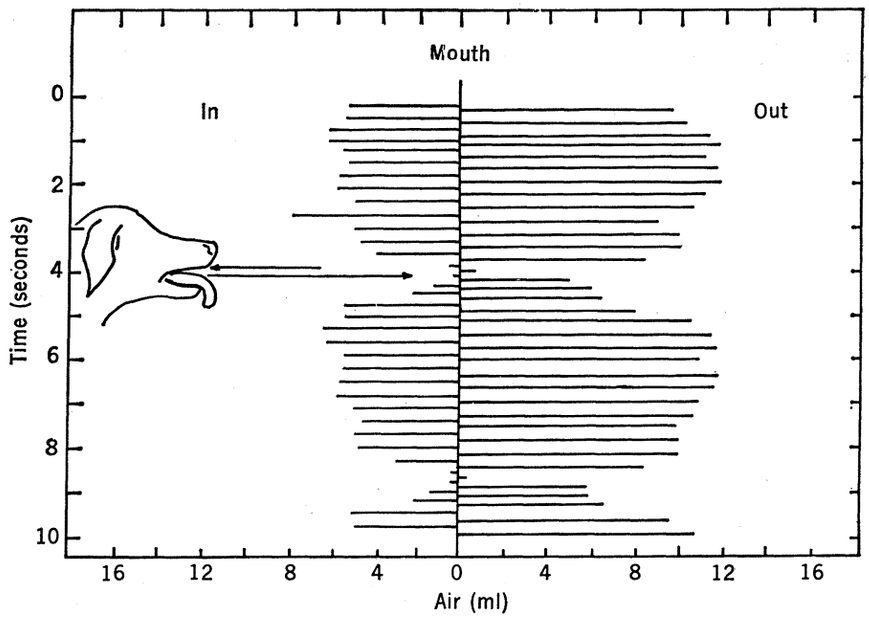

Fig. 2. Volume of air entering and leaving the mouth during "deep" panting (see text). Mean inspired and expired volumes for 10 seconds are indicated by vectors adjacent to the dog head. (Volumes expressed as milliliters of dry air at 23°C and 760 mm-Hg.)

more distressed, the panting tended to change to a somewhat lower frequency (from 300 to 220), and inhalation through the mouth began to appear in the recordings (Fig. 2). At the same time the total tidal volume increased, and the volume exhaled through the mouth was now approximately twice as large as that inhaled through the mouth. Thus air inhaled through the nose was still exhaled through the mouth. The statement of Negus (*3*) that man and anthropoid apes are the only mammals capable of inhalation through the mouth is therefore incorrect.

The importance of the described pattern of air flow can be estimated from the temperature of the exhaled air. A dog may pant with closed mouth, both inhalation and exhalation taking place through the nose. Under these circumstances the temperature of the exhaled air was 29°C (*4*). If the same dog, while still panting at the same frequency, changed to exhalation through the mouth, the temperature of the exhaled air was nearly identical to body temperature (38°C). For a given volume of air, exhalation at 38°C instead of 29°C substantially increases the amount of heat carried away. In our experiments the amount of heat carried away by 1 liter of air was 14.9 cal for exhalation through the nose and 27.7 cal from exhalation through the mouth (calculated from the water added to saturate air and the heat added to warm the air to exhaled air temperature). By changing the relative amounts of air exhaled via the nose or via the mouth, the dog can thus, without changing frequency or tidal volume, modulate the amount of heat dissipated. The advantage of a constant frequency of panting was discussed by Crawford (*5*), who emphasized that dogs seem to pant at the resonant frequency of the respiratory system, thus reducing the energy expenditure (and heat load) of panting. Modulation of tidal volume, especially an increase in tidal volume, may be undesirable because of its effect on hyperventilation of the lungs and the ensuing alkalosis. It seems to us that a modulation of evaporation by a mere change in the flow pattern of the air is, within its limitations, a simple and effective mechanism.

