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The Metabolism of DL-1-Deoxy-1-Fluoroglycerol

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(Received 23 December 1957)

The study of fluorine-substituted metabolites may give information about the pathways of normal metabolism or lead to the discovery of compounds suitable for therapeutic or pesticidal use. The compound 3-fluoro-DL-propane-1,2-diol (DL-1-deoxy-1-fluoroglycerol) was first described by Gryskiewicz-Trochimowski (1947), and its physical properties were given by Taylor & Kent (1956). Dr R. M. C. Dawson (personal communication) observed that the compound was toxic to mice and induced citrate accumulation, particularly in heart and kidney, and to a lesser extent in liver. Since the present work was started, Pattison & Norman (1957) have reported that DL-1-deoxy-1-fluoroglycerol is toxic to the mouse, and have suggested that it is oxidized *in vivo* to fluoroacetate or fluoroacetaldehyde, on the grounds of a comparison of its toxicity with that of various possible metabolites of DL-1-deoxy-1-fluoroglycerol or of their derivatives.

MATERIALS AND METHODS

DL-1-Deoxy-1-fluoroglycerol (FG) and epifluorohydrin were synthesized by Drs Taylor and Kent and generously presented to us by them. [Dr Kent found (for FG): C, 38.0%; H, 7.6%. Calc. C, 38.3; H, 7.45%.] Sodium

fluoroacetate was purchased from the Monsanto Chemical Co. The disodium salt of adenosine triphosphate (ATP) was used, purchased from the Pabst Laboratories, Milwaukee, Wisc., U.S.A. Citrate was determined by the method of Taylor (1953), except that the step of drying the light petroleum was omitted. Phosphate was determined by the method of Rockstein & Herron (1951). Acid-labile phosphate was determined following 10 min. hydrolysis in N-HCl at 100°. Glycolysis was measured according to LePage (1948). Kidney particles were prepared and assayed, with fumarate as substrate, as described by Peters & Wakelin (1953, 1957); brain particles were used as described by these authors. Metabolic products were examined for organic acids, by two-dimensional paper and by column chromatography.

Paper chromatography. The two-dimensional hydroxamate system of Bergmann & Segal (1956) was used; the first solvent was absolute ethanol-pyridine-water-ammonia (60:20:6:14, by vol.) and the second was absolute ethanol-dioxan-water-acetic acid (60:20:19:2, by vol.). At the suggestion of Dr D. B. Lindsay, the free acid for chromatography was obtained by evaporating a solution of the sodium salt to dryness, taking up in 75 μl . of 30% H_3PO_4 , adding to a micro-column (0.6 cm. $\times$ 1.5 cm.) of anhydrous Na_2SO_4 topped with Celite 545, and washing through with ether.

Column chromatography. The material to be chromatographed was made alkaline and evaporated to dryness on the steam bath, then taken up in 0.5 ml. of 0.5N- H_2SO_4 , and chromatographed by the silica-gel-column procedure of Bulen, Varner & Burrell (1952), eluting only with butanol-chloroform (5:95, v/v). Successive 2 ml. fractions were

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collected and neutralized. In fractionating a heart-liver perfusate, the neutral emulsions were evaporated to dryness on the steam bath, each was dissolved in 1.25 ml. of 0.25M-phosphate buffer (pH 7.2), and 1 ml. was assayed, by the standard kidney-particle preparations, for compounds which could be converted into fluorocitrate.

Perfusions. The coronary-perfused heart preparation of Bleehen & Fisher (1954) was used upon female rats anaesthetized with Nembutal (48 mg./kg.) and injected with heparin (2000 units). The heart-liver preparation was essentially a combination of the Bleehen & Fisher perfused heart with liver perfused according to Trowell (1942), with adaptations based on Brauer, Pessotti & Pizzolati (1951) and Miller, Bly, Watson & Bale (1951), the organs being arranged in parallel. Both organs were obtained from a single heparinized rat under Nembutal anaesthesia, the heart being stored in iced Ringer's solution until the liver was in the apparatus. Fig. 1 shows the apparatus. The ischaemic interval was not more than 10 min. Penicillin G sodium (Glaxo Laboratories Ltd.) (65 000 units) was added to the 55–70 ml. of perfusion fluid, which was that used by Bleehen & Fisher.

