freshly distilled water, kept for no longer than a working day and employed for preparation of hospital solutions, if no extensive exogenous contamination occurs during processing. If bacterial contamination does occur, the extent is mirrored by the relatively simple methods of membrane filter culture. The membrane filter procedure thus may be employed as a screen test for pyrogenicity. Details of such a procedure will be published.

Summary. Multiple tests on solutions to which have been added known numbers of various bacterial species, suggest that 1000 or more organisms/ml must be present before the U.S.P. rabbit pyrogen test will yield a positive result. The potentiality is inferred of a simple culture procedure as a screen test for pyrogenicity of solutions to be employed for parenteral infusion.

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Relation of Ethyleneimmonium Ring Stability to Adrenergic Blocking Activity in Two Haloalkylamines.* (24351)

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A previous report(1) described the adrenergic blocking and cholinergic properties of N, N-dimethyl, 2 - chloro - 2 - phenylethylamine (DMEA). Comparative study of the 1-methyl analog (N,N-dimethyl,2-chloro-2-phenyl-1-methylethylamine) (M-DMEA) has raised questions concerning the metabolic fate of these compounds.

Methods. The experimental procedures were the same as used in the original study (1).

Results. Intramolecular rearrangement. In water solution, M-DMEA and DMEA liberated a full equivalent of chloride, indicating formation of an ethyleneimmonium ring, which in turn reacted with an equivalent of thiosulfate ion. The reactivity with thiosulfate declined with time, as the ring presumably was hydrolyzed to an alcohol. Comparison was made of speed with which M-DMEA and DMEA underwent these changes, by serial titration of chloride liberation and thiosulfate uptake. Since reaction rates varied with pH and temperature, tests were made in buffered solution (pH 7.4) at 37°C. As shown in the Table, formation of the ring form of DMEA was virtually complete within minutes and hydrolysis was complete within 1½ to 2 hours. In the case of M-DMEA, formation of the ethyleneimmonium ring was again rapid, at the same rate as for DMEA. How-

TABLE I. Rates of Intramolecular Transformations *In Vitro*.

	DMEA		M-DMEA	
Time after sol.	Chloride liberation	Thiosulfate uptake	Chloride liberation	Thiosulfate uptake
	(eq.)			
2 min.	.90	.98	.94	.98
5	.96		.97	
10	.98		.96	
30		.46		
60		.19		.89
90		.03		
2 hr		.00		.82
4				.76
6				.70
18				.41

In buffered solution (pH 7.4) at 37°C.

* Supplies of DMEA and M-DMEA were furnished by Eli Lilly Research Labs. All doses of DMEA and M-DMEA are given in terms of hydrochlorides.

ever, the ring was strikingly resistant to hydrolysis and thiosulfate uptake fell only slowly. After 18 hours in solution, approximately half the compound still remained in the intermediate stage. The time required for complete hydrolysis was not determined.

Samples containing the compounds in each of the 3 separate molecular forms were easily obtained. When M-DMEA was dissolved in distilled water, chloride liberation after 5 minutes was 0.03 meq and thiosulfate uptake 0.96; thus doses used within 5 minutes contained most of the compound in the original form. To obtain the ring form, M-DMEA was dissolved in 0.16 M sodium bicarbonate and administered within 5 minutes; samples at this time yielded 0.95 meq of chloride and reacted with 0.99 meq of thiosulfate. The hydrolyzed compound was formed by boiling 8 hours in a reflux condenser; thiosulfate uptake was then zero. Preparation of DMEA samples was described previously(1).

Adrenergic blockade. Experiments on circulation of the cat demonstrated that M-DMEA shared the adrenergic blocking property of DMEA. The blockade was specific for vasopressor activity and did not affect vasodepressor or chronotropic responses. The action appeared to be a function of the intermediate ring form, for the original molecule and the ring form were equally potent while the hydrolyzed form was inert. Furthermore, prior administration of sodium thiosulfate in a few experiments prevented development of adrenergic blockade, provided the M-DMEA was injected slowly. Blockade did appear if M-DMEA was injected rapidly in the presence of thiosulfate. This differed from observations made with DMEA; prior administration of thiosulfate prevented blockade no matter how rapidly DMEA was injected. Presumably, there were differences in reaction rates of the compounds with thiosulfate. Once blockade had been established by either DMEA or M-DMEA the effect was not altered by subsequent injection of either thiosulfate or epinephrine.

