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Effect of 2-*N*-Mono- and 2-*N*-Diethylaminoethanol on Normal and Choline-deficient Rats

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Abstract—In rats, the acute oral LD_{50} value of neutralized 2-*N*-monoethylaminoethanol (MEAE) was found to be 1·0 (0·68–1 35) g/kg. The acute oral toxicity of MEAE, but not that of 2-*N*-diethylaminoethanol (DEAE) could be largely reversed by simultaneous or subsequent ingestion of choline. MEAE partially prevented the development of fatty liver in choline-deficient rats. No reversal of depressed growth rate was noted in choline-deficient animals whose diets were supplemented with either MEAE or DEAE.

INTRODUCTION

The *N*-substituted aminoethanols (ethanolamines) are excellent curing agents, flotation agents, dispersants and emulsifiers. As such, they find wide application in the manufacture of cosmetics, shampoos, waxes, polishes, lubricants, resins and other related materials.

The continuous inhalation toxicity of the parent compound, 2-aminoethanol was the subject of a report by Weeks, Downing, Musselman, Carson & Groff (1960) and two *N*-substituted aminoethanols, 2-*N*-monoethylaminoethanol (MEAE) and 2-*N*-diethylaminoethanol (DEAE), are the subjects of the present paper. The biological activity of *N*-ethylaminoethanols is of considerable interest because of their possible action as antimetabolites due to the structural relationship of these compounds to choline (*N*-trimethylaminoethanol). Choline has vitamin-like activity in animals and deficiencies may result in fatty liver, anaemia and hypoproteinaemia. Moyer & du Vigneaud (1942) demonstrated that *N,N*-dimethylethylaminoethanol functioned as choline in promoting the growth of rats on a diet deficient in choline and supplemented with homocysteine. *N,N*-Diethylmethylaminoethanol was quite toxic. Both these two choline analogues could be formed by the *in vivo* methylation of MEAE and DEAE. Thus, if the acute toxicities of MEAE and DEAE are related to their rapid conversion to *N,N*-dimethylethyl- and *N,N*-diethylmethylaminoethanol respectively, one would anticipate, on the basis of the work by Moyer & du Vigneaud (1942), that DEAE would be considerably more toxic than MEAE. *N*-Triethylaminoethanol has been extensively investigated (Stekol & Weiss, 1950). It is lipotropic and prevents renal haemorrhage in young choline-deficient rats. However, animals also exhibit muscular weakness and convulsions. These toxic signs can be prevented by the simultaneous consumption of equivalent amounts of choline. Thus, *N*-triethylaminoethanol appears to substitute for choline as a lipotropic agent but by other mechanisms also produces a toxic effect. Hence, there is considerable evidence to indicate that *N*-ethyl analogues of choline may interfere with the normal metabolism of choline. The present studies were undertaken to determine the relationship of MEAE and DEAE toxicity to metabolic functions of choline. The LD_{50} and 95% confidence level of neutralized DEAE (5·6 (3·5–9·1) g/kg) has been previously reported (Cornish, 1965).

METHODS

Animals and materials. Sprague–Dawley strain (Rawley Farms) male rats were utilized in all studies. MEAE and DEAE were procured from Eastman Organic Chemicals (Rochester, N.Y.) and redistilled prior to use. Since the primary interest was in the metabolic effects of MEAE and DEAE all solutions of these compounds were adjusted to pH 7 with HCl prior to dosing.

Experimental design and conduct. Acute oral single dose studies were carried out to determine appropriate working levels and to provide tissues for histopathological examination. The LD_{50} value of neutralized MEAE was determined by feeding geometrically graded doses to groups of five male rats at each dosage and observing mortality over a period of 14 days. Calculations of the LD_{50} and 95% confidence level were made utilizing the tables of Weil (1952).

To determine the effect of choline on the acute oral toxicity of MEAE and DEAE, choline (80 mg/rat) was given by stomach tube or in the drinking water (4 mg/ml) to animals which had previously received an oral LD_{50} or LD_{100} dose of the *N*-substituted aminoethanol. Ten male rats, 150–175 g were used in each group.

