METABOLIC CONTROL IN PERFUSED RAT HEART DURING FLUOROACETATE POISONING.*

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The inhibitory effect of fluoroacetate on the citric acid cycle is due to the synthesis of fluorocitrate, which is a competitive inhibitor of aconitase (Peters, 1957). Consequently, citrate accumulates in fluoroacetate poisoned tissues. Margreth and Azzone (1964) have recently reported that rat heart homogenates have a large endogenous fluoroacetate insensitive respiration, and on the basis of studies with metabolic inhibitors concluded that it was largely supported by the utilization of glutamate and glycolytically generated DPNH.

The present investigation with the isolated perfused rat heart has failed to substantiate this suggestion. In this paper we report data on the mechanical performance of the heart during fluoroacetate poisoning, and metabolic control points have been identified by applying the cross-over theorem of Chance et al (1958) to the glycolytic system and the citric acid cycle.

METHODS. Perfusion and analytical techniques were similar to those previously described (Williamson, 1964a, Williamson and Jones, 1964). Male, fed, albino rats of Wistar strain (240-260 g.) were used. Sodium fluoroacetate (20 mg/kg body wt.) was injected intraperitoneally 30 min.

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before the hearts were removed. Contractile force was measured with a Decker transducer (Model 902-1) and the fluorescence intensity of the intact beating heart was measured by means of a microfluorimeter (Chance, Cohen, Jöbsis and Schoener, 1962) focussed on the surface of the heart through the window in the heart chamber.

RESULTS. In Fig. 1. are reported the levels of the glycolytic intermediates in hearts from fluoroacetate poisoned rats under two perfusion conditions, the values being expressed as a percentage of the appropriate levels in hearts from normal rats.

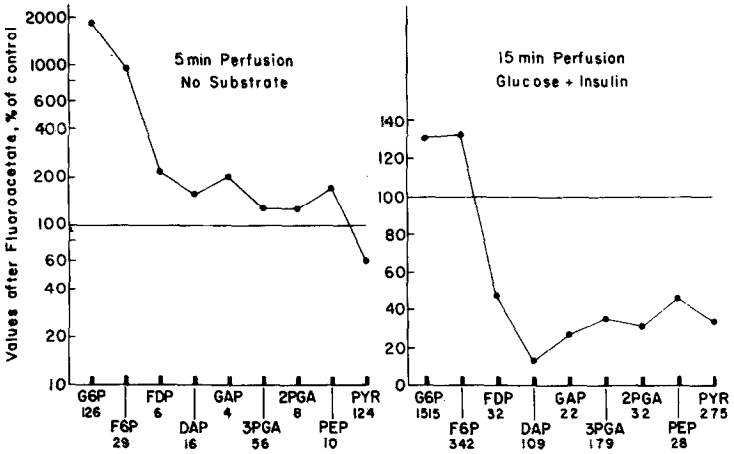

Fig. 1. Tissue levels of the glycolytic intermediates in perfused hearts from normal and fluoroacetate poisoned rats. The glycolytic intermediates are shown in sequence along the abscissa, and the figures beside each intermediate are the mean tissue levels found in control hearts, expressed in mμmoles/g. dry wt.

Outstanding in the glycolytic pattern is that in hearts from fluoroacetate poisoned rats there was a large accumulation of hexose monophosphates. In the absence of added substrate pyruvate was the only glycolytic intermediate which was decreased relative to the controls. After 15 minutes perfusion with glucose and insulin, glucose 6-P and fructose 6-P increased 12-fold to new stationary state levels, while the other intermediates increased only 2 to 6-fold. In hearts from

fluoroacetate poisoned rats perfused with glucose and insulin, Fig. 1 shows that there was a relative accumulation of the hexose monophosphates and a relative depletion of all the other intermediates. Since glycolytic flux (measured by the rate of glucose uptake and lactate formation) was decreased by 50 o/o under these conditions, the control point in the glycolytic sequence is identified at the phosphofructokinase step (Ghosh and Chance, 1964). Clearly glucose is not a major metabolic fuel in the fluoroacetate poisoned rat heart. The citrate content of hearts from fluoroacetate poisoned rats after 5 minutes of perfusion in the absence of substrate was 22.8 μmoles/g.dry wt. compared with 0.4 μmoles/g. dry wt. in the controls. On further perfusion for 15 minutes with glucose and insulin, the citrate content of control hearts showed little change, while that of the poisoned hearts fell gradually to about 50 o/o of the initial value.