Functional hepatectomy. 'Eviscerations' (removal of the alimentary canal and those tissues between the rectum and the lower oesophagus which are connected to it) were carried out under urethane anaesthesia (1.5 g./kg.). When total functional hepatectomy was desired, the following were tied: rectum; oesophagus; coeliac artery; inferior and superior mesenteric arteries; and finally the portal vein. The rat was then eviscerated and sewn up. Subcutaneous injections of penicillin G sodium (33 000 units) were made immediately after the operation (Markowitz, Rappaport & Scott, 1949) and of glucose (200 mg.) at 2–3 hr. intervals. When the hepatic arterial supply was to be retained, the same procedure was used except that, instead of the coeliac artery, its non-hepatic tributaries (lineal, gastroduodenal and left gastric) were tied; in this case penicillin, but no glucose, was injected. Tracheotomy was performed when necessary. FG (dissolved in 0.9% NaCl) was injected into the femoral vein.

Rats. Rats were either those bred at this Institute from a Wistar strain obtained originally from the National Institute for Medical Research, Mill Hill, London, N.W. 7; or they were purchased from A. Tuck and Sons, Rayleigh, Essex. The former rats were used for the perfused hearts and livers. About equal numbers of each were used for the hepatectomy experiments.

RESULTS

Since the amount of the substance available was small, experiments had to be designed with a view to economy of DL-1-deoxy-1-fluoroglycerol.

Effect in vivo. DL-1-Deoxy-1-fluoroglycerol (FG) was toxic when injected into the rat, mouse and guinea pig, as shown in Table 1. The values obtained represent the order of toxicity. As the amounts of fluoroglycerol were so limited, no attempts was made to get an exact LD₅₀. Death was associated with considerable citrate accumulations (Table 2). The symptoms of poisoning in rats injected with an approximate LD₅₀ are as follows: no signs for about 1 hr., then a brief extreme hyper-

Fig. 1. Heart-liver perfusion apparatus. The rubber tubes with screw clamps inserted in the tubing between A and B, B and C, C and D, A and G, H and D are not shown. Chambers A, B, C and G are jacketed with water pumped by a centrifugal pump from a thermostat bath at 38°. Perfusion fluid in the reservoir (A) may flow through B into the cannula on which is tied the heart, in chamber C. After passing through the heart, it flows through the bottom of D into E (a Büchner funnel packed with glass wool and with a Whatman no. 351 paper at the bottom), and then through the pump F. The pump is provided with two dental valves, at entry and exit, and a gum-rubber tube in the centre, which responds to the alternating pressure created in the outer chamber of F by connexion to a Dale-Schuster pump (not shown). The fluid then returns to A, which is treated with Silicone M.S. Antifoam A (Hopkin and Williams Ltd.). Other fluid from A passes into the cannula in G, on which the liver is tied, perfusion being through the thoracic vena cava and out of the portal vein (the abdominal vena cava is tied). The liver lies in G supported by perfusion fluid, which overflows down the outflow tube. H is used to measure the flow rate through the liver, by clamping the H-D clamp and removing the stopper of H. Normally, fluid flows straight from G into D, the gassing tower, flowing down the wall and into E. Tower D is gassed with O₂ + CO₂ (95:5, v/v) in the direction shown by the arrows. The heart flow-rate is measured by introducing an air bubble with polythene cannula tubing, and measuring its speed through B, by using calibrated marks on B. Important dimensions: liquid level in A is 76 cm. above the heart in C and 5.5 cm. above the liver in G. Chamber sizes (internal diameter × internal depth in cm.): A and C, 2.6 × 5.0; G, 4.3 × 3.0. In later experiments a filter of stainless-steel mesh (Baxter N14 Filter-drip Assembly; Baxter Lab. Inc., Morton Grove, Illinois) was inserted between A and B.

