replacement of the hydrogens of ammonia with alkyl radicals results in a decrease in the acute toxicity. Beyond this it is somewhat difficult to make generalizations because of the variations in test animals, modes of administration, etc. It appears that there is some decrease in toxicity among the primary amines as the length of the chain increases. In the cases of the methylamines, there is a slight decrease in toxicity in passing from the primary to the secondary to the tertiary amine (1); whereas the reverse is reported for the *n*-butylamines, the mono-*n*-butylamine being least toxic (12). Among the di-alkylamines there appears to be an increase in acute toxicity with increase in molecular weight up to C_5 (1). These toxicity data are not particularly significant since they do not take into consideration the relative molecular weights of the respective amines.

Only a few studies which indicate a chronic toxicity as the result of continued administration of comparatively small doses are reported. For example, when the methylamines, diethyl-, dipropyl-, or isoamylamine (13) were added to the otherwise non-hemorrhagic diet of 7-day-old chicks, hemorrhages, as well as other marked blood changes, occurred. The addition of materials rich in cystine or cysteine to the diet caused a very definite alleviation of the symptoms. Upon subcutaneous injection of 0.5 cc. of *M*/15 solutions of methyl-, ethyl-, propyl- or isobutylamines (14) on five consecutive days into guinea pigs, extensive gastric ulcers were found when the animals were sacrificed on the sixth day. The amines tested showed nearly equal potency in producing the ulcers. When the amines were administered orally with the stomach in the postabsorptive state, the ulcers were more severe. According to Barbour (15), small subcutaneous doses of ethylenediamine, administered daily to rabbits, produce a hypothermia which fails to appear after the fourth day; over a period of ten days such injections have no significant effects on body weight, kidney, heart or blood.

The daily oral administration of 1 Gm. propylamine hydrochloride (about 102 mg./Kg.) to dogs (16) produced no noticeable symptoms while the daily injection of 1-Gm. doses subcutaneously (neutralized) led to necrosis at the site of injection in about 10 days. Propylamine is completely destroyed in the body.

The development of liver lesions in mice has been shown to arise from the continued oral administration of isoamylamine or choline-HCl (17).

On the whole, it appears that there is an absence of characteristic difference between the primary, secondary and tertiary amines. Any differences which appear between the three classes are in degree rather than type of action. When, however, the fourth alkyl is introduced and the quaternary ammonium salts and bases formed, a rather distinct change in character of action appears. The physiological effect becomes predominantly curare-like. The typical action of the amines on the central nervous system is reduced or lost. This loss of central activity on alkylation is also observed among certain of the alkaloids as, for example, strychnine and morphine (1). Recently the high bacteriostatic and bactericidal activities of the quaternary ammonium salts have been emphasized with the marketing of the zephirans, etc. (18).

While speaking of the antiseptic action of the quaternary bases, it might be well to mention that in 1928 Tilley and Schaffer (19) determined the "phenol coefficients" using *B. typhosus* and *S. aureus* for the normal amines from propyl to heptyl, the dialkylamines from ethyl to *n*-butyl and triethylamine. While the phenol coefficient method may be open to question when applied to amines, it can be said that there was an increase in activity in the primary and in the secondary series with increasing chain length, the dialkylamines being more active than the corresponding monoalkylamines. During the past ten years, patents (20) have been issued for the higher aliphatic amines such as octylamine, octyldiethylamine, oleylamine, etc., as disinfectants.

The effect of the presence of halogens in the aliphatic chain has not been investigated fully. It has been shown that tri-(β-chloroethyl)-amine is a very powerful vesicant (21), quite analogous to mustard gas, although the di- and mono-(β-chloroethyl)-amines are not vesicant. Tri-(γ-chloropropyl)-amine and tri-(δ-chlorobutyl)amine (22) are also vesicants.

The introduction of the hydroxyl leads to a decrease in toxicity and activity as compared to the non-hydroxylated amine. Thus ethalolamine is less toxic than ethylamine (1). The very interesting

and useful properties of triethanolamine make it seem desirable to investigate more extensively its physiological properties. Salts of triethanolamine with various iodinated acids (23, 24) have been administered to animals in experiments in iodine therapy without apparent untoward symptoms attributable to the amine.

The introduction of the second amino group in the chain leads to a reduction in the toxicity of the amine (1). This is of interest since the compounds putrescine and cadaverine were at first called ptomaines and thought to be the materials responsible for the severe poisoning resulting from partly decayed food.

