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THE INFLUENCE OF BODY TEMPERATURE ON THE ELIMINATION OF OXOTREMORINE IN THE MOUSE

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Abstract—The influence of body temperature on the elimination of oxotremorine (OTMN) from the mouse was studied by following the disappearance of tritiated OTMN. In a dose of 0·025 mg/kg, i.p. OTMN does not affect body temperature and the drug disappears exponentially with a half-life of 35 min. A dose of 0·5 mg/kg of OTMN depresses body temperature by 10 to 15°, 60–90 min after administration. The degree of hypothermia is dependent upon the environmental temperature. At this dose, OTMN is initially eliminated exponentially ($T\ \frac{1}{2} = 100$ min), however, during maximal hypothermia the rate approaches zero. When the body temperature starts to recover (150–180 min after administration) the rate of elimination increases. Disappearance of OTMN from the brain is at all times more rapid than from the carcass. If the hypothermia is prevented by maintaining the environmental temperature at 32 to 35°, or by pretreating the animals with atropine or amphetamine, OTMN is eliminated exponentially throughout the experiment. These findings are discussed in relation to the use of OTMN as a tool for screening anti-Parkinson drugs.

TREMORINE (1,4-dipyrrolidino-2-butyne), a drug which produces tremor in animals, has been used to screen compounds for anti-Parkinson activity.[1] The observation[2, 3] that drugs may counteract the effects of tremorine by decelerating the formation of its active metabolite,[4] oxotremorine (1-pyrrolidino-4(2-oxopyrrolidino)-2-butyne), made the validity of this screening test questionable. Therefore tremorine (TMN) has been replaced by oxotremorine (OTMN) in drug-screening programs. It is now generally believed that compounds which counteract or shorten the effects of OTMN in animals have central anticholinergic actions. However, the pronounced hypothermia which is evoked by OTMN in the mouse is antagonized both by anticholinergic and amphetamine-like drugs.[5]

While studying the metabolism of OTMN in the mouse, we observed that its rate of elimination* was decelerated as the body temperature of the animals declined. The data reported here show that the administration of atropine or amphetamine, as well as non-pharmacological procedures which counteract the hypothermic effect of OTMN, also enhance its elimination and may thereby shorten the duration of the over-all syndrome produced by OTMN.

MATERIAL AND METHODS

Animals

Male Swiss albino mice (N.M.R.I., Bethesda) were used. Their weights varied from 17 to 23 g in different experiments but by no more than 2 g in any single experiment.

* In this paper the term "elimination" will be used to represent the overall disappearance of OTMN, i.e. both by conversion to more polar metabolites and by urinary excretion.

Drugs and their administration

^{3}H-Oxotremorine (S.A. = 730 mC/m-mole) was synthesized according to Karlén and Telč.[6] The radiochemical purity was established by paper chromatography (n-butanol:acetic acid:water, 4:1:5). The radioactive compound was diluted with unlabelled OTMN to yield a sp. act. which was convenient for analysis in the experiments described below. Thirty minutes before the i.p. administration of OTMN oxalate (equivalent to 0·5 mg/kg of the base), mice were injected with either saline i.p., atropine sulphate (10 or 20 mg/kg, i.p.) or amphetamine sulphate (15 mg/kg, s.c.).

Recording of body temperature

The rectal temperature of the mice was recorded with an electrothermometer at intervals of 30 or 60 min throughout the experiments. Except when otherwise stated, the experiments were run at room temperature (18–20°).

Urinary excretion of OTMN

Following administration of OTMN, single mice were placed into 100 ml beakers, the bottoms of which were covered with fine wire mesh. After 3 hr the animals were removed, wire mesh and beakers were carefully rinsed with 3 ml of water and an aliquot of the dilute urine was analyzed for OTMN as described for the homogenates.