excitability ending in clonic convulsions which often terminate in a tonic convulsion. During the next 3-4 hr., several similar convulsions occur, often within a few minutes of each other, the tonic phase becoming more dominant and severe. In between convulsions there is lassitude, although the animals are lively on handling, and fur staring; rectal temperatures are normal at 33-35°, as taken under the conditions in this Laboratory; hearts are normal at 500 beats/min. After this 3-4 hr. phase there are seldom any more convulsions until death, but rectal temperatures and heart rates decline slowly until at 28 hr. they average 25° and 150 beats/min. respectively. The heart beat is then very faint and irregular, the animals sprawl, with hind legs particularly splayed out. They are weak and completely lethargic, even on handling when they are apt to appear resentful. Often a little red secretion is observed at eyes and nostrils. They do not eat or drink. By 44 hr. those that are going to survive are recovered with heart rates at about 450 beats/min., temperatures 34°; they eat, drink and are lively. Those that will die eventually may make a partial recovery (e.g. to temperature 31°, heart 150 beats/min.), but they do not eat and become colder once more; they may convulse on

handling and eventually die at about 44 hr. with temperatures of 20-25°, about half of them with a mild tonic convulsion immediately before death.

Citrate analyses upon the tissues are given in Table 2. The citrate content of all four tissues studied was increased, the heart being most affected, but there was very considerable individual variation in response. At the presumed LD₅₀ of 10 mg./kg. the survivors had less citrate in all tissues than had those which succumbed, and this difference was especially striking with respect to the heart. The survival time after injection decreased sharply with increasing dosage. When citrate determinations were made on the tissues removed 3 hr. or more after death, the values for poisoned animals were radically reduced. Thus the citrate level in freshly removed hearts of animals that had succumbed to 10 mg. of FG/kg. averaged 1173 µg./g.; but, in two similar animals in which tissues were removed 3 and 5 hr. after death, values of 97 and 136 µg./g. respectively were obtained. A somewhat similar phenomenon was observed by Buffa & Peters (1949).

Paper chromatography. The sample of FG was examined chromatographically for possible contamination with fluoroacetate. The conditions were

Table 1. *Toxicity of DL-1-deoxy-1-fluoroglycerol to the mouse, guinea pig and rat*

Injections were intraperitoneal.

Animal	Sex	Average wt. and range (g.)	Dose (mg./kg.)	No. injected	No. died	Estimated LD ₅₀
Mouse	M.	36 (26-42)	50	2	2	20-40
			45	4	4	
			40	4	4	
			20	4	0	
			10	4	0	
Guinea pig	F.	596 (450-666)	20	4	4	<20
Rat (Tuck)	M.	174 (139-228)	100	2	2	25
			50	3	3	
			25	3	2	
			20	2	0	
			10	4	0	
Rat (Babraham)	M.	141 (80-206)	100	2	2	10
			20	5	5	
			10	8	5	

Table 2. *Effect of intraperitoneal injection of DL-1-deoxy-1-fluoroglycerol in male rats*

Rats were those bred at Babraham. Citrate was determined on extracts treated with trichloroacetic acid, 5% final concentration. Figures are means ± S.D.

Dose (mg./kg.)	No. of animals	Time to death (hr.)	Citrate (µg./g. wet wt.)			
			Heart	Liver	Kidney	Brain
0	3	—	54 ± 9	18 ± 1	37 ± 1	43 ± 7
10 (survivors)*	2	—	41, 93	31, 33	79, 93	45, 46
10 (succumbed)	2	23, 42	1688, 760	70, 57	217, 147	136, 108
20	4	25 ± 2	2537 ± 658	164 ± 80	926 ± 296	388 ± 131
100	1	8	1245	224	1140	160

* Killed 44 hr. after injection.

such that 1% of fluoroacetate would have been detected. None was found.

At high levels of FG (e.g. 250 μ g.) a purple spot with R_f 0.81 in the alkaline and 0.78 in the acid solvent was noted. This was certainly not due to fluoroacetate, which had R_f values of 0.057 and 0.63 in alkaline and acid solvents respectively. No attempt was made to identify the purple spot, which may have been some product of FG.