The response of the perfused rat heart to fluoroacetate in vitro was studied kinetically by simultaneously observing the force of contraction and the level of reduced pyridine nucleotides (as determined by direct fluorimetric measurement). Addition of fluoroacetate (1 mM) to a heart perfused in the absence of substrate decreased the contractile force by 30 o/o within 5 minutes, and the pyridine nucleotides became more oxidised as shown by a decrease of the fluorescence (Fig. 2A). Fig. 2B is a continuation of the trace shown in Fig. 2A after an interval of 5 minutes when the force had decreased to a constant value 55 o/o of the initial. Addition of 100 μmoles acetate had no effect on the force of contraction over a period of 7 minutes. Fig. 2C shows that the addition of 20 μmoles pyruvate fully restored the contractile force, but had no effect on the state of oxidation-reduction of the pyridine nucleotides. In the absence of fluoroacetate, pyruvate invariably produces an increase of mitochondrial DPNH which is reoxidised upon the addition of 0.1 mM arsenite, thus locating the DPNH generating step at pyruvic oxidase (Williamson, 1964b).

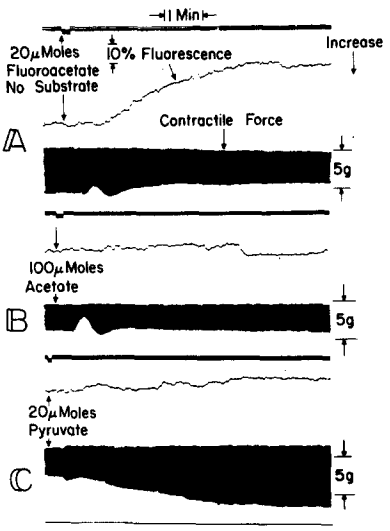

Fig. 2. Changes in the contractile force and fluorescence intensity of the perfused rat heart after the addition of fluoroacetate. The heart was perfused initially with 20 ml. of buffer containing no substrates.

The uptake of pyruvate and conversion of pyruvate to lactate were found to be the same in hearts from normal and fluoroacetate poisoned rats, indicating that the restoration of the contractile force after pyruvate addition was associated with the ability of pyruvate to increase energy production by overcoming the block in the citric acid cycle. This was confirmed in the experiment shown in Fig. 3. Addition of fluoroacetate produced the expected crossover at the site of aconitase as a result of fluorocitrate inhibition. Glutamate and aspartate levels were decreased by 13 and 18 o/o respectively. Pyruvate when present alone greatly increased the levels of all the intermediates of the citric acid cycle, and decreased those of glutamate and aspartate. When pyruvate was added to hearts previously perfused with fluoroacetate, the citrate content increased to values 100 times the control, and the level of intermediates beyond the aconitase step were also increased above the control values, indicating that the inhibition of aconitase has been overcome by the increased levels of citrate.

