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The Lipotropic Action of some Halogen Derivatives of Acetic Acid

By C. C. KRATZING AND G. M. WINDRUM

Department of Physiology, University of Queensland, Brisbane, and Department of Pathology, Brisbane Hospital, Brisbane, Australia

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In previous work it was found that ethyl trichloroacetate was effective in lowering the high amount of liver lipid which accumulated when rats were fed a choline-deficient diet (Kratzing & Windrum, 1957, 1959). It seemed likely that other halogen derivatives of acetic acid might also possess this lipotropic action.

The investigation reported here shows that ethyl trifluoroacetate was able to prevent accumulation of excess of lipids in the liver of choline-deficient rats. Ethyl acetate, ethyl dichloroacetate and ethyl tribromoacetate were without demonstrable lipotropic action.

METHODS

Animals. The present experiments were carried out in separate laboratories; two different locally bred strains of young rats were used. The animals were housed separately in wire cages with wire-mesh floors. Strain 'A' rats were from the Physiology Department colony and were the same strain used for the experiments described in the initial papers (Kratzing & Windrum, 1957, 1959). Strain 'B' rats were from the Brisbane Hospital colony and had been used for experiments described in another paper (Windrum & Kratzing, 1960).

Diet. Strain 'A' rats were fed *ad lib.* on the diet used in previous experiments (Kratzing & Windrum, 1959). Strain 'B' rats were fed on the same diet to which 0.4% of cystine was added in order to increase the requirement of choline as discussed previously (Windrum & Kratzing, 1960). The amount of food fed to strain 'B' rats was adjusted to maintain the growth of all groups at an approximately uniform rate. Choline was fed to the control groups as choline chloride.

Esters. Ethyl trichloroacetate was prepared as described by Kratzing & Windrum (1959). Ethyl tribromoacetate,

ethyl trifluoroacetate and ethyl dichloroacetate were prepared from the corresponding acids and the fractions which boiled between 120° and 125° (at 35 mm. Hg), 62° and 64°, and 156° and 159° respectively were used for injection. Ethyl acetate was a redistilled commercial sample.

Treatment. The esters were given for a 3- or 4-week period from the time of commencement of the deficient diet, except that strain 'A' rats, given ethyl dichloroacetate, were fed the choline-deficient diet for 3 weeks before commencing injections. The animals were injected subcutaneously once daily 5 days a week. Series 'B' rats were given the ethyl acetate and ethyl trichloroacetate as a 1:1 dilution in liquid paraffin for 4 weeks. Ethyl trifluoroacetate was injected as such because it was not completely miscible with paraffin. In order to minimize any lipotropic effect of the injection itself (see Kratzing & Windrum, 1959), strain 'A' choline-deficient control rats received injections of 0.9% sodium chloride and strain 'B' animals were given subcutaneous injections of liquid paraffin.

The animals were killed 24 hr. after the last injection and exsanguinated, and the livers and kidneys were excised and weighed.

Analysis. The liver-lipid content of strain 'A' rats was determined by the method described by Wheeldon & Collins (1957). The lipid content of strain 'B' rat liver was determined by the method used by Windrum & Kratzing (1960).

RESULTS

Ethyl acetate. Expt. 1 of Table 1 shows that strain 'A' rats fed on the choline-deficient diet did not respond with lowered lipid content of the liver when given repeated doses of ethyl acetate. The dosage given was that previously found effective with ethyl trichloroacetate (on an equimolar basis) in reducing excess of liver lipids (Kratzing & Windrum, 1959).

In this experiment the administration of either ethyl acetate or trichloroacetate did not result in any significant change in the rate of growth of animals in the different groups. The ratio of liver weight to body weight was significantly ($P < 0.01$) greater in choline-deficient and ethyl acetate-treated rats than in the choline-fed controls. It was also significantly greater in the ethyl trichloroacetate-treated group. The ratio of kidney weight to body weight was significantly increased in the ethyl trichloroacetate-supplemented groups compared with the choline-deficient group ($P < 0.01$). The choline-deficient group had a kidney to body weight ratio that did not differ significantly from the choline-supplemented group. However, the ethyl acetate group had a kidney to body weight ratio

that was significantly ($P < 0.001$) higher than the comparable choline-deficient controls.

