mechanism. The present results may thus allow the conclusion that DAQ exhibits a DPN-like type of effect on the mitochondrial ATPase, whereas DSQ and AAT appear to interact primarily with some component or reaction governing the structural integrity of the particles.

In view of the reverse relationship between water uptake by and structural integrity of isolated mitochondria, it is of interest that DNP has been shown[4, 5] to counteract the spontaneous and thyroxine-induced swelling of liver mitochondria incubated in the absence of oxidizable substrate and cofactors, as measured by (changes in) the optical density at 520 mμ of such suspensions. In similar experiments carried out with DAQ and DSQ a small increase, respectively a decrease, of the $o.d._{520}$ of the mitochondria, as compared with that of the controls, was observed. However, an interpretation of the results in terms of mitochondrial shrinkage or swelling was difficult, if not impossible, in view of the limited solubility of the compounds (approximately one-third of the added $1{\cdot}5 \times 10^{-4}$ was in real solution); part of the finely-dispersed compound may become attached to the particles and thus change the optical density of the suspension (measured at 660 mμ in the present case in view of the absorbency of DAQ at 520 mμ). However, in the case of AAT, which was in complete solution, a marked swelling (decrease of 30–50 per cent in $o.d._{520}$) has been noted previously.[3] It has also been reported[2] that DSQ causes a notable swelling of liver mitochondria when Mg^{2+} was present. This effect, i.e. fall in optical density which was interpreted as swelling, has now been found by phase contrast microscopical examination to be due to an agglutination of the particles. Addition of Mg^{2+} (as chloride, 5×10^{-3} M) to the control suspensions resulted in many very small aggregates of mitochondrial bodies without causing a change in the optical density of the suspension as compared with the controls. Addition of DSQ and Mg^{2+} gave rise to the formation of elaborate aggregates of particles and a marked fall in the optical density (60 per cent decrease). In the presence of DAQ and Mg^{2+}, however, no change in the optical density of the mitochondrial suspension was observed, the agglutination of the particles being less than in the former case but still more extensive than in the controls receiving Mg^{2+} only. Since DSQ or DAQ did not cause an agglutination of the mitochondria in the absence of Mg^{2+} these compounds, and DSQ especially so, appear to accelerate the agglutination of mitochondria incubated in the presence of Mg^{2+}.

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REFERENCES

1. P. SIEKEVITZ, H. LÖW, L. ERNSTER and O. LINDBERG, *Biochim. Biophys. Acta* **29**, 378 (1958).
2. P. EMMELOT and C. J. BOS, *Biochem. Pharmacol.* **1**, 105 (1958).
3. P. EMMELOT and C. J. BOS, *Biochim. Biophys. Acta* **24**, 442 (1957).
4. D. F. TAPLEY, *J. Biol. Chem.* **222**, 325 (1956).
5. P. EMMELOT and C. J. BOS, *Exp. Cell Res.* **12**, 191 (1957).

Variations in toxicity of some halogen derivatives of acetic acid in rats

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SOME halogen derivatives of acetic acid have been used extensively in biological systems and show a degree of toxicity. For example monoiodoacetate acts as an alkylating agent for sulphydryl groups[1] and monofluoroacetate is toxic because it is a precursor of fluorocitrate, which in turn is an inhibitor of aconitase.[2] Because of the lipotropic properties of ethyl trichloroacetate[3, 4] other halogen derivatives of acetate were examined for this property, and in this study their relative toxicity in young rats has been noted.

Young rats (60–80 g) of both sexes were kept on a choline deficient diet which provided 8 per cent casein and 12 per cent gelatin in the food.[4] From the commencement of the experiment (unless otherwise stated) the rats received subcutaneous injections of the ethyl ester of one of the di- or tri-halogen derivatives of acetic acid. The ethyl esters were used rather than the neutralized acids, since the esters should not be excreted as quickly as the salts and hence would provide a higher level of halogen compound in the animal for a longer time. In addition the use of the esters prevented the difficulties involved in injecting large volumes of water soluble salts.