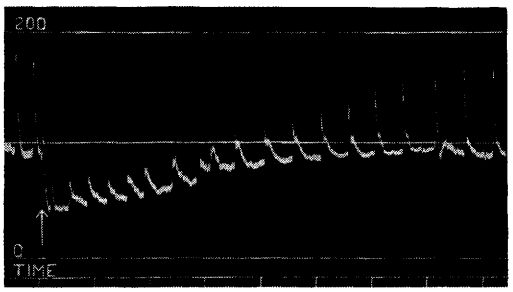

FIG. 1. Continuous recording of blood pressure of cat, anesthetized (Dial-urethane) and atropinized (2 mg/kg). Vertical excursions represent response to epinephrine (0.01 mg/kg) given I.V. every 15 min. At arrow, rapid I.V. injection of M-DMEA (5 mg/kg). Calibration in mm of mercury. Time interval 20 min. Note that adrenergic blockade is incomplete, is more marked at second than at first epinephrine dose after injection of M-DMEA, and disappears rapidly.

The 1-methyl substitution drastically weakened adrenolytic potency. Intravenous administration of about 0.5 mg/kg of DMEA reversed the pressor effects of small amounts (0.01 mg/kg) of epinephrine and 3 mg/kg produced complete blockade, reversing the effect of any amount of epinephrine. Far greater amounts of M-DMEA were needed to give equivalent results. The responses to 0.001-0.004 mg/kg of epinephrine were reversed by 2-6 mg/kg of M-DMEA. When the epinephrine dose was increased to 0.01 mg/kg, M-DMEA doses in the order of 2-3 mg/kg were required to produce any discernible reduction in the pressor response and reversal occurred only occasionally after administration of 10-15 mg/kg of M-DMEA, in divided doses. Ordinarily, these high doses were prohibited by the toxicity of the analog. It could not therefore be determined whether M-DMEA was capable of producing complete adrenergic blockade, but it appeared to be at most only 1/20 as potent a blocking agent as DMEA.

The adrenergic blocking activity of the 2 compounds differed in a second characteristic. The block by DMEA was almost immediately effective. With M-DMEA, no immediate action was apparent and an appreciable interval, usually 5-15 minutes, elapsed before the maximum blocking effect of a dose developed. Despite these differences in speed of onset, duration of adrenegic blockade was essentially the same for both. After induction of complete blockade by DMEA, pressor responses to epinephrine returned to control levels in an average of 3 hours. Comparison

with M-DMEA was of course difficult since this chemical only rarely produced epinephrine reversal. However, rate of recovery of pressor activity was approximately the same with both drugs and the blocking action of substantial amounts (3-6 mg/kg) of M-DMEA disappeared in 2 to 4 hours. A typical recovery curve is shown in the figure.

Cholinergic actions. The muscarinic actions of DMEA and M-DMEA were compared on the cat submaxillary gland preparation. Despite the demonstrated differences in adrenergic blocking potentialities, there was close parallelism in cholinergic actions. The intra-arterial threshold dose was about the same for each (0.04-0.08 mg/kg) and intensity and duration of salivation after equal doses was also the same. Despite the marked difference in speed of onset of adrenergic blocking effects, both compounds produced cholinergic effects with equal rapidity. In other respects too, M-DMEA mimicked DMEA; repeated doses showed cumulation, atropine antagonized the effect of single doses, potentiation of acetylcholine was observed, the ring form produced more intense effects more quickly than the parent structure, and the hydrolyzed form was inert.

There was but a single difference in the actions on the salivary gland. DMEA could produce prolonged salivation, lasting for hours, which was not halted by subsequent atropinization. This occurred only with large amounts given after repeated small doses and was likened to the discontinuous salivation found by Hunt and Philips(2) with a nitrogen mustard. Three attempts were made to produce this effect with M-DMEA, giving 0.6-1 mg/kg intra-arterially after serially increasing smaller doses. Prolonged salivation always resulted but this could always be stopped by atropine even after a linear flow had persisted for 30 to 60 minutes after the last dose.