In more prolonged feeding studies, rats were fed neutralized MEAE or DEAE in the drinking water (10–15 mg/rat/day) and were maintained on either normal or choline-deficient diets (Nutritional Biochemical Corp., Cleveland, Ohio) for periods of 2 or 4 wk. At sacrifice, liver and kidney weights and body weight were recorded. Blood was collected for the determination of total serum lipids, cholesterol, cholesterol esters, and phospholipids. Tissues were taken for histopathological studies. Cholesterol and cholesterol esters were determined by the colorimetric method of Schoenheimer & Sperry (1954). Phospholipids were digested and determined by the phosphate method of Fiske & Subbarow (1925). Liver lipids were determined gravimetrically after homogenizing the tissue in chloroform–methanol (3:1, by vol.), filtering, evaporating the extract to dryness on a steambath and re-extracting with petroleum ether. The petroleum ether extracts were evaporated to dryness in tared beakers and the lipid determined by weighing.

RESULTS

Acute toxicity

The acute oral LD_{50} of neutralized MEAE was found to be 1·0 (0·68–1·35) g/kg. Histopathological studies of lung, liver, kidney, and spleen revealed no demonstrable organ damage in animals sacrificed 24 hr after a single LD_{50} dose.

Effect of choline on MEAE and DEAE toxicity

The mortality data of rats fed a single oral dose of 2 g/kg of MEAE with and without added choline are shown in Table 1. In three separate trials, the mortality rate of rats receiving 2 g/kg of MEAE was 7/8, 10/10, and 9/10 (total: 26/28; 7% survival). Deaths occurred 2–4 days after ingestion of the compound. Among animals which received the same oral dose of MEAE, but subsequently received 4 mg/ml of choline in the drinking water, approximately 50% survived (mortality 3/8, 6/10, and 4/10; total: 13/28). Similarly, rats which received a single 80 mg oral dose of choline simultaneously with a 2 g/kg oral dose of MEAE experienced a death rate of only 2/8, 4/10, and 3/10; total 9/28 (60% survival). In addition, most of the deaths were delayed to day 4 and 5 in the choline-supplemented rats.

Table 1. *Effect of choline on mortality rate of rats given a single oral dose (2 g/kg) of MEAE*

Days after MEAE treatment		No. of deaths		
		MEAE	Choline* plus MEAE	Choline† plus MEAE
		Trial 1		
2		3	–	–
3		2	1	1
4		1	1	1
5		1	1	–
	Total....	7/8	3/8	2/8
		Trial 2		
2		4	–	–
3		3	1	–
4		3	2	4
5		–	3	–
	Total....	10/10	6/10	4/10
		Trial 3		
3		6	–	1
4		2	1	–
5		1	3	1
6		–	–	1
	Total....	9/10	4/10	3/10
	Total....	26/28	13/28	9/28

*Choline was administered at a level of 4 mg/ml in the drinking water.
†Choline was administered as a single oral dose (80 mg/rat) at the time of MEAE administration.

When choline and DEAE were administered together or when choline was added to the drinking water after DEAE ingestion, there was no apparent effect on the mortality rate when compared to DEAE administration alone (Table 2). When given a dose of 8 g/kg of DEAE, approximately 50% of all animals died including those groups receiving choline. Dosages of 10 and 12 g/kg of DEAE resulted in the death of all animals including those receiving choline.

Table 2. *Effect of choline on mortality rate of rats given a single oral dose (8 g/kg) of DEAE*

Days after DEAE treatment		No. of deaths		
		DEAE	Choline* plus DEAE	Choline† plus DEAE
		Trial 1		
1		3	2	5
2		–	–	–
3		–	1	–
	Total....	3/10	3/10	5/10
		Trial 2		
1		5	3	5
2		1	1	1
3		–	–	–
	Total....	6/10	4/10	6/10
	Total....	9/20	7/20	11/20

*Choline was administered at a level of 4 mg/ml in the drinking water.
†Choline was administered as a single oral dose (80 mg/rat) at the time of DEAE administration.