An important consequence of the described pattern of panting is that the nasal mucosa, rather than the oral surfaces and the tongue, is the primary site of evaporation. Consequently, these surfaces must be supplied with sufficient quantities of moisture. It is quite possible that the large serous-type nasal gland which is found in the dog is the major source of the necessary secretion. This gland was first described by Steno in 1664 and has since received considerable attention from anatomists (*6*), but no specific function has been ascribed to it. If this gland is indeed of importance for supplying the water required for heat dissipation during panting, its function is in a sense analagous to that of sweat glands in man. It can therefore be expected that secretory activity may be under control of the thermoregulatory system, and this should be investigated.

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Alcohol Addiction and Tetrahydropapaveroline

Davis and Walsh (*1*) proposed that heavy consumption of alcohol may result in a diversion of dopamine (DA) metabolism in the central nervous system from a pathway producing dihydroxyphenylacetic acid to a nonenzymatic condensation of dihydroxyphenylacetaldehyde with DA to produce tetrahydropapaveroline (THP). They further hypothesized that THP has addictive liability or is further metabolized to morphine-like compounds which are then responsible for the development of the syndrome of alcohol addiction. In support of this formulation, they have (i) extended to rat brain homogenates the findings in vitro of Holtz *et al* (*2*) as well as our own (*3*) with guinea pig liver preparations with respect to the formation of THP from milligram quantities of DA; (ii) shown a slight increase in the amount of THP formed as a per-

centage of the DA deaminated with increasing concentrations of acetaldehyde in such homogenates; and (iii) shown that when exogenous [^{14}C]THP is administered to rats, it is metabolized to unidentified compounds appearing in the urine.

Acceptance of their hypothesis concerning the etiology of alcohol addiction requires the demonstration that THP is formed under physiological conditions in vivo. Experiments to demonstrate such formation have been inconclusive. We have shown that even in vitro, milligram quantities of DA are required for significant THP formation. However, if concentrations more consistent with tissue concentrations of DA are used (micrograms), then the formation of THP in liver mitochondrial suspensions is at best only 3 percent of the original amount of DA (*3*). Furthermore, after the intravenous administration of 250 μg of [^{14}C]DA per kilogram to guinea pigs, an estimate of the maximum amount of THP that was formed was only 1 to 2 percent of the total radioactivity present in the peripheral tissues. This was neither a consistent finding nor was THP formed in tissues other than the liver (*3*).

The failure to find THP synthesis from DA in vivo has been further substantiated by Langeneckert and Palm (*4*). They showed that even after inhibition of aldehyde dehydrogenase by disulfiram, presumably producing optimum conditions for the formation of THP, no THP could be found in the liver, heart, and urine of rats and guinea pigs. Thus, even supplying exogenous DA to the animal does not result in THP formation.

Although the brain was not examined for THP in either of these investigations, Rutledge and Jonason (*5*) were able to recover over 95 percent of the DA added to slices of rabbit cortex as DA or known metabolites. Had any significant amount of THP been formed, a much lower recovery would have been obtained. Thus, all evidence currently available indicates that THP is not formed from physiological quantities of DA under physiological conditions. It would, therefore, seem that the high amount of THP formed in the rat brainstem homogenate (46.65 percent of the DA deaminated) in the experiments of Davis and Walsh (*1*) is found only in a highly artificial system, and extrapolation to the complex situation of alcohol addiction is unwarranted.

It might be argued that only minute quantities of THP or its unidentified metabolites would be required to establish addiction. This would imply that these compounds have a high addictive liability. Davis and Walsh cite the report of Fraser *et al.* (*6*) as evidence for their statements that "it is feasible that THP may have intrinsic addiction liability of its own because alkaloids of this type have been shown to produce analgesia and dependence." However, Fraser *et al.* (*6*) conclude that "I-K-1 [1-(*p*-chlorophenethyl) 2-methyl-6,7-dimethoxy-1,2,-3,4-tetrahydroisoquinoline] has substantially less addiction liability than morphine and codeine and even less addictiveness than D-propoxyphene." In addition, they state that "In these trials, I-K-1 and D-propoxyphene were not better than a placebo, but codeine and aspirin were more effective than placebos." This conclusion would indicate that these compounds do not possess the requisite addictive liability.