Taken with the observation below of the inactivity of the FG against kidney particles, it is certain that no significant amount of fluoroacetate can be present in the sample, and that the effects of FG are not due to contamination of the sample with fluoroacetate.

Results with tissue homogenates and slices. The symptoms of poisoning and the great increases in citrate levels in the tissues suggested that the critical biochemical lesion occurred with aconitase, as is the case for fluoroacetate (Lotspeich, Peters & Wilson, 1952; Morrison & Peters, 1954). If this were so, the FG was probably being converted into fluoroacitrate, and fluoroacetate or fluoroacetyl coenzyme A were possible intermediates.

All attempts to duplicate the citrate accumulation with simple enzymic preparations *in vitro* were unsuccessful. At 2 mM, FG had no effect on oxidation of fumarate or pyruvate plus oxaloacetate by kidney or brain particles, nor was any citrate accumulation found in the preparations. Such particles are very sensitive to fluoroacetate at 0.1 mM. Since it was possible that a preliminary conversion into (say) fluoroacetate took place *in vivo* in some other tissue, mixed preparations of kidney with liver, heart or voluntary muscle were tried, both homogenates and slices. Also di- and tri-phosphopyridine nucleotide, nicotinamide and fluoride along with simple or mixed slice and homogenate preparations were studied. No citrate accumulations were found.

At 7 mM, FG does not affect the extra oxygen uptake obtained by kidney slices on the addition of glycerol, citrate or pyruvate. No oxidation of FG by liver slices could be observed manometrically. Much time was spent on reaching this negative conclusion and many variations of media were used, i.e. Krebs-Henseleit bicarbonate and phosphate,

and three improved media suggested by Krebs and colleagues (Krebs & Henseleit, 1933; Krebs, 1950). Extracts of liver, kidney or heart slices incubated for up to 3 hr. with FG did not contain any material that gave citrate accumulation after incubation with kidney particles. It might be thought that any fluoroacetate could be detected by paper chromatography, but in this Laboratory it has proved very difficult in the products of biochemical incubation mixtures to distinguish fluoroacetate and especially to separate this from acetate. Hence the failure to induce citrate accumulation in kidney preparations is more reliable evidence for the absence of fluoroacetate.

The possibility that FG might influence glycerol metabolism or glycolysis was investigated. With FG at 7 mM (21 μ moles/flask) guinea-pig-brain glycolysis was inhibited slightly as follows: (a) no added substrate: CO_2 evolution inhibited 22%; lactate formation 13%; phosphorus incorporation increased by 0.8 μ mole; (b) glucose as substrate: CO_2 evolution inhibited 7%; lactate formation 5%; phosphorus incorporation increased 0.7 μ mole. (Results are means of three experiments.) This suggested the possibility of some phosphorylation of FG, perhaps with small subsequent interference with glycolysis. However, with purified rat-liver glycerokinase (Bublitz & Kennedy, 1954) FG was not phosphorylated nor did it interfere with phosphorylation of glycerol (4 mM-FG with 10 mM-glycerol).

Perfused heart. The failure of the sliced or homogenized tissues to show the citrate accumulation with FG that they did *in vivo* suggested that an intact perfused organ might repay study. The heart is easy to perfuse, and showed considerable increases of citrate with FG *in vivo*. Also it survives for many hours, and one might observe effects in it (due to an enzyme system of low concentration or of low affinity for FG) that sliced tissue would not demonstrate. The coronary-perfused heart was examined: heart beat and perfusion rate were noted for 5 hr. (or less if the heart stopped sooner), then it was removed and ground with trichloroacetic acid, and citrate determined on the extract. Table 3 shows that although 1.2 mM-fluoroacetate caused marked citrate accumulation, 4 mM-FG had no effect at all.

Table 3. *Effect of fluoroacetate and DL-1-deoxy-1-fluoroglycerol on the coronary-perfused rat heart*

The citrate content of the tissue was determined after 5 hr., or when the heart had stopped (if before 5 hr.). The figures represent means $\pm$ S.D.