4-Wk feeding studies with MEAE and DEAE

Male rats (150–175 g) maintained on normal diets were fed neutralized MEAE and DEAE in drinking water at a level of 4 mg/ml. The total intake of compound was in the range of 80–120 mg/rat/day. The only effect noted after 4 wk of DEAE feeding was a slight elevation in kidney weight to body weight ratios ($P<0.05$). The addition of MEAE to the drinking water at a level of 4 mg/ml was markedly toxic. Nine animals died during the first week and five were sacrificed at the end of 1 wk. The surviving animals had lost approximately 25 g in weight. Kidney weight ratios of 1·06 (0·99–1·13) were well above the control value of 0·76 (0·70–0·87). Actual kidney weights were only slightly elevated and the ratio is primarily affected by the loss of body weight. Liver lipids, serum lipids, cholesterol levels and liver weight to body weight ratios of rats given MEAE or DEAE were within normal ranges. No significant histopathological changes were seen in the lung, liver, kidney and spleen of treated rats.

Effect of MEAE or DEAE on rats placed on a choline-deficient diet

To study the relationship of these compounds with choline metabolism, animals on choline-deficient diets received supplements of MEAE or DEAE in the drinking water over periods of 2 and 4 wk (Table 3). Body weight gains of all animals on choline-deficient diets were 50–60 g below those of control animals at the end of 2 wk. During the second 2-wk period, choline-deficient animals gained weight at the same rate as controls but remained approximately 50 g below the weight of animals on the normal diet. Organ to body weight ratios of choline-deficient animals receiving MEAE or DEAE supplement were not significantly different from choline-deficient controls at both the 2- and 4-wk intervals. After 2 wk on the choline-deficient diet, liver lipids were double those of control animals ($P<0.05$) while serum cholesterol levels were depressed ($P<0.01$). Serum total lipid and phospholipid levels were not different from those of normal rats. The presence of MEAE or DEAE in the diet during the 2-wk period on the choline-deficient diet was without apparent effect other than a slight further depression of mean cholesterol levels ($P<0.01$). After 4 wk on the

Table 3. *Effect of MEAE† or DEAE† on choline-deficient rats*

Group	Week on test	Body weight gain (g)	Relative organ weight (g/100 g body weight)		Liver lipid (%)	Serum constituents (mg/100 ml)		
			Liver	Kidney		Total lipid	Total cholesterol	Phospholipids
Control	2	81 ±3	0·76±0·01	4·3±0·1	3·2 ±0·2	329±40	69 ±1	78±4
	4	121 ±13	0·76±0.01	3·4±0·2	3·2 ±0·1	263±50	59 ±7	64±14
Choline deficient								
No addition	2	29 ±3**	0·81±0·02	4·3±0·2	6·9*±0·8	249±21	42**±6	96±22
MEAE		31 ±4**	0·84±0·01	4·3±0·1	6·4*±1·3	360±22	30**±2	78±12
DEAE		24 ±6**	0·82±0·04	4·6±0·1	7·3*±1·3	269±19	24**±2	66±10
Choline deficient								
No addition	4	75**±4	0·78±0·06	3·7±0·3	9·5*±2·6	257±23	56 ±11	70±3
MEAE		84**±5	0·75±0·03	3·6±0·1	4·6‡±0·5	247±17	59 ±4	90±27
DEAE		78**±7	0·74±0·02	4·1±0·1	5·7 ±0·8	281±27	38 ±7	81±14

Results are expressed as the means ±SE for groups of five rats. Values marked with asterisks differ significantly from those of controls: *$P<0.05$; **$P<0.01$. With the exception of the liver lipid value (wk 4) for the MEAE/choline-deficient group, no differences were seen at wk 2 or 4 between the three choline-deficient groups.

†Given in the drinking water to provide a level of 10–15 mg/rat/day.