In a confirmatory experiment (Expt. 3, Table 1) in which rats with established choline deficiency were given 2-3 times the previous dose of either ethyl acetate or trichloroacetate, only the latter was effective in clearing the liver of excess of lipid.

Strain 'B' rats also did not respond to ethyl acetate supplements. A group which had received 20 doses of 360 μ moles of ethyl acetate in paraffin during 28 days on the deficient diet had a mean liver-lipid content of 15%, which was not significantly different from that of the paraffin-injected controls (Table 2).

Ethyl tribromoacetate. This was not as effective as ethyl trichloroacetate in removing excess of lipid

Table 1. *Effect of halogen-containing esters on organ weights and liver-lipid content*

Strain 'A' rats while on a choline-deficient diet were given the following esters by subcutaneous injection. Choline, when added, was given at 20 mg./10 g. of diet. The rats in Expts. 1 and 4 were all males. In Expts. 2 and 3 the sexes were mixed. Figures represent the average values $\pm$ s.e.m. Abbreviations: EDCA, ethyl dichloroacetate; ETCA, ethyl trichloroacetate; ETFA, ethyl trifluoroacetate; ETBA, ethyl tribromoacetate; EA, ethyl acetate. Choline: +, present; -, absent.

Expt. no.	Choline	Ester	No of rats	Dosage of ester (μ moles)	Initial wt. (g.)	Final wt. (g.)	Kidney wt. as % of body wt.	Liver wt. as % of body wt.	Lipid content of wet liver (%)
1	-	.	10	.	70 $\pm$ 1	96 $\pm$ 4	0.96 $\pm$ 0.02	4.68 $\pm$ 0.21	13.6 $\pm$ 2.5
	-	EDCA	10	360	70 $\pm$ 1	90 $\pm$ 2	1.11 $\pm$ 0.02	4.93 $\pm$ 0.12	6.6 $\pm$ 0.6
	-	ETCA	10	540	70 $\pm$ 1	93 $\pm$ 3	1.05 $\pm$ 0.02	5.19 $\pm$ 0.12	5.9 $\pm$ 0.3
	-	EA	10	720	70 $\pm$ 1	92 $\pm$ 2	1.08 $\pm$ 0.04	5.42 $\pm$ 0.35	12.9 $\pm$ 1.5
	+	.	10	.	70 $\pm$ 1	97 $\pm$ 3	0.94 $\pm$ 0.02	4.01 $\pm$ 0.11	4.9 $\pm$ 0.2
2	-	.	10	.	73 $\pm$ 3	89 $\pm$ 4	0.93 $\pm$ 0.02	5.65 $\pm$ 0.18	23.9 $\pm$ 2.3
	-	ETCA	10	360	72 $\pm$ 3	86 $\pm$ 6*	0.91 $\pm$ 0.02	4.98 $\pm$ 0.14	8.0 $\pm$ 0.9
	-	ETFA	10	360	73 $\pm$ 2	80 $\pm$ 4	1.09 $\pm$ 0.04	5.52 $\pm$ 0.15	6.7 $\pm$ 0.3
	+	.	10	.	73 $\pm$ 3	89 $\pm$ 4	0.87 $\pm$ 0.03	3.80 $\pm$ 0.12	4.8 $\pm$ 0.2
3	-	.	8	.	68 $\pm$ 4	87 $\pm$ 4	.	.	11.7 $\pm$ 1.5
	-	EA	7	1440	69 $\pm$ 4	72 $\pm$ 8†	.	.	9.1 $\pm$ 1.4
	-	ETCA	6	1440	66 $\pm$ 4	86 $\pm$ 9†	.	.	5.0 $\pm$ 0.1
4	-	.	9	.	74 $\pm$ 3	106 $\pm$ 3	1.11 $\pm$ 0.05	5.50 $\pm$ 0.28	16.9 $\pm$ 2.0
	-	ETBA	10	520	85 $\pm$ 3	86 $\pm$ 3†	1.29 $\pm$ 0.07	6.71 $\pm$ 0.32	7.8 $\pm$ 1.0
	+	.	10	.	84 $\pm$ 3	118 $\pm$ 3	0.93 $\pm$ 0.02	4.27 $\pm$ 0.12	3.5 $\pm$ 0.3

* Two male rats died in this group.