Other comparisons of cholinergic activity were made on the circulation, measuring vasodepressor effects after intravenous injection in non-atropinized cats, and on autonomic ganglia, measuring the contractile response of the nictitating membrane to intra-carotid injections. In all respects the drugs were identical. However, some minor differences were noted on the myoneural junction, utilizing the cat gastrocnemius preparation. Both drugs produced paralysis but in an occasional animal M-DMEA seemed less potent than DMEA. Additionally, the ring form of DMEA was a potent stimulant to the junction and in appropriate doses produced a contracture before onset of paralysis, whereas the immonium form of M-DMEA produced only minor fasciculations. It was evidently the ring form which mediated the myoneural actions of both substances for in each instance cyclization before injection yielded more intense effects while hydrolysis of the ring abolished all myoneural activity.

Discussion. The haloalkylamines can undergo several possible transformations in solution but it has been assumed that the primary rearrangement is formation of an ethyleneimmonium ring which is subsequently hydrolyzed to an alcohol. Harvey and Nickerson (3) have confirmed that, with Dibenamine at least, the major part of dissolved drug does undergo these changes *in vitro*. Whether these reactions occur *in vivo* is unsettled. Most workers feel that the immonium ring is formed under physiological conditions and does in fact mediate activity; this view has been summarized by Graham(4). With regard to the drugs in this study, there are no data on adrenergic blocking activity incompatible with this opinion and strong support could be derived from the cholinergic actions; to explain these effects it seems necessary to postulate formation of the quaternary nitrogen immonium ring.

There is still question whether hydrolysis of the immonium compound to an alcohol occurs *in vivo*. The alcohol would be the expected metabolite of the ring, as remarked by Brodie, Aronow and Axelrod(5). Yet they were unable to detect an alcohol after administration of Dibenamine(6) or phenoxybenzamine (Dibenzyline)(5). The present data may bear indirectly on this question. Both DMEA and M-DMEA apparently form an active ring but rates of hydrolysis of the rings *in vitro* differ greatly. Yet the duration of effects is approximately the same for both, longer than the time required for spontaneous hydrolysis

of DMEA, shorter than that required for hydrolysis of M-DMEA. There is no reason to suppose that reactions rates would be grossly different in the concentrations involved in the pharmacologic effects and while an enzymatic mechanism could speed hydrolysis of M-DMEA, one would then have to assume that hydrolysis was slowed for DMEA. There is a complete lack of correlation between duration of effects and *in vitro* life of what is assumed to be the active forms. This situation makes it seem doubtful that *in vivo* metabolism of these compounds is simply a matter of spontaneous ring hydrolysis, analogous to the reactions observed *in vitro*.

Specific points of difference between DMEA and M-DMEA may be mentioned. There was considerable difference in adrenergic blocking potency. While the potency of this action of haloalkylamines cannot be predicted solely upon rates and degrees of intramolecular change, there has not previously been reported such marked difference in activity between compounds with such similar structures and rates of imine formation. Rate of development of adrenergic blockade also varied, with DMEA producing maximum effects in seconds while M-DMEA required minutes. Finally, both compounds produced long-continued salivation but only with DMEA was this effect resistant to atropine antagonism.

Summary. Acute pharmacologic comparisons have been made of N, N-dimethyl, 2-chloro-2-phenylethylamine (DMEA) and N, N-dimethyl, 2-chloro-2-phenyl-1-methylethylamine (M-DMEA). The 2 differed in immonium ring stability *in vitro,* speed of onset and degree of adrenergic blocking activity, and production of atropine-resistant salivation. There was no relationship between duration of effects and *in vitro* life of intermediate ethyleneimmonium forms.

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Absorption and Distribution of Cholesterol-4-C^{14} in the Rat.* (24352)

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The peak in the specific activity-time curve for blood cholesterol after feeding a single dose labelled cholesterol to humans(1,2,3) and to rats(4) has been reported to occur during the second or third day. This delay in the postabsorptive peak compared to most other ingested material, has not been adequately explained. The finding fo Biggs(2) that in the rat the activity of intravenously injected chyle cholesterol-H^3 is selectively taken up by the liver points to this organ as one site of delay of newly absorbed cholesterol. The present investigation was undertaken in an attempt to analyse the dynamics of absorption and distribution of dietary cholesterol in the intact rat. The results obtained indicate that in the rat dietary cholesterol is rapidly absorbed from the intestinal lumen but is held up in the intestinal mucosa cells for a considerable time before being transferred into the thoracic duct lymph over a period of days. Further delay of appearance of newly absorbed cholesterol in the blood stream is caused

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