Determination of ^{3}H-OTMN

The mice were killed by cervical dislocation. After the brain had been removed for separate analysis, the animal (=carcass) was homogenized in a Waring–Blendor with 4 parts of ice-cold water. Two ml of the homogenate, filtered through gauze, was made alkaline with 1 ml of 0·5 ml of 0·5 N NaOH and extracted with 6 ml of toluene, containing 1·5% isoamylalcohol. The brain was homogenized with 7 parts of water and 1 ml was alkalinized with 0·2 ml of 0·5 N NaOH and extracted with 3 ml of toluene (1·5% isoamylalcohol). All samples were centrifuged and 2 ml of the organic phase were counted in a Liquid Scintillation Spectrometer. Internal standards were prepared as follows: *Carcass*; animals were killed and homogenized immediately after the administration of ^{3}H-OTMN. These homogenates were considered to contain 0·1 μg/OTMN/ml. *Brain*; known amounts of ^{3}H-OTMN in a volume of 10 μl were added to 1 ml of brain homogenates from untreated mice. The standards and the samples of unknown concentrations were analyzed simultaneously.

Specificity of extraction procedure

The identity of the extracted radioactive material was authenticated by the technique of comparative distribution ratios.[3, 7] The distribution ratio of the material extracted from the homogenates of animals killed at different time points after the administration of ^{3}H-OTMN was compared with that of authentic ^{3}H-OTMN in a two phase-system, consisting of toluene and aqueous buffers of different pH-values. The solubility characteristics were similar (Table 1).

The identity of the extracted material was further ascertained by TLC of the toluene extract on silicagel G (heptane:chloroform:ethanol:diethylamine 12:2:1:1). Subsequent scanning showed the radioactivity to be located in only one spot with an average R_f-value of 0·30, identical with that of authentic ^{3}H-OTMN.

RESULTS

I. *Disappearance of OTMN from the carcass*

(1) *Elimination of a small dose of OTMN.* A small dose of OTMN (0·025 mg/kg), insufficient to lower the body temperature of the mouse, disappeared exponentially throughout the experiment. The half-life was about 35 min (Fig. 1).

TABLE 1. DISTRIBUTION OF ^{3}H-OTMN AND APPARANT ^{3}H-OTMN BETWEEN TOLUENE AND WATER AT VARIOUS PH-VALUES

pH	Authentic OTMN	Apparant OTMN	
		30 min	120 min
7·5	0·19	0·16	0·18
8·5	0·36	0·37	0·38

The apparant ^{3}H-OTMN was extracted from alkalinized tissue homogenates of mice killed at the indicated times after administration of the drug. The extraction was performed as described under 'Methods'. Aliquots of the toluene phase and of a solution of authentic ^{3}H-OTMN in toluene were shaken with equal volumes of 0·1 N Tris buffer at the indicated pH-values. The results are expressed as the ratio of the amount of radioactivity in the organic phase to total radioactivity.

(2) *Elimination of a dose of OTMN producing hypothermia.* When a dose of 0·5 mg/kg of OTMN was given to mice and the room temperature kept at 18–20°, three distinct periods of elimination of the compound from the carcass were observed (Fig. 2). During the initial period which lasted for 60–90 min, the levels of the drug declined exponentially with a half-life of approximately 100 min and the rectal temperature of the animals decreased to less than 30°. During the second period which lasted for another 60–90 min, and was the time of maximal hypothermia, the amount of OTMN in the carcass remained relatively constant. A third period followed during which the body temperature started to rise and the rate of elimination increased again. At the same time the appearance of the animals returned to normal. The described pattern of disappearance of OTMN from the carcass was consistently found in five more experiments.

(3) *Effect of environmental temperature on the elimination and hypothermic effect of OTMN.* Separate groups of mice were injected with OTMN (0·5 mg/kg) and kept at environmental temperatures of 4° (cold room), 20° (laboratory) or 35° (oven). At 35°, OTMN produced a very small drop in rectal temperature and the drug levels declined exponentially with a half-life of about 65 min (Fig. 3). At 20°, OTMN markedly lowered the body temperature and the elimination of the compound was decelerated as described above. In mice placed in the cold room of 4° the elimination of OTMN was further slowed down. Within 1 hr the rectal temperature fell to less than 15° and the animals finally died. The results of these experiments are presented in Table 2.