† One rat died in this group.

Table 2. *Effects of halogen-containing esters on liver weight and lipid content*

Strain 'B' rats received the following halogen-containing esters while on a choline-deficient diet. All groups were of mixed sexes. Choline-deficient control groups received subcutaneous injections of paraffin. Choline, when added, was given at 10 mg./10 g. of diet. Abbreviations are as in Table 1.

Choline	Ester	No. of rats	Dosage of ester (μ moles)	Mean rat wt.		Liver wt. as % of body wt.	Lipid content in wet liver (%)
				Initial (g.)	Final (g.)		
-	.	10	.	52	71	4.58	17.7
-	ETCA	10	220	53	68*	5.06	7.3*
-	ETFA	10	360	51	70*	5.52	6.1*
-	EA	10	360	58	77	4.46	15.0
+	.	8	.	65	90	3.29	4.2

* Two rats died in this group.

from the liver of choline-deficient rats (Table 1). There was a significant fall in the total lipid content of the liver when ethyl tribromoacetate was given to choline-deficient rats, but the lipid content was still significantly ($P = 0.001$) higher than that of rats given choline. At the dosage of ethyl tribromoacetate given, the rats appeared listless and failed to gain weight. There was a significant increase in both kidney and liver weights when expressed as a ratio to body weight ($P < 0.05$ and $P < 0.02$ respectively).

Ethyl trifluoroacetate. In Expt. 2 (Table 1) it was found that this was as effective as ethyl trichloroacetate in lowering excess of liver lipids in choline-deficient rats. In addition, ethyl trifluoroacetate-treated rats had significantly increased ratios of both kidney and liver weights when expressed as a ratio of body weight. It was noticeable that at the site of injection there was more tissue breakdown with ethyl trifluoro- than with ethyl trichloro-acetate.

The 'B' strain of rats also responded to ethyl trifluoroacetate supplements by having a liver-lipid content significantly lower than the paraffin-injected controls (Table 2).

The addition of choline to the diet was more effective in reducing the lipid content of the liver than was either the trichloro or the trifluoro compound.

Ethyl dichloroacetate. This, when given to rats with established choline deficiency, was more toxic than an equimolar amount of ethyl trichloroacetate. With strain 'A' rats (four males and three females of approx. 65 g. body wt.) one dose of 1440 μ moles of ethyl dichloroacetate/100 g. produced a moribund condition in all seven in less than 24 hr. The mean liver-lipid weight per g. of wet liver for the group was 15.9%.

Eight female rats of similar body weight received five daily doses of 1440 μ moles of ethyl dichloroacetate/100 g./day. These animals were fed on the rat-colony breeding ration throughout the period of injection. All animals survived with slightly increased body weight.

Eight strain 'B' rats received a dosage of 360 μ moles of ethyl dichloroacetate/100 g./day for 5 days per week during the administration of the choline-deficient diet. Seven animals died during the second and third week, and one animal, which survived for 26 days, had a liver-lipid content of 21%.

DISCUSSION

In previous papers (Kratzing & Windrum, 1957, 1959) it was reported that ethyl trichloroacetate was able to decrease excess of liver-lipid content when given to choline-deficient rats. The present experiments confirmed earlier results and showed that ethyl trichloroacetate consistently and signifi-

cantly lowered the concentration of liver lipids of the choline-deficient rats.

Ethyl trifluoroacetate was now found to act as a lipotropic factor and appeared to lower the concentration of liver lipids as effectively as ethyl trichloroacetate. On the other hand, neither ethyl acetate nor ethyl dichloroacetate showed any lipotropic effect at comparable dosage.

Ethyl tribromoacetate injections decreased the lipid content of the liver but the treatment stopped the growth of the rats. The relationship between growth and choline requirement of rats has been established (Best, Lucas & Ridout, 1954). In addition, Hartroft (1954) has stated that rats which become sick and listless will be able to mobilize much of the excess of lipid in the liver to produce 'the pseudolipotropic effect of caloric restriction' (Best, Hartroft & Sellers, 1952; see also Best & Ridout, 1938). It is likely that for ethyl tribromoacetate the lowering of the lipid content was due to a failure to grow and consequently these experiments do not indicate whether it is a true lipotropic factor or not.