While not many unsaturated amines have been investigated, evidence indicates that the double bond leads to an increase in toxicity (1). About 1932, reports on the antispasmodic action of octin (6-methylamino-2-methyl-2-heptene) appeared in the literature and more than 20 references to studies of its action have appeared since that time. The compound is claimed to have strong antispasmodic properties (25, 26), and has been promoted and sold for that purpose. The reports are conflicting. On intravenous injection (27) it causes a loss in tone, a decreased force of rhythmical contraction and a decreased peristaltic activity of loops of the jejunum, ileum or duodenum. This antispasmodic or relaxing action of octin is unexpected in view of the fact that the lower saturated amines are generally reported as stimulating intestinal tissue, causing increased tone and activity. It produces a temporary fall in blood pressure (28) followed by a prolonged rise, as the result of a cardiac stimulant action. The blood-pressure effect is not observed when the drug is given subcutaneously or orally (29), but a rise (30) is reported on intramuscular injection. It has poor bronchodilator activity (31). According to some more recent work (32), the action is weak and irregular on artificial cardiospasm in rabbits, and on the acetylcholine spasm (33) of rabbit intestine. Comparative studies (34) with a series of related saturated and unsaturated amines, either containing or devoid of an *N*-methyl group and having 6 to 9 carbon atoms, indicate that octin is not the optimum compound of the group.

In connection with antispasmodics, some of the recent work of Blicke (35) deserves to be mentioned. Much of his work has dealt with cyclic or mixed amines, but he has studied some aliphatic amines. Methyl-di-β-cyclohexylethylamine [CH_3N—(CH_2—CH_2—C_6H_{11})$_2$] is a strong antispasmodic. If the rings are opened in the 1-2 position, methyl-dioctylamine is obtained. It, as well as methyl-di-*n*-hexylamine, are only weak antispasmodics while methyl-di-(2-ethylhexyl)- and methyl-di-(2-ethylbutyl)-amine have no antispasmodic properties.

Some studies of the effects of amines on various enzymic processes have been made. In general, the alcoholic fermentation of sugar by yeast (36) is retarded by the free amine but may be unaffected or accelerated by the hydrochloride depending on the amine. Similar studies (37) on the effects on the hydrolysis of starch by ptyalin, pancreatin or malt extract indicate that again the free amines retard fermentation while the hydrochlorides accelerate the process, the effect decreasing with increasing length of the carbon chain. However, no mention is made whether or not these effects are associated with the pH of the solution.

A large series of amines, among them 8 aliphatic amines ranging from C_1 to C_{17}, was investigated (38) for their antigenic properties. When the amines were injected into rabbits, they caused the formation of precipitating and complement-fixing antibodies which reacted more or less specifically with the amine used for injection. The effect was weak with amylamine, increased with hexyl- and heptylamines and was pronounced for heptadecylamine.

The toxicity of a large number of aliphatic amines to paramecia, as a measure of their effectiveness against protozoa, has been determined (39) and some of the generalizations follow. Methylamine is only slightly more toxic than ammonia, the toxicity increasing with the number of carbon atoms. Of the amines with the same number of carbon atoms, the isomer with the longest possible primary alkyl chain is more toxic than either the branched chain primary amine or than the secondary or tertiary amine with the same number of carbon atoms; for example, *n*-hexylamine is many times more toxic than isohexylamine, than di-*n*-propylamine or than triethylamine, etc. Most of the aliphatic amines are, however, inferior to quinine in this respect. On the other hand, in a limited series of amines, the compounds with the smallest chain were the most toxic to amoeba (40). Recently, 1,10-tetrabutyl- and 1,10-tetraamyl-diaminodecane (41) have been patented as amœbacides.

Various other effects are attributed to aliphatic amines in the literature as the following selected references indicate. Some aliphatic amines (42) tend to inhibit the growth of tumors and to prolong the life of tumor-bearing animals. Colamine, or ethanolamine, and diethylamine are indifferent whereas choline, triethanolamine, cadaverine and putrescine delayed the appearance of the tumor and prolonged the life period.

Under certain conditions, ethanolamine is reported to prevent the convulsions caused by a vitamin B_1 deficiency (43), whereas methylamine, diethylamine and isoamylamine are entirely ineffective.

The anti-coagulative action of diethylamine is interesting; it is reported to be one-seventh as active as heparin, and, like heparin, its effect is neutralized by hydrochloric acid (44).

Trimethylamine and ethanolamine, when given in doses of 100 mg. per day for twelve days had no effect on the liver fat content of animals with fatty livers as the result of improper diet (45).

The injection of trimethylamine causes a slight fall in the blood sugar level of rabbits (46) whereas methylamine, diethanolamine, ethylamine and ethanolamine are ineffective.

Various amines as methyl-, ethyl-, propyl-, butyl-, dimethyl-, trimethylamines cause swelling, softening and solution of pure casein (47). The effects are attributed to compound formation in consequence of which proteinates possessed of a higher degree of solvent power for water are produced. It is suggested that such a mechanism may account for swellings in pathological states.