The hypothermic effect of OTMN becomes more marked as the environmental temperature is decreased from 25 to 20° and 15°, respectively (Fig. 4). To make

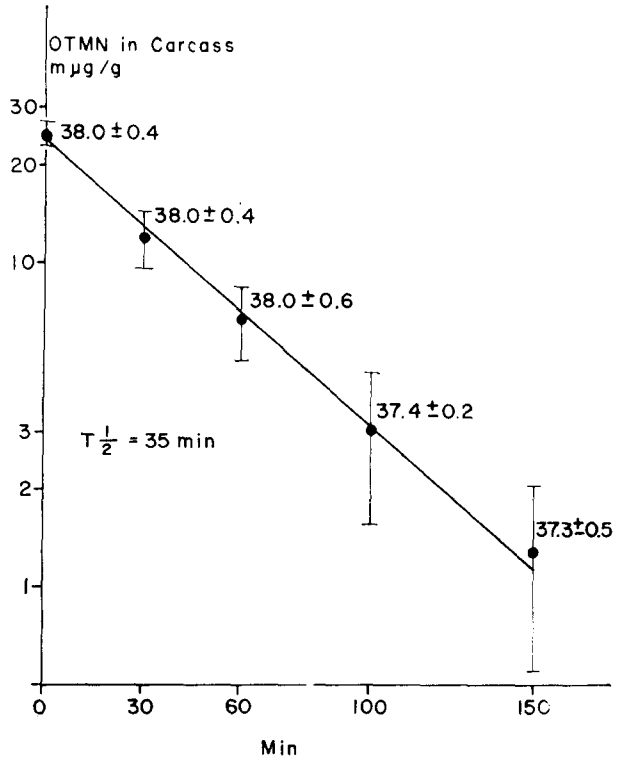

FIG. 1. Elimination of a small dose (0·025 mg/kg, i.p.) of OTMN, insufficient to lower the body temperature. First order rate of disappearance. In this and subsequent figures all data are given as means ± S.D. (n = 5). The numbers in the graph refer to the rectal temperature.

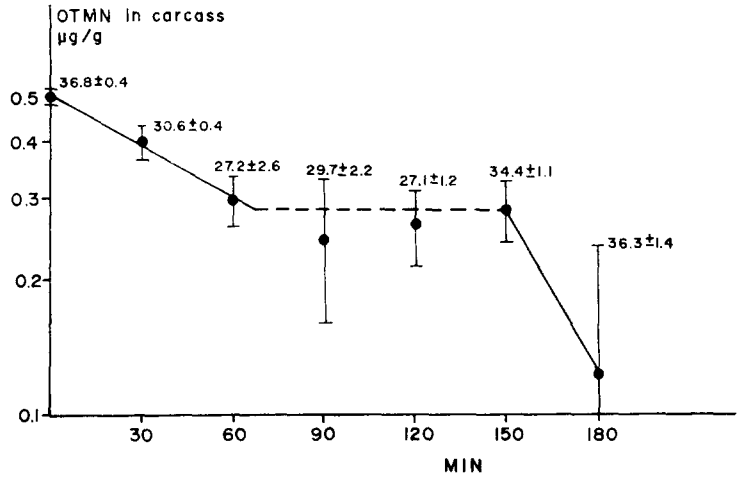

FIG. 2. Elimination of OTMN (0·5 mg/kg, i.p.) in mice kept at 20°. Note marked hypothermia.

experiments reproducable a rigorous control of the environmental temperature is therefore absolutely necessary.