The choline-deficient groups had consistently heavier livers than the choline-supplemented groups and this appeared to be related to the higher lipid content of the former. The esters produced liver weights which were comparable with the choline-deficient groups, and this effect was independent of their lipotropic effect as it was produced by ethyl acetate as well as by the trichloro and trifluoro compounds.

The relatively heavier kidneys produced by all the esters, whether lipotropic or not, suggest that they increased renal damage originally produced by the choline deficiency (Griffith, 1940; Moore, 1957). Previous work has suggested that ethyl trichloroacetate did not prevent renal degeneration in rats on a choline-deficient diet (Windrum & Kratzing, 1960).

With experiments of the type described in this paper, changes in metabolism are more likely to be recognized if the causative agent can exert its effects over long periods of time. For this reason, the ester forms of the halogen acids were chosen rather than the water-soluble salts, which are likely to be more rapidly excreted from the body. In addition, administration of the esters avoided the difficulties involved in injecting large amounts of water-soluble material.

Because of the similarity in chemical structure between ethyl trichloro- and trifluoro-acetate, it is likely that both compounds exert their effect in a similar way. The large energy of the C-F bond makes it unlikely that ethyl trifluoroacetate has lipotropic action as a result of any loss of fluorine atoms. Similarly, it is most probable that ethyl trichloroacetate does not lose chlorine atoms in the animal

body but rather that both ethyl trichloro- and tri-fluoro-acetate are effective as lipotropic agents because of the CCl_3 or CF_3 group. These groups are larger than the CH_3 group and may exert their effects through a steric blockage of an enzyme system.

Ethyl dichloroacetate was not only without lipotropic action but was highly toxic when given to rats in an established choline-deficient state. Rats fed on a ration which supported the breeding colony tolerated the same dosage with no noticeable impairment of function. It is probable that it is more toxic when the quality of protein in the diet is poor since it was also toxic when choline was added to the experimental diet (Kratzing, 1960). Laug (1946) stated that dichloroacetate was a more effective inhibitor of mouse-liver respiration than corresponding concentrations of trichloroacetate, whereas acetate, at the same concentration range, was not inhibitory. But Woodard, Lange, Nelson & Calvery (1941) found that rats tolerated larger oral doses of dichloroacetic acid than they did of trichloroacetic acid or acetic acid before showing signs of toxicity. In general the non-toxicity of the poly-halogen derivatives of acetic acid is in marked contrast to the toxicity of the monohalogen derivatives (Kratzing, 1960).

SUMMARY

1. Ethyl trifluoroacetate appeared as effective as ethyl trichloroacetate in decreasing excess of

liver-lipid content of choline-deficient rats; whereas ethyl acetate and ethyl dichloroacetate were without effect.

2. Because of failure of the rats to grow, no lipotropic properties could be attributed to ethyl tribromoacetate.

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Studies in the Biosynthesis of Fungal Metabolites

2. THE BIOSYNTHESIS OF ALTERNARIOL AND ITS RELATION TO OTHER FUNGAL PHENOLS*

By R. THOMAS†

Department of Biochemistry, London School of Hygiene and Tropical Medicine, University of London, Keppel Street, London, W.C. 1

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The mode of biosynthesis of certain types of fungal phenols involves the condensation of acetate units (for a review see Birch, 1957). However, the biosynthesis of alternariol, $\text{C}_{14}\text{H}_{10}\text{O}_5$ (I), a metabolite of *Alternaria tenuis* auct. (Raistrick, Stickings & Thomas, 1953), was of particular

interest since, in addition to its rarity among known fungal products as a diphenyl derivative, it appeared to constitute a classical example of the acetate hypothesis. Thus it can in theory be derived solely by the head-to-tail condensation of seven acetate units without requiring additional stages involving oxidation, reduction, decarboxylation, etc. Another example of a fungal phenol, exclusively derived from acetate, is the key substance orsellinic acid, $\text{C}_8\text{H}_8\text{O}_4$ (II), isolated from *Chaeto-*

* Part 1: Chaplen & Thomas (1960).

† Present address: Department of Microbiology, Squibb Institute for Medical Research, New Brunswick, New Jersey, U.S.A.