According to a recent report (48) solutions of trimethylamine fed to young tomato plants through a cut in the stem for 36 days led to an increase of about 22% in the number of flowers produced. In fish and frogs, the presence of small amounts of trimethylamine in the water leads to the changes in colors associated with the mating period, to moulting, awakening of the sexual instinct and other secondary effects as long as four months after the normal mating season. However, no cases of actual mating were observed. It is suggested that these effects are indicative of hormonal influence.

In view of the fact that the importance of optical isomerism in certain types of physiological action is so well known, it seems remarkable that no reference was found to any studies in which optically active aliphatic amines were prepared and studied physiologically.

It may be seen that while many varied investigations on the biological properties of simple amines have been made, it is impossible to correlate or tabulate the available data and information because of their motley and heterogeneous character. It is obvious that if systematic knowledge in these fields is to become available, properly conducted experiments, carried out under comparable and uniform conditions, must be performed. It is the aim of subsequent papers in this series of "Aliphatic Amines" to record such information.

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Aliphatic Amines II. The *n*-Heptylamines, Preparation and Toxicity*

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With the exception of the primary heptylamine, no studies on toxicity have been made on the C_7 amines. The work on heptylamine is limited to pressor studies (1), studies on the oxidation of heptylamine by tissues (2) and by amine oxidase (3), studies on the antibacterial efficiency (4) of the compound, the production of specific precipitating and complement-fixing antibodies when injected in rabbits (5), and its toxicity for paramecia (6). As a preliminary experiment, it was thought interesting to prepare the amines in pure form and determine whether the position of the amino group in the chain had any effect on the toxicity of the compounds.

The amines were administered intraperitoneally in neutral solutions to white mice. According to the results obtained in comparative experiments, the 1- and 4-aminoheptanes seemed to be the least toxic, the 2- and 3-aminoheptanes being approximately equal but more toxic than the 1-aminoheptane.

EXPERIMENTAL

The amines were prepared free of secondary or tertiary amines by the reduction of the oximes. The necessary aldehyde and ketones were obtained as follows. Eastman technical heptaldehyde was fractionated twice and the cut boiling 152–156° used. Methyl *n*-amylketone and dipropylketone were generously supplied by Carbide and Carbon Company. The samples were fractionated before use. Methyl *n*-amyl ketone boiling at 150–151° and dipropylketone boiling at 142–144° were used. The heptanone-3 was prepared by the dry distillation of an intimate mixture of calcium propionate and calcium valerate. The product was fractionated and a fraction boiling 147–153° used for the preparation of oximes. The oximes were all prepared according to the method given in the literature (7) and illustrated below.

Preparation of Oximes.—To 87 Gm. (1.25 mol) of hydroxylamine hydrochloride in 125 cc. water in a 1-L. 3-neck flask equipped with a reflux condenser, mechanical stirrer, thermometer and dropping funnel were added 114 Gm. (1 mol) heptaldehyde and the mixture was stirred vigorously. A solution of 66 Gm. (0.63 mol) sodium carbonate in 175 cc. water was added slowly so that the temperature did not rise above 45°. The stirring was continued for an hour after the complete addition of the solution. On standing over night, the oxime solidified in large lumps which were filtered out and dried in a desiccator. The yield was quantitative. The crystals melted at 53–55° and after crystallization from 60% alcohol melted at 53.5–54.5°. The preparation of the three ketoximes was carried out in like manner and the properties are given in Table I.

Reduction of Oximes.—Test experiments carried out on the reduction of heptaldoxime in either glacial acetic acid or alcoholic hydrochloric acid in the presence of palladium on charcoal at atmospheric pressure or pressures up to an initial pressure of 750 lb. were not successful in the production of primary amine.

Since the catalytic reduction did not work successfully under the conditions investigated, the reductions were carried out with sodium and absolute alcohol (8). Nine-tenths mol methyl *n*-amyl ketoxime was dissolved in 1.8 L. absolute alcohol and the solution heated to boiling in a water bath under a reflux condenser. The sodium, cut in thin strips (9.8 mols or 225 Gm.), was added slowly through the condenser. Near the end, the sodium melted to a ball which settled to the bottom and it was necessary to continue the heat to retard the formation of a crystalline mass of sodium ethoxide which trapped globules of sodium. When the sodium appeared to be gone, the cautious addition of water was begun and finally 1.5 L. of water were added. The flask was arranged for distillation and the distillate collected in a mixture of 135 cc. concentrated hydrochloric acid and 135 cc. water. The alcohol and most of the water were then distilled from a water bath under reduced pressure. To the amine hydrochloride crystals were added 500 cc. of 40% solution of sodium hydroxide while cooling. The aqueous layer was withdrawn and the amine dried

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