‡Significantly different from choline-deficient rats ($P<0.05$).

choline-deficient diet, liver lipids were markedly elevated averaging 9·5% ($P<0·05$). The presence of MEAE in the diet for the full 4 wk appeared to prevent partially this marked elevation of liver lipid in choline-deficient rats, the mean liver lipid value being 4·6% ($P<0·05$). DEAE in the diet for 4 wk also appeared to prevent partially liver lipid accumulation (5·6%) although this is not statistically different from the choline-deficient animals (9·5%). Serum lipid, total cholesterol, and phospholipid levels in animals on 4-wk diets supplemented with DEAE or MEAE and deficient in choline were not significantly different from those of the choline-deficient group.

Histopathological sections of lung, liver, kidney and spleen of MEAE- or DEAE-treated choline-deficient rats at 2 and 4 wk were not different from the choline-deficient controls. Moderate to heavy lipid infiltration was present in the liver sections of all choline-deficient animals.

DISCUSSION

The acute oral LD_{50} values in rats indicate that neutralized MEAE is more acutely toxic than DEAE. However, deaths due to MEAE occurred several days after ingestion of the compound while those from DEAE usually occurred within 24 hr. Thus the acute toxicity of MEAE and DEAE parallels that of *N*-mono- and *N*-dimethylaminoethanol (du Vigneaud, Chandler, Simmonds, Moyer & Cohn, 1946) in that the mono-substituted aminoethanol is considerably more toxic than the di-substituted compound.

When rats were fed 80–120 mg/day of MEAE most of them succumbed by the end of the first week. Animals which received the same daily dose of DEAE survived the 4-wk feeding schedule, developing only slight elevations in kidney weight to body weight ratios. Thus the greater toxicity of the mono-substituted compound was also evident in these feeding studies.

Choline-deficient rats, whose diets were supplemented with relatively low doses of *N*-mono- and *N*-diethylaminoethanol had mean values of liver lipids considerably below those of choline-deficient rats. However, this decrease is statistically significant at the 95% level only in rats receiving the 4-wk MEAE-supplemented diets.

Bell, Davis & Strength (1964) indicated that MEAE and DEAE are incorporated into the phospholipids of choline-deficient rats. In the present study the ability of MEAE to prevent partially the development of fatty liver in choline-deficient animals indicates that this compound may also have some lipotropic activity.

The ability of choline to reduce the mortality rate of rats fed an approximate oral LD_{100} dose of MEAE suggests that these two compounds enter into similar metabolic pathways. This protective effect of choline was not evident in animals fed acutely toxic doses of DEAE.

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Effet du 2-*N*-mono et 2-*N*-diéthylaminoéthanol sur des rats normaux et à déficience de choline

Résumé—On a trouvé que le taux de la DL_{50} orale aigué de la 2-*N*-monoéthylaminoéthanol (MEAE) neutralisée était chez les rats de 1,0 (0,68 à 1,35) g/kg. La toxicité orale aigué du MEAE, mais non celle due 2-*N*-diéthylaminoéthanol (DEAE), pouvait être inversée de manière importante par l'ingestion simultanée ou consécutive de choline Le MEAE empêche en partie le développement de la dégénérescence graisseuse du foie chez les rats carencés en choline. On n'a pas noté de réversion de la diminution de la vitesse de croissance chez les rongeurs carencés en choline dont le régime avait été supplémenté soit en MEAE, soit en DEAE.

Einfluss von 2-*N*-Mono- und 2-*N*-Diäthylaminoäthanol auf normale Ratten und Ratten mit Cholinmangel

Zusammenfassung—Bei Ratten wurde der akute orale LD_{50}-Wert fur neutralisiertes 2-*N*-Monoathylaminoathanol (MÄAÄ) mit 1,0 (0,68–1,35) g/kg festgestellt. Die akute orale Toxizitat von MÄAÄ, aber nicht die von 2-*N*-Diathylaminoathanol (DÄAÄ) konnte grosstenteils durch gleichzeitige oder anschliessende Verfütterung von Cholin aufgehoben werden. MÄAÄ verhinderte teilweise die Entwicklung einer Fettleber bei Cholinmangel im Futter von Ratten. Keine Verbesserung der Wachstumsrate war bei Cholinmangel der Tiere zu bemerken, deren Futter durch MÄAÄ oder DÄAÄ ergänzt wurde.