1969), NO$_3^-$ (cf. Williams & Evans, 1975) or SO$_4^{2-}$ the bacteria which dissipilate the aromatic structures under these conditions must rely on some system provided by the methane bacteria as their source of electron acceptors.

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Clarke, F. M. & Fina, L. R. (1952) *Arch. Biochem. Biophys.* 36, 26-32
Williams, R. J. & Evans, W. C. (1975) *Biochem. J.* 148, 1-10

**The Susceptibility of Rainbow Trout to Fluoroacetate**

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Fluoroacetate poisons most mammals, examples of its LD$_{50}$ (in μmol/kg) being 0.6 for the dog, 20-50 for man and 50 for the albino rat (Chenoweth, 1949). It is toxic because it is converted into fluorocitrate, which inhibits aconitate hydratase (EC 4.2.1.3) and thus blocks the tricarboxylic acid cycle. The critical organs are usually the central nervous system or heart (Peters, 1957). By contrast, plants are relatively immune to fluoroacetate, possibly because their aconitate hydratase is only slightly inhibited by fluorocitrate (Treble *et al.*, 1962). Certain poikilothermic animals are also resistant to fluoroacetate, examples being the South African clawed toad (*Xenopus laevis*) (LD$_{50}$ = 5mmol/kg; Chenoweth, 1949) and fish such as the bass and the bream (King & Penfound, 1946). Since no-one seems to know how these poikilotherms can tolerate fluoroacetate, we have examined some of its effects on the rainbow trout (*Salmo gairdnerii*) and, for comparison, on the albino rat. For experimental convenience the liver was chosen as the representative organ to be studied.

Young Wistar-strain rats fed on a standard diet and artificially reared rainbow trout weighing about 100g were used. The trout were kept in the laboratory in a 40-litre aerated tank at 8°C for up to 6 days, during which period they did not seem to feed. Sodium fluoroacetate and barium fluorocitrate were purchased from the Sigma Chemical Co. The fluorocitrate concentrations given below refer to total fluorocitrate and not the inhibitory isomer.

The LD$_{50}$ of fluoroacetate for trout was determined (by injecting fish intraperitoneally with 100-1000μmol/kg of fluoroacetate, avoiding the swim-bladder) to be approx. 500μmol/kg. Our fish therefore seem to be less sensitive to fluoroacetate than are rats. It is noteworthy that the trout probably refused all food during the 6 days the experiment lasted. Since fasting diminishes the sensitivity of liver slices to fluoroacetate (see below), the LD$_{50}$ may also depend on dietary status.

The effect of fluoroacetate on the respiration of liver slices was investigated by incubating slices (0.3mm thick) in pH7.4 Krebs–Ringer phosphate buffer (Umbreit *et al.*, 1964), containing 20mm-glucose plus fluoroacetate at the concentrations indicated below, and following respiration manometrically. At 37°C, 2mm-fluoroacetate decreased the respiration of rat liver slices by approx. 50%, whereas for trout the comparable...
Fig. 1. Effect of fluoroacetate on respiration of slices of trout and rat liver

Slices were incubated at 37°C (see the text). The concentration of fluoroacetate is shown by each curve. (a) Trout: ▲, fed; △, starved for 4 days. (b) Rat: ●, fed; ○, starved for 1 day.

concentration was about 20mM (Fig. 1). At 10°C neither preparation was affected appreciably by 20mM-fluoroacetate over a period of 5h (by which time 110μl of O₂/100mg wet weight had been consumed), after which respiration declined, whether or not fluoroacetate was present (data not shown). Thus trout liver at 10°C is less susceptible to fluoroacetate than is rat liver at 37°C, partly because it is intrinsically less susceptible and partly because for both species susceptibility declines with temperature. Fig. 1 also shows that, at 37°C, susceptibility declined dramatically with starvation, whereas respiration increased with it. A possible explanation is that, in the liver of the starved animal, citrate synthetase is inhibited (Newsholme & Start, 1973), so less fluorocitrate is formed.

To establish whether the relative insensitivity of trout liver to fluoroacetate is paralleled by a similar insensitivity of its aconitate hydratase to fluorocitrate, the oxygen consumption of liver homogenates was measured in the presence or absence of fluorocitrate. Livers were homogenized in 4vol. of medium (0.25m-sucrose/10mM-3-(N-morpholino)propanesulphonic acid and 1mM-EGTA titrated to pH7.2 with NaOH) and oxygen uptake was monitored after the sequential addition of 1.7mM-citrate and 0.17mM-ADP by using a Clark-type oxygen electrode. At 37°C, State-3 respiration (typically 0.7–2.4nmol of O₂/min per mg of liver) was not inhibited so long as fluorocitrate was added after citrate, whereas it was inhibited when fluorocitrate was added 1 min before citrate (Fig. 2a). The trout homogenate was the less affected, and fluorocitrate was about 1000 times more potent than fluoroacetate. In similar experiments at 10°C (when State-3 respiration was typically 0.2–0.4nmol of O₂/min per mg of liver), both homogenates were insensitive to approx. 20μM-fluorocitrate (Fig. 2b). However, when the preincubation with 20μM-fluorocitrate at 10°C was extended to 4h, oxygen uptake by the rat liver homogenate in the presence of citrate and ADP was only 20% of its control value, whereas that of the trout liver homogenate was the same as it (data not shown). The results with fluorocitrate therefore parallel those with fluoroacetate, in that at a given temperature the trout liver is the one less readily poisoned by these agents, and that sensitivity declines with temperature. [The increased potency of fluorocitrate with time is consistent with the data of Dummel & Kun (1969),]
Fluorocitrate was added 1 min before citrate. O₂ uptakes (State-3 respiration) are given as means±s.E.M. of three to five observations, expressed as percentages of State-3 respiration in the absence of fluorocitrate. ▲, Trout; ●, rat; (a) 37°C; (b), 10°C.

who postulated that the inhibitor initially acts in a reversible competitive manner, but is then converted into fluoroacetoacetate which alkylates the active site of the enzyme.

Comparison of the LD₅₀ values for fluoroacetate with the concentrations of it which inhibit hepatic respiration suggests that in neither species is the liver the critical organ. Nevertheless, it is tempting to speculate that trout are relatively immune to fluoroacetate partly because their aconitate hydratase is only poorly inhibited by fluorocitrate, and partly because their low body temperature further diminishes the potency of this inhibitor.

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King, J. E. & Penfound, W. T. (1946) Science 103, 487

Effect of Riboflavin Deficiency on Activity of NADH–FMN Oxidoreductase (Ferriductase) and Iron Content of Rat Liver

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NADH–FMN oxidoreductase (ferriductase) has been proposed for the mobilization of iron from ferritin (Osaki & Sirivech, 1971). We studied the effect of riboflavin deficiency on the activity of this enzyme and on the storage iron contents of rat liver.

Random-bred male Wistar rats (21 days old) of about equal body weights were kept on a normal diet or a riboflavin-free diet (Ogunmodede & McCormick, 1966). After 24 weeks some of the rats were intravenously injected with plasma-bound ⁵⁹Fe (20 μCi) only, or plasma-bound ⁵⁹Fe (20 μCi) and 2 mg of Fe (as Imferon; Fisons, Loughborough, U.K.) per 100 g body wt., 2 days before being killed. After death the livers