Also of some concern is the identity of the THP formed by rat brainstem homogenates. Although the authors verified the identity of THP by three separate methods, they did not mention whether these methods were sufficient to discriminate between THP and the tetrahydroisoquinoline condensation product which is formed from DA and acetaldehyde, 1-methyl-6,7-dihydroxy-1,-2,3,4-tetrahydroisoquinoline (*7*). This is of significance since the authors added increasing concentrations of acetaldehyde to the incubation mixture and found parallel increases in the amounts of THP.

In the absence of direct evidence that THP is formed in vivo, or that either THP or one of its metabolites has high addictive liability, we find it difficult to accept the suggestion that THP is involved in the development of alcohol addiction.

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Halushka and Hoffmann have raised several objections to our hypothesis (*1*) which offered a possible mechanism for alcohol addiction. They state that "acceptance of the hypothesis concerning the etiology of alcohol addiction requires the demonstration that THP (tetrahydropapaveroline) is formed under physiological conditions in vivo." However, this interpretation may not be entirely correct. A major point of our paper was that experimental verification of this proposal will depend on the demonstration of THP or its metabolites under pharmacological conditions in vivo. Specifically, we suggested that, under the influence of long-term ethanol ingestion, the normal metabolism of dopamine (DA) might be diverted with the resultant formation of THP. We did not intend to imply and would not anticipate that THP would be of any consequence as a metabolite of dopamine in vivo under physiological conditions. Nevertheless, even this possibility may still be tenable, since experiments in vivo to date (*2*, *3*) are decidedly "inconclusive."

In this regard, Langeneckert and Palm (*2*) refer to unpublished observations that THP was undetectable in the liver, heart, and urine of rats and guinea pigs after pretreatment with disulfiram and administration of DA. This comment is difficult to evaluate without greater knowledge of experimental detail—for example, the time and dose used for pretreatment with disulfiram. The inhibition in vivo of aldehyde dehydrogenase by disulfiram is quite slow, requiring 16 hours for maximum inhibition of the supernatant enzyme and 40 hours for the mitochondrial enzyme (*4*). Of greater importance, however, may be the fact that THP is further metabolized and might preclude the ready detection of THP in vivo by the extraction procedure of Holtz *et al.* (*5*). Similarly, Halushka and Hoffmann (*3*) were unable to detect significant formation of THP in peripheral tissues at 2 and 5 minutes after intravenous administration of [^{14}C]DA to normal guinea pigs. Although these authors state that "other possibly important metabolites of DA, norepinephrine and 3-methoxydopamine, did not migrate with THP in any solvent systems," they failed to show the presence of 3-methoxytyramine or of norepinephrine and its known metabolites in their autoradiograms. On the basis of these results, however, one could hardly conclude

that norepinephrine or 3-methoxytyramine are of no consequence as metabolites of endogenous DA. Thus, the statement that THP is of little import as a metabolite of DA in vivo may also be a premature conclusion.

Furthermore, these authors (*3*) have confirmed the original work of Holtz *et al.* (*5*) which demonstrated that THP is formed when milligram quantities of DA are incubated with a mitochondrial preparation equivalent to 900 mg of liver. In extending these studies, we have shown in both liver and brainstem homogenates that the formation of THP is highly dependent on the substrate concentration as well as on the rate at which the intermediate aldehyde is oxidized or reduced (*6*). We used concentrations of DA required for maximum monoamine oxidase activity and an incubation period of 30 minutes during which a linear deamination rate was observed. Under these conditions the formation of THP in rat liver homogenates was essentially abolished when exogenous nicotinamide-adenine dinucleotide was added. In rat brainstem homogenates, however, THP formation remained substantial even with added cofactor. It is this tissue which is relevant to DA function and biotransformation and which was found to have a limited pathway for reduction of the aldehyde derivative of DA as well as a low capacity to oxidize aromatic aldehydes when compared with liver (*1*, *6*). While homogenates are of course "artificial" systems, experimental data derived from them have focused on possible events in vivo and support our contention that brainstem should be exquisitely sensitive to compounds that inhibit the aldehyde-acid pathway of amines (*1*, *7*). It is implicit in our proposal that in discrete areas of the brain where DA is localized in high concentrations and functions as a neurotransmitter, diversion of DA metabolic pathways may occur as a result of alcohol metabolism. So while we would not anticipate THP to occur as a normal metabolite of DA, we would be particularly surprised to find it in peripheral organs after intravenous administration of the amine (*3*).