Addition to perfusion fluid	No. of experiments	Citrate (μ g./g.)	After 2 hr.	
			Heart rate (beats/min.)	Flow rate (ml./min.)
Nil	4	42 $\pm$ 15	181 $\pm$ 37	2.3 $\pm$ 0.9
Sodium fluoroacetate (1.2 mM)	3	179 $\pm$ 72	74 $\pm$ 58	1.3 $\pm$ 0.8
FG (4.0 mM)	3	50 $\pm$ 13	213 $\pm$ 37	3.3 $\pm$ 0.9

Since the perfused beating heart is a more 'physiological' preparation, its failure to act when isolated as it did in the whole animal could most readily be explained by the assumption that, *in vivo*, FG must be altered or 'activated' by some other tissue than heart, before it was capable of accumulating citrate in the heart.

Functional hepatectomy. As attempts to obtain comparable results *in vivo* and *in vitro* had failed, it was decided to attempt the opposite approach: rather than obtain an enzymic preparation *in vitro* complex enough to metabolize FG, an attempt would be made to eliminate various organs in the intact animal in order to establish their importance by observing the effect of FG injected subsequently. The liver was selected as the first organ to study.

There are two problems in connexion with hepatectomy in the rat, owing to the facts that (a) the venous return from the alimentary canal goes through the liver, by the portal vein, (b) part of the vena cava is in intimate contact with the liver. In order to remove the liver, a preliminary operation would be required in which the vena cava and portal vein are constricted in order to develop collateral circulation. Six weeks must elapse before the hepatectomy can be performed (Cheng, 1951). Even so, the animals survive only 6 hr.

A method was therefore sought to accomplish a rapid functional hepatectomy with reasonable survival. The details of the procedure eventually evolved are given under Materials and Methods. The warnings of Markowitz (1949*a*) were, however, borne in mind: tying off the portal vein and hepatic

artery does not completely eliminate the liver from the circulation (presumably owing to diffusion into and out of the vena cava), and dogs treated in this way, even though provided with an Eck fistula, survive only 10–12 hr. and do not show the typical symptoms of hepatectomy.

If the portal vein is tied the animal dies rapidly (e.g. 1.5 hr.) owing to loss of blood from the general circulation into the vessels of the portal system. Therefore to improve survival the blood supply to the alimentary canal must be stopped; and since decomposition of the canal presumably begins when the circulation stops it was considered best to remove the canal and its adherent organs altogether (this is termed 'evisceration' in this paper, although the term would usually imply removal of the liver as well). As Table 4 (*a* and *b*) shows, animals so treated survive for over 12 hr., a period longer than that required for intact animals to be killed by 85 mg. of FG/kg. Yet, as Table 4 shows, this very high dose of FG had no effect on the survival time or the citrate levels of kidney or heart of these eviscerated and functionally hepatectomized animals. This proved that either the liver or the alimentary canal is essential in order to metabolize FG to a compound capable of inducing citrate accumulation, and suggested that the citrate accumulation is associated with the toxic effect.

It remained to be shown that it was the liver and not the alimentary canal that was responsible for the conversion of FG into a substance capable of inducing citrate formation. The simple experiment of removing the canal whilst retaining a normal liver was of course impossible without extensive

Table 4. *Effect of DL-1-deoxy-1-fluoroglycerol on the urethane-anaesthetized female rat and the influence of functional hepatectomy*

Surgical treatment	Without FG			With FG (85 mg./kg.)		
	Survival (hr.)	Citrate (μ g./g.)		Survival (hr.)	Citrate (μ g./g.)	
		Heart	Kidney		Heart	Kidney
(a) None	3 (killed)	124	175	3 (killed)	755	629
	4 (killed)	157	220	3 (killed)	738	610
	20 (killed)	115	80	33 (died)	960	73
	20 (killed)	64	32	—	—	—
(b) Evisceration plus functional hepatectomy	12	51	41	7*	64	29
	12	30	18	13	86	77
				13	27	22
	18	53	115	17	90	38
	20	71	182	—	—	—
(c) Evisceration with maintenance of hepatic arterial circulation	4*	125	85	5	122	85
	5*	81	56	—	—	—
	8	90	56	9	125	38
	—	—	—	9	1190	101
	12	204	69	11	2690	121
	—	—	—	12	230	34
	18	104	38	18	108	63
	—	—	—	22†	348	62

* Asphyxiated by tracheal mucus.