(4) *Effect of atropine and amphetamine on the elimination of OTMN.* When the hypothermic effect of OTMN (0·5 mg/kg) was blocked by either atropine or amphetamine, the disappearance curve of OTMN became monophasic and the rate of elimination was markedly enhanced as compared to hypothermic mice (Table 3). After pretreatment with atropine, the half-life of OTMN was 62 min and 75 per cent was

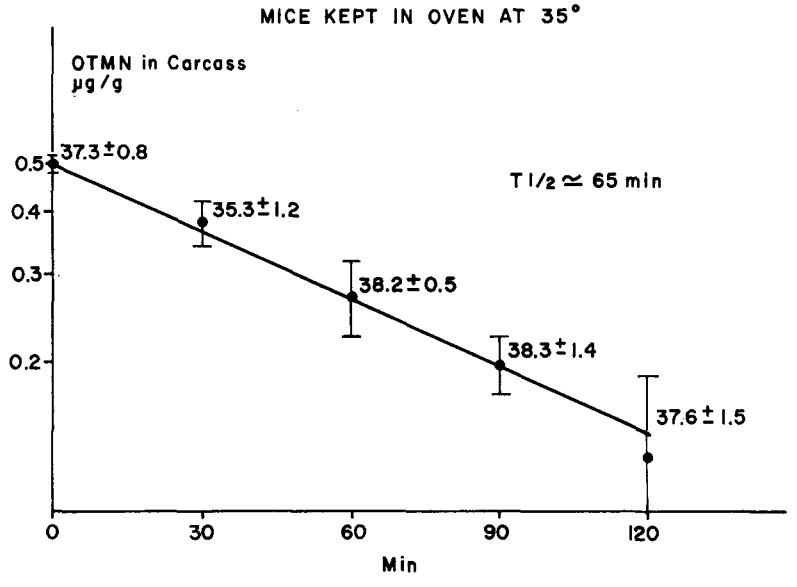

FIG. 3. Elimination of OTMN (0·5 mg/kg, i.p.) in mice kept at 35°. No hypothermia. First order rate of disappearance.

TABLE 2. EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE ELIMINATION OF OTMN IN MICE*

Environmental temperature \ Minutes after administration	Per cent OTMN eliminated			
	30 min	60 min	120 min	180 min
4°	17 ± 9 (22·0 ± 0·4)	(a) 27 ± 7 (<15)		
20°	21 ± 7 (30·6 ± 0·4)	(b) 41 ± 7 (27·2 ± 2·6)	(d) 48 ± 10 (27·1 ± 1·2)	76 ± 20 (36·3 ± 1·4)
35°	24 ± 8 (35·3 ± 1·2)	(c) 46 ± 9 (38·2 ± 0·5)	(e) 75 ± 12 (37·6 ± 1·5)	

* The experiments were run on the same day in mice randomly selected from a population of mice which, prior to the experiment, were kept under identical conditions. Figures within parenthesis are rectal temperatures at the time of killing. All data are means ±S.D. (n = 5).

Statistical significance: a–b; $P < 0·05$. a–c; $P < 0·01$. b–c; N.S. d–e; $P < 0·01$.

eliminated within 2 hr after the administration. Following pretreatment with amphetamine, OTMN disappeared with a half-life of 85 min, corresponding to an elimination of 60 per cent during the first 2 hr. By contrast, in controls pretreated with saline only 38 per cent had disappeared during the same time. The difference between treated animals and controls was significant ($P < 0.01$).