Some typical results are summarized in Table 1, which shows that rats tolerated relatively large doses of di- or tri-halogen esters. In the doses given both the di- or tri-fluoroacetate esters were

TABLE 1. TOXICITY OF SOME HALOGEN DERIVATIVES OF ACETIC ACID IN RATS

Derivatives	No. Rats	Sex	Dosage (mM/100 g)	Total dosage over injection period (mM)	Deaths within injection period
ethyl trifluoroacetate	10	M + F	0·72	10·9	0
	10	M + F	0·72	14·5	0
ethyl trichloroacetate	13	F	1·45	29	2 after 4 injections
	10	M	0·72	10·9	0
ethyl tribromoacetate	10	M	0·52	4·7	1 after 3 injections
ethyl difluoroacetate	10	M	0·66	13·2	1 after 15 injections
ethyl dichloroacetate	5	F	1·44	8·6	2 after 2 injections 2 after 4 injections
	5	M + F	1·44*	1·4	5 after 1 injection
ethyl dichloroacetate†	5	F	1·44	8·6	0
	8	F	1·44	7·2	0

* Rats were in established choline deficiency.
† Rats fed a commercial rat cube which was used for breeding colony.

non-lethal, and were in marked contrast to the lethal oral dose of 0·06 mM/kg for monofluoroacetate reported by Peters.[2] The trichloroacetate was tolerated without signs of distress at doses which were in agreement with the low oral toxicity found by Woodard, Lange, Nelson and Calvery.[5] These authors found that the oral LD_{50} dose for monochloroacetate was 0·08 mM/kg about 1/40 of the lethal dose of trichloroacetate.

The toxicity of the dichloroacetate ester was interesting, for on the experimental dietary regime, with or without added choline, the ester was lethal at the dose given. When the same amount of ethyl dichloroacetate was given to rats of the same size fed the usual diet given to the breeding colony, the ester was not lethal. The difference between the two diets was particularly marked in the quality of protein provided; the breeding diet provided protein which supported rapid growth and reproduction of rats. The toxicity appeared to be depressed when an adequate level of essential amino acids was supplied in the diet. Whether protein was needed to form a detoxication complex with the acid, or for replacement of damaged tissue, has not been investigated. An alternative possibility is that liver halidase[6] may remove one chlorine atom to form monochloroacetate which would combine with tissue-SH groups.

The fluoro derivatives produced more necrotic tissue at the site of injection than was produced in animals given the chloro derivatives. This damage might result from the local accumulation of free acid which could result from the hydrolysis of the ester. Gorin, Pierce and McBee[7] have shown that the ethyl esters of both di- and tri-fluoroacetic acid break down rapidly even in an initially neutral aqueous medium. On the other hand ethyl dichloroacetate has been shown to be relatively difficult

to hydrolyse,[8] so that local concentrations of free acid may not build up with the chloroacids. However, despite this necrotic damage, the ester-treated animals with the exception of those given ethyl tribromoacetate, increased in body weight during the period of injections at rates which were only slightly less than those of uninjected controls.

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REFERENCES

1. E. LUNDSGAARD, *Biochem. Z.*, **217**, 162 (1930).
2. R. A. PETERS, *Proc. Royal Soc. London*, Ser. B, **139**, 143 (1952).
3. C. C. KRATZING and G. M. WINDRUM, *Nature*, **180**, 859 (1957).
4. C. C. KRATZING and G. M. WINDRUM, *Aust. J. Exp. Biol. Med. Sci.*, **37**, 321 (1959).
5. G. WOODARD, S. W. LANGE, K. W. NELSON and H. O. CALVERY, *J. Indust. Hyg. and Toxicol*, **23**, 78 (1941).
6. L. A. HEPPEL and V. T. PORTERFIELD, *J. Biol. Chem.*, **176**, 763 (1948).
7. G. GORIN, O. R. PIERCE and E. T. MCBEE, *J. Amer. Chem. Soc.*, **75**, 5622 (1953).
8. P. M. NAIR and E. S. AMIS, *J. Amer. Chem. Soc.*, **77**, 3452 (1955).

Dimethanesulphonic Acid Esters of Sugar Alcohols

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THE finding of an interesting degree of tumour inhibiting activity in 1,6-dimethanesulphonyl-D-mannitol (I)[1] and the possibility that this activity might depend upon stereochemical and other structural factors led us to investigate some appropriate isomers and analogues in the sugar alcohol series. In brief, all the following compounds were found to be inactive or very feebly active as inhibitors of growth of the Walker tumour, in comparison with (I).

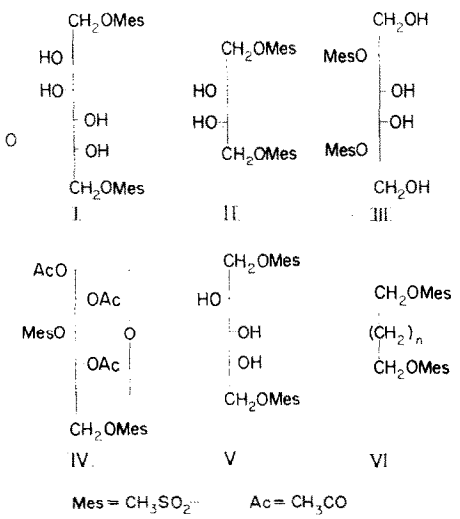

FIG. 1