† FG 47 mg./kg.

Table 5. *Effect of DL-1-deoxy-1-fluoroglycerol on the perfused rat-heart-liver preparation*

Addition	Expt.	Heart flow rate (ml./min.) at				Liver flow rate (ml./min.) at				Citrate (μ g./g.)*	
		0 hr.	1 hr.	2 hr.	3 hr.	0 hr.	1 hr.	2 hr.	3 hr.	Heart	Liver
None	1	5.0	1.4	0.8	0.6	16	16	8.0	8.0	18	9
	2	5.6	3.0	0.9	—	37	37	35	—	16	25
	3	14.0	8.6	4.3	1.3	11	8.9	12	14	15	6
FG (3 mM)	4	10.9	1.0	—	—	12	9.0	7.2	7.6	45	15
	5	4.9	—	—	—	24	6.0	5.0	4.5	18	20
	6	1.4	1.1	1.3	—	7.3	4.0	8.0	5.0	46	27
	7	7.1	4.0	0.2	—	15	0.6	3.1	4.0	80	25
	8	5.6	4.6	2.5	—	48	48	48	48	123	32
	9	8.9	1.0	—	—	27	19	16	16	61	89
	10	8.6	8.9	6.0	4.4	30	27	27	25	467	251

* Estimated at the end of the experiment.

cannulation, since the principal blood supply to the liver comes from the portal system. However, it was possible to maintain a partial circulation to the liver of the eviscerated animal by retaining the hepatic arterial circulation, which normally contributes about 20 % of the total blood supply to the liver (Markowitz, 1949b). One would expect that in a rat treated in this way: (i) if the alimentary canal alone were responsible for the conversion of FG, injected FG would have no effect; (ii) if the liver alone were responsible, injected FG would cause citrate accumulations, but the effect would be far less extensive (by about 80 % perhaps) than in the intact animal.

The results are given in Table 4 (c). The control levels of citrate were high, as in Table 4 (a); the full evisceration and functional hepatectomy [Table 4 (b)] had reduced the elevated citrate levels that urethane produces in the tissues, mainly heart. Elevated citrate levels had previously been noticed with urethane dosing in this Laboratory (R. A. Peters, unpublished work). Thus it seems that the presence of the liver is a prerequisite for this urethane effect. Further, it proves that the technique in Table 4 (c) was in fact effectively different from that in Table 4 (b). This is relevant to consideration of the results of FG treatment with hepatic arterial maintenance. Out of seven experiments, only three produced clear-cut increases in heart citrate as a result of injecting FG. In two of these three the increases are so very large as to suggest that there was some fundamental difference in the success of their operation compared with the other supposedly identical ones. There is no evidence about what this difference can be. However, the three animals which did show citrate increases were 'eviscerated' and therefore it is certain that the increases can take place in the absence of the alimentary canal and its associated organs.

Perfused heart-liver. The above experiments showed that FG in the 'eviscerated' animals does

not cause citrate accumulation in the heart unless the liver is included in the system, and explain the finding that the perfused heart is insensitive to FG. As a corollary, the perfusion of the liver along with a heart should produce citrate accumulation in the heart on addition of FG. This proved to be the case, as Table 5 shows. Considerable citrate increases in the livers were also observed. Substantial accumulations were found only if both the heart and the liver were in good enough condition to be perfused fairly rapidly for over 1 hr. When either was in poor condition, FG induced small citrate accumulation in the heart. If the liver only was in good condition, citrate accumulated in the liver but not in the heart. This suggests that one or more of the systems responsible for metabolizing FG to a citrate-accumulating compound is either in low concentration in the tissue or has a low affinity for FG, and this could account for the failure with preparations of slices or homogenates of mixed liver and kidney to demonstrate the change of FG to the inhibitor of citrate metabolism.