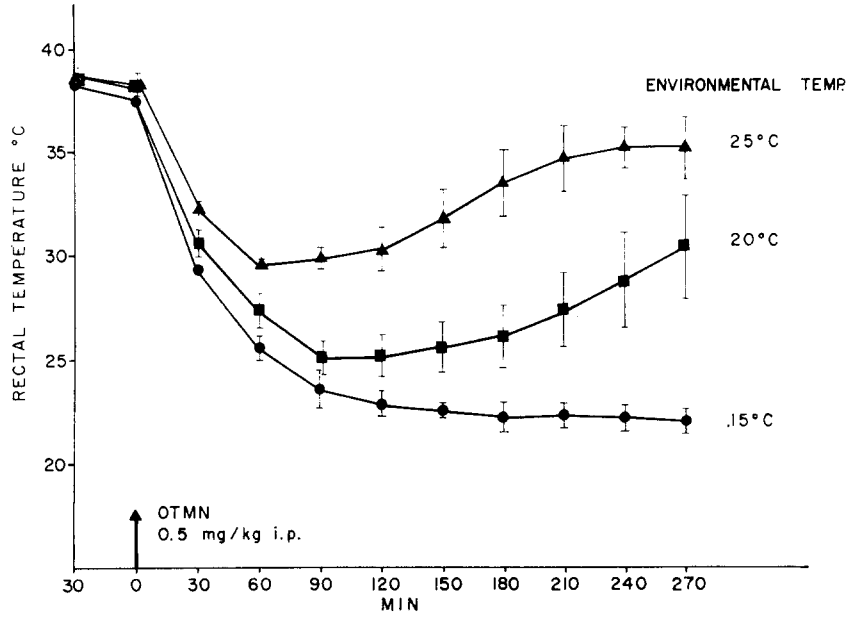

FIG. 4. Hypothermic effect of OTMN (0·5 mg/kg, i.p.) in mice kept at different environmental temperatures.

II. *Disappearance of OTMN from the brain*

The experiments described above raised the question of why the body temperature of the mouse should start to recover at a time when the amount of OTMN in the

TABLE 3. EFFECT OF ATROPINE AND AMPHETAMINE ON THE ELIMINATION OF OTMN IN MICE*

Pretreatment \ Minutes after administration	Per cent OTMN eliminated			
	30 min	60 min	90 min	120 min
None	18 ± 3 (31·3 ± 0·6)	34 ± 7 (29·8 ± 1·8)	—	(a) 38 ± 14 (27·5 ± 2·3)
Atropine sulphate 20 mg/kg i.p.	28 ± 16 (37·2 ± 0·7)	57 ± 17 (37·0 ± 0·2)	63 ± 6 (37·9 ± 0·2)	(b) 75 ± 9 (38·0 ± 0·2)
Amphetamine sulphate 15 mg/kg s.c.	26 ± 8 (37·6 ± 1·8)	33 ± 3 (38·1 ± 1·9)	55 ± 14 (38·2 ± 0·8)	(c) 61 ± 6 (39·0 ± 0·9)

* For legend see Table 2.

Statistical significance: a–b; P < 0·001. a–c; P < 0·01. b–c; N.S.

carcass was practically constant. Since the hypothermic effect of OTMN is evoked centrally, the concentration of OTMN in the brain should determine its onset and duration. It was found that the drug disappeared more rapidly from the brain than from the carcass, suggesting a redistribution from central to peripheral tissues. Two representative results of a number of experiments are presented in Figs. 5 and 6.

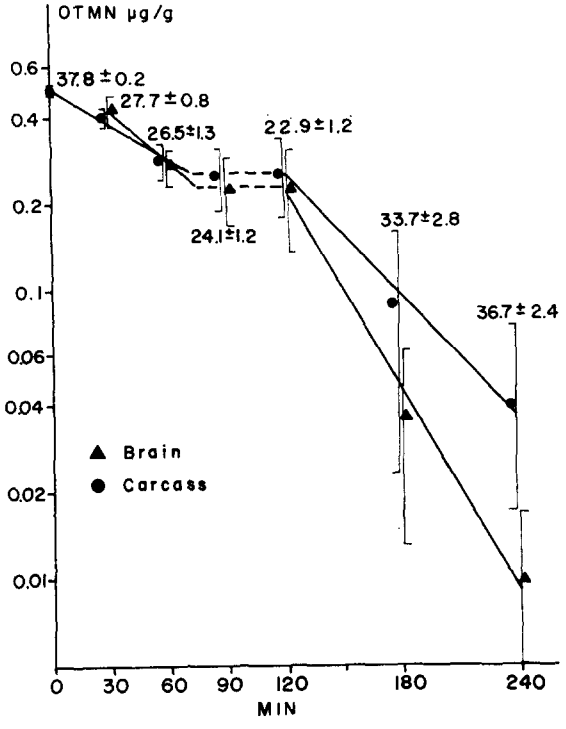

FIG. 5. Elimination of OTMN (0·5 mg/kg, i.p.) in mice kept at 18°. In this particular experiment OTMN has produced an excessive hypothermia. During the period of maximal hypothermia the disappearance of OTMN is decelerated both in carcass and brain.