The possibility of the formation of THP from DA added to brain cortex slices must await further experimental verification. The isolation procedures used by Rutledge and Jonason (*8*) would not permit the elution of THP since this compound would be retained on the cation-exchange resin. In their experiments with rabbit cortex slices incubated with DA, the radioactivity isolated as DA or its known metabolites represented approximately 90 percent of the radioactivity present as DA in tissue control samples. Recalculation of these data reveals that only 80 percent of the DA metabolized was recovered as known metabolites of DA. In contrast, the recovery of known metabolites rose to more than 92 percent of the DA metabolized in incubation mixtures of brain cortex slices from animals given prior treatment with an inhibitor of monoamine oxidase. Therefore, we would suggest that these data may also reflect the possibility that the aldehyde derivative of DA may traverse alternate metabolic pathways.

Also, we are aware of the facility with which acetaldehyde condenses with DA to form the simple tetrahydroisoquinoline, salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline). As we have shown, this reaction is proportionate to the concentration of acetaldehyde (*9*). Consequently, the methodology was developed to assure that the THP was uncontaminated by salsolinol (*7*). The methods used to verify the identity of THP did discriminate between THP and salsolinol since these two alkaloids demonstrate completely different infrared and gas chromatographic characteristics (*10*).

The question of the potential addiction liability of THP is an important aspect of this concept. It must be remembered, however, that we are talking about alcohol, a compound which in and of itself does not have a high addiction liability. Nevertheless, ethanol causes many millions of persons to become victims of alcoholism. While the addiction liability of I-K-1 [1-(*p*-chlorophenethyl)2-methyl-6,7-dimethoxy-1,2,-3,4-tetrahydroisoquinoline] studied by Fraser *et al.* (*11*) is quite low, this particular compound is a phenethyltetrahydroisoquinoline; whereas, THP is a benzyltetrahydroisooquinoline. The limited investigation of the benzyltetrahydroisoquinolines, both structurally and biogenetically related to morphine, prevents much speculation concerning their analgesic and addictive potencies. One report states that the fully methylated derivative of THP, laudanosine, was tested in this regard and was found "not to have any distinct analgesic efficacy." However, the suggestion was made that "it would be worthwhile to extend this testing program to include some of the optically active and partially *O*-methylated alkaloids of [this] type" (*12*). This is indeed an important point, because it is only through very specific methylation and demethylation steps that THP undergoes oxidative phenol coupling reactions in plants for transformation into the aporphine and phenanthrene alkaloids.

As far as a specific role for THP in the development of alcohol addiction, we certainly recognize that nature may not be as simplistic and direct as we have depicted. Our purpose was not only to offer a possible relation of a hypothetical biosynthetic chain of events to the pharmacology of the simple molecule ethanol, but also to explore a scheme involving drug-induced aberrations in the metabolic disposition of neuroamines based on reaction sequences which are known and understood in the laboratory and which serve as routes for the biosynthesis of several alkaloids in living plants. The demonstration in mammalian systems in vitro of the formation of alkaloids from amine-aldehyde reactions involving condensation of aromatic amines with either a drug-derived aldehyde or amine-derived aldehyde could serve as a framework for future investigations. These investigations might focus on the possible formation and metabolism of alkaloids derived from DA and other neuroamines under certain pharmacologic conditions.

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