After the heart-liver had been perfused, the solution perfused was collected and its volume reduced upon the steam bath. The resultant solution was potent in inducing citrate accumulation in respiring kidney particles, and most potent in those cases where the heart-citrate levels showed the perfusion to have been most successful. Thus 0.1 ml. of four-times concentrated perfusion solution from Experiment 10 (Table 5) increased the citrate in kidney homogenate by 2.3 μ moles/Warburg flask, that from Expt. 8 increased it by 1.1, and the control (from Expt. 2) by 0.3 μ mole. These findings showed that the heart-liver preparation released into the perfusion fluid a 'citrate-accumulating factor'. Experiments were now undertaken to discover if this factor could be fluoroacetate, or other carbon-fluorine substance capable of stopping the metabolism of citrate. It was realised that this would be difficult owing to the small amounts present.

The factor was not removed by passing through a short (2.0 cm. $\times$ 0.6 cm.) column of De-Acidite FF, a strong anion-exchange resin; this was found to be due to the presence of phosphate. Eventually a longer column (5.5 cm. $\times$ 0.7 cm.) of the anion-exchange resin IRA-400, mesh 100-200, was found to remove both fluoroacetate from a phosphate-containing solution, and citrate-inducing factor from perfusion fluid.

The silica-gel column-chromatographic procedure of Bulen *et al.* (1952) was found to be capable of separating fluoroacetate from other acids. Any fluoroacetate would be eluted by the butanol-chloroform (5:95, v/v) solvent. Concentrated perfusion fluid from Expt. 10 (Table 5) was made alkaline and evaporated to dryness, then taken up in sulphuric acid (as in Bulen *et al.* 1952) and chromatographed in such a system. Fractions (2 ml.) were collected, neutralized, evaporated to dryness, dissolved in buffer and assayed against kidney particles. The results (Fig. 2) showed that a peak of citrate-accumulating activity appeared in the fraction corresponding to the peak for pure fluoroacetate. Fig. 2 represents the second of two similar experiments on the same perfusion fluid. Fluorocitrate is not eluted by butanol-chloroform (5:95, v/v) (P. F. V. Ward & R. A. Peters, unpublished work).

A portion (0.5 ml.) of the four-times concentrated perfusate from Expt. 10, Table 5, also induced citrate accumulation (0.23 μ mole) in a pigeon-brain-particle preparation. Such a preparation is insensitive to fluoroacetate (Peters & Wakelin, 1953) but sensitive to fluorocitrate, which may therefore be present in the perfusion fluid, and would contribute to the property of inducing

citrate accumulation in kidney particles. Fluoro-citrate is exceedingly potent in citrate accumulation in brain particles; 2 μ g. (enzymically synthesized)/Warburg flask may give 1 μ mole excess of citrate over the control (Peters & Wakelin, 1953). Therefore the above effect of perfusate against brain, if due to fluorocitrate, implies only about 0.2 μ g./ml. in the original perfusion fluid. The evidence from the columns is consistent with the presence in the perfusion fluid of fluoroacetate and fluorocitrate. However, other degradation products of FG might be responsible for the observations with brain-particles.

Toxicity of epifluorohydrin. Epifluorohydrin is the anhydride of FG. Chemically, hydration of such epoxy compounds is easily achieved by diverse reagents. However, when injected into mice (intraperitoneally, in propylene glycol) the compound was found to have a very low toxicity. Doses up to 100 mg./kg. had no visible effect, nor did they give increased citrate levels in heart or kidney. At 200 mg./kg. death occurred in about 16 hr.: convulsions were not observed (but occasional mild ones could have escaped notice after the first few hours). The animals after 3 hr. were either badly sprawling or prostrate, with lowered body temperatures, and remained more or less prostrate until death. The citrate levels in heart and kidney were not significantly raised in the two animals whose tissues were analysed.