In all experiments performed, brain levels of OTMN declined exponentially for the first 60 min. The half-life during this period was approximately 35 min, i.e. markedly shorter than in the rest of the body under the same experimental conditions. The further disappearance of OTMN from the brain was dependent on the degree of hypothermia produced. In the case that OTMN produced a marked hypothermia (body temperature falling below 25°), the rate of disappearance of the compound from the brain decreased markedly (Fig. 5). In the case that the hypothermia was less pronounced, the brain levels of OTMN continued to decline exponentially with a half-life of 35 min (body temperature remaining above 28°) (Fig. 6). In both cases the fractional rate of elimination increased in the terminal phase of the experiment.

III. *Urinary excretion of OTMN*

Mice injected with a hypothermia producing dose of OTMN (0·5 mg/kg) did not excrete any urine during the first 3 hr after administration, possibly due to a pronounced

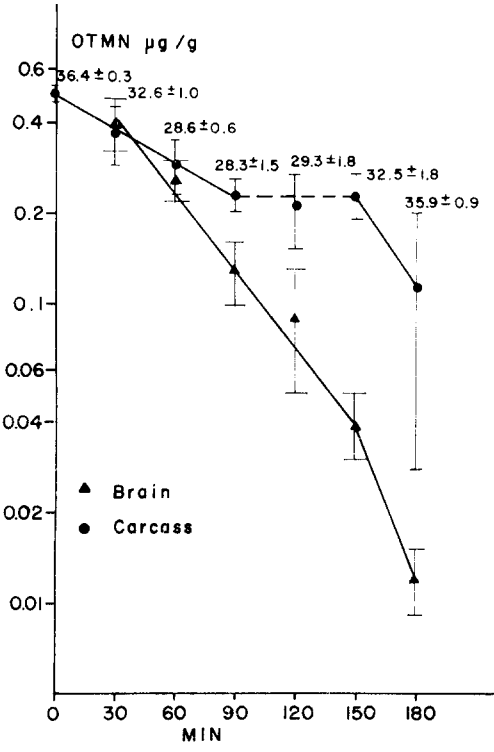

FIG. 6. Elimination of OTMN in mice kept at 22°. OTMN produced a less marked drop in body temperature. At the time of maximal hypothermia the rate of elimination of OTMN from the carcass becomes negligible, while the elimination from brain is little, if at all, decelerated.

constriction of the bladder (Table 4). In mice pretreated with atropine, about 16 per cent of the injected OTMN was recovered in the urine within this time period, while totally about 90 per cent had disappeared from the body. A somewhat higher percentage of the injected OTMN was excreted unchanged in mice given 0·025 mg/kg. Thus, urinary excretion accounts for a minor portion of the total amount of drug which is eliminated from the body.

TABLE 4. URINARY EXCRETION OF OTMN IN MICE WITHIN 3 HR AFTER ADMINISTRATION

Treatment	Per cent OTMN excreted
Saline + OTMN (0·025 mg/kg)	22 ± 8 (10)
Saline + OTMN (0·5 mg/kg)	None (10)
Atropine + OTMN (0·5 mg/kg)	16 ± 6 (10)

DISCUSSION

Our experiments demonstrate that the elimination of OTMN in the mouse is decelerated by the hypothermia which is induced by the compound. During the period of lowest body temperature, OTMN disappears from the carcass very slowly, if at all.

The question arose why the animals start to recover normal body temperature at a time when the elimination of OTMN from the carcass is negligible. When the rectal temperature did not fall below 28–30°, the disappearance of the compound from the brain was still rapid, while the levels in the carcass remained constant (Fig. 6). However, in the case of a more drastic hypothermia the disappearance curves of OTMN from both carcass and brain showed an apparent plateau between 60 and 120 min. Still the body temperature started to return to normal soon thereafter (Fig. 5). A number of reasons may be offered to explain this: The elimination from the brain may continue, though at a reduced rate, during the apparent plateau period. The rather large variation of the experimental values would permit such an assumption. Another possibility is redistribution within the brain from active to inactive sites. Also tachyphylaxis of the temperature center to the sustained OTMN levels is possible. Refractoriness to the pharmacological and electrophysiological changes produced by OTMN has been reported to occur after repeated microinjections into the lower brain stem of the cat.[10] The relationship between the pharmacological effects and brain levels of OTMN is now being studied in detail in this laboratory.

The disappearance of OTMN from the brain should depend primarily on its rate of diffusion, which in turn is proportional to the blood flow. By contrast, the elimination of OTMN from the carcass involves active enzymatic processes, which are likely to be impared at a low body temperature. Consequently, brain and carcass should be expected to dispose of OTMN at a similar rate only if blood flow and body temperature are unaffected. In accord with this view, preliminary experiments indicate that the half-life is the same in carcass and brain when OTMN is given in a dose of 0·025 mg/kg, insufficient to cause any apparant pharmacological effects. The differences in the rate constants of the first order processes of elimination of OTMN from the carcass after a dose of 0·025 mg/kg (Fig. 1) and after 0·5 mg/kg in animals pretreated with atropine or amphetamine (Figs. 4, 5) can not be explained at present. In hypothermic animals, the rate constant appears higher after the plateau period than before it and the fractional rate of elimination of OTMN from the brain seems to increase as drug levels decline.

In rats, OTMN normally evokes only a relatively slight hypothermia (1–2°) and 75 per cent of the compound is eliminated within the first 20 min after its administration. If however, the half-life of the drug is prolonged by blocking its metabolism in the liver, a drop in body temperature of several degrees is produced also in this species.[8, 9] When OTMN is incubated with liver preparations from rats or mice in the presence of a NADPH generating system, the *in vitro* metabolism of the compound is considerably faster with rat preparations.[9] The urinary excretion of OTMN is of minor quantitative importance both in rats[8] and mice. The species difference in the hypothermic response to OTMN is therefore probably explained by the quoted differences in the rate of metabolism of the drug. The slow metabolism of OTMN in the mouse is therefore not the result of the hypothermia but rather the cause of it.

Drugs which block the hypothermia produced by OTMN thereby enhance its metabolism. Such compounds, quite independent of their mechanisms of action, influence the duration and intensity of other effects of OTMN as well, e.g. tremor, salivation and lacrimation. Such substances need not necessarily be anticholinergic. Accordingly, amphetamine inhibits the hypothermic effect of OTMN and also shortens

the duration of central cholinergic symptoms such as tremor.[5] We have found that this is at least partly due to the fact that the levels of OTMN in the brain are lower in animals pretreated with amphetamine than in controls (to be published).

Observations with hexobarbital also indicate that the body temperature is an important determinant for the rate of metabolism of drugs. Thus, hexobarbital sleeping time in mice increases as the room temperature is decreased.[11] Mullen and Fouts[12] report a prolongation of hexobarbital sleeping time in mice at room temperature after pretreatment with a number of adrenergic blocking agents. These authors feel that hexobarbital metabolism was inhibited by hydralazine, phenoxybenzamine, azapetine and phentholamine both by a direct action on the microsomal enzymes (shown *in vitro*) and a depression of body temperature (2–4° below control values). However, the action of yohimbine and tolazoline was correlated only with a depression of body temperature. It is known that hypothermic children have prolonged sleeping times after anaesthesia with barbiturates. Hypothermia is now produced clinically in connection with certain surgical procedures; yet little is known about its effects on the metabolism of drugs. OTMN may be a useful tool for further studies of the effect of lowered body temperature on drug metabolism.

Our experiments illustrate the complex pharmacokinetic events which may occur when one drug is used to investigate the action of another, and which must be controlled in order to avoid artefacts in drug-screening programs. As far as the anti-OTMN test is concerned, one must control the environmental temperature and ascertain that the "anti-OTMN-drug" does not act by decreasing the brain levels of OTMN.

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