DISCUSSION

The results show that unaltered FG is a metabolically inactive compound, ineffective against all the enzymic systems tested *in vitro*, which included the pathways of glycolysis and of the tricarboxylic acid cycle. Liver slices and homogenate did not convert FG into detectable amounts of a metabolic inhibitor; but the intact liver in a perfused system converted it into a compound which could cause citrate accumulation in the intact heart or in kidney particles. In some experiments also in the 'eviscerated' animal, the liver also caused citrate accumulations from FG, whereas in the absence of the liver no extra citrate was found. The obvious hypothesis is that the compound formed is fluoroacetate or fluorocitrate. The evidence from silica-gel chromatography is that fluoroacetate is present in the perfusion fluid, and the finding with the anion-exchange resin is consistent with this. However, as Fig. 2 shows, there may be other citrate-accumulating factors, eluted by butanol-chloroform (5:95, v/v), in the perfusate. Fluorocitrate may also be present in small amounts. The results of Fig. 2 would be given if the liver produced from FG some compound which in the course of preparation for the column gave rise to fluoro-

Fig. 2. Column chromatograms of fluoroacetate and heart-liver perfusate. O, Sodium fluoroacetate (1 mg.); 2 ml. fractions of eluate were titrated with 0.01 N-NaOH; ●, heart-liver perfusate (20 ml.) from Table 5, Expt. 10; 2 ml. fractions were collected and assayed against kidney particles.

acetate. Fluoroacetyl-CoA would give fluoroacetate in these circumstances; in fact even in the mild conditions of the perfusion it would be converted into fluoroacetate to a very large extent (Brady, 1955).

The difference in the behaviour of FG in the slice and in the perfusion is remarkable; it might be thought that it was due to failure to use bicarbonate media, and it should be stressed therefore that these were also used. The simplest explanation would be that there are still unknown co-factors which may escape from slices, but there are other possibilities. In any case, it indicates that there can still be no complacency about negative biochemical results.

As to the pathway of conversion of FG, no evidence is available. A plausible guess would be oxidation (possibly via the aldehyde) to 3-fluoro-3-deoxyglyceric acid (1-fluoro-2-hydroxypropane-3-carboxylic acid), followed by decarboxylation and further oxidation to fluoroacetate. Rudney (1954) showed that propane-1:3-diol could give rise to lactate or to formate; in the latter case a C_2 fragment was presumably formed. If FG followed precisely the same pathway, fluoroacetate would be the C_2 fragment.

The results reported here demonstrate that not only can the animal body perform 'lethal synthesis' (e.g. with fluoroacetate) to transform a compound, which is itself inactive, into a potent anti-metabolite, but that it can also degrade an inactive compound to provide the starting material for a lethal synthesis. The animal is thus prepared to go to some lengths to poison itself.

SUMMARY

1. DL-1-Deoxy-1-fluoroglycerol is toxic to the rat, mouse and guinea pig. Death is associated with convulsions, bradycardia, lowering of body temperature and accumulation of citrate, particularly in heart and kidney.

2. DL-1-Deoxy-1-fluoroglycerol did not affect respiration or citrate level in homogenates or slices of kidney, brain, liver or muscle, nor did it increase the citrate level in the perfused heart.

3. Functional hepatectomy made the rat insensitive to DL-1-deoxy-1-fluoroglycerol.

4. Perfused rat liver converted DL-1-deoxy-1-fluoroglycerol into a compound (or compounds)

capable of causing citrate accumulation in a perfused heart or in kidney particles.

5. Chromatography on silica gel of the perfusate from a heart-liver preparation indicated that fluoroacetate was the principal citrate-accumulating factor.

We are indebted to Dr N. F. Taylor and Dr P. W. Kent for providing the DL-1-deoxy-1-fluoroglycerol and the epifluorohydrin; to Dr D. B. Lindsay for advice on perfusion techniques; to Dr P. F. V. Ward for advice on column chromatography, and to Miss P. Ivall for technical assistance. One of us (R.D.O'B.) was in receipt of a Canadian National Research Council Post-Doctoral Fellowship.

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