DISCUSSION. Fluoroacetate poisoning in the intact rat is characterised

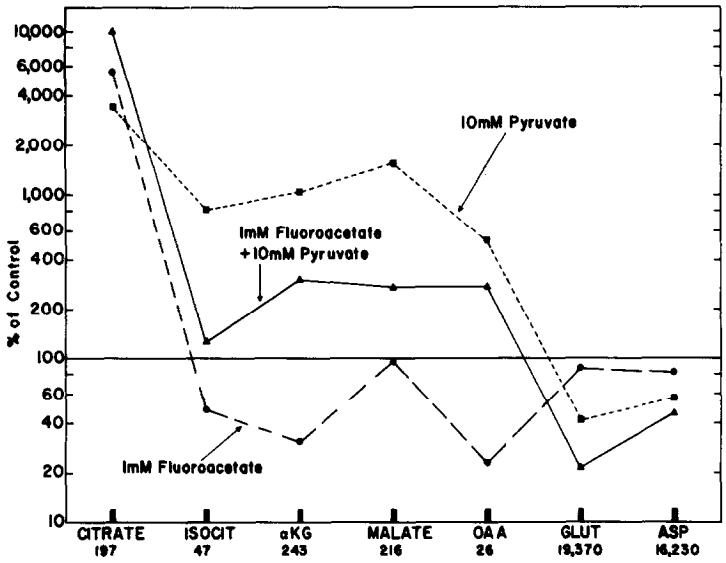

Fig. 3. Effects of fluoroacetate and pyruvate on the levels of citric acid cycle intermediates, glutamate and aspartate in perfused rat heart. All hearts were pre-perfused for 10 minutes with substrate free buffer and transfered to recirculation circuits containing 15 ml of medium for further perfusion as shown. Control hearts were perfused for 10 minutes in the absence of substrate, and the figures shown beside each intermediate are the mean tissue levels expressed in mμmoles/g. dry wt. Hearts were perfused either with fluoroacetate alone for 5 minutes, with pyruvate alone for 10 minutes, or with fluoroacetate for 5 minutes followed by pyruvate addition and perfusion for a further 10 minutes.

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by a diabetic-like syndrome involving effects on the nervous system, glycemia and ketonemia (Peters, 1957). The elevated blood sugar levels could theoretically be caused either by decreased glucose utilisation or by suppression of insulin release from the beta cells of the pancreas. A possible specific effect of fluoroacetate on insulin production has recently been ruled out by the work of Karam and Grodsky (1962). A decreased rate of glucose uptake and phosphorylation by the rat heart has been confirmed in the present investigation, and the site of inhibition located at the phosphofructokinase step. The mechanism for the inhibition of glycolysis is undoubtedly related to the large accumulation of citrate, which has recently been shown to be a potent inhibitor of phosphofructokinase in cell-free systems (Lowry and Passonneau, 1964). Furthermore, it would appear that the mechanism responsible for decreased

glucose utilization during fluoroacetate poisoning is similar to that in the acute alloxan diabetic state (Garland,Randle and Newsholme, 1963; Parmeggiani and Bowman, 1963). The reason for the accumulation of citrate in hearts from diabetic rats is not at present clear.

The complete oxidation of pyridine nucleotides after the addition of fluoroacetate even in the presence of pyruvate indicates that DPNH oxidation by the respiratory chain is faster than DPNH production. This is an expected consequence of the block in the citric acid cycle at the aconitase site. Apparently, however, this block is not complete in cardiac muscle since neither glycolysis nor amino acids serve as major hydrogen donors to the respiratory chain. Pyruvate may be especially effective in overcoming the block in the citric acid cycle by providing oxaloacetate, acetyl CoA and DPNH. Oxaloacetate formation can be stimulated in the presence of pyruvate either by direct carboxylation, or by transamination reactions as indicated by the decreased levels of glutamate and aspartate. Condensation of oxaloacetate with acetyl CoA will elevate citrate until it can compete effectively with fluorocitrate at the aconitase active site. Acetate is unable to overcome the aconitase inhibition since it cannot provide a new source of oxaloacetate, and unlike pyruvate requires ATP for activation.

These results suggest that although the initial effect of fluoroacetate is to give rise to fluorocitrate, the secondary inhibition of phosphofructokinase by the accumulated citrate is actually lethal since it deprives the cell of pyruvate which would eventually overcome the inhibition of aconitase.

REFERENCES.

Chance, B., Cohen, P., Jöbsis, F., and Schoener, B. (1962), Science, 137, 499.
Chance, B., Higgins, J., Holmes, W. and Connelly, C.M. (1958). Nature, 182, 1190.
Garland, P.B., Randle, P.J. and Newsholme, E.A. (1963), Nature, 200, 169.
Ghosh, A., and Chance,B. (1964). Biochem. Biophys. Res. Comm. 16, 174.
Karam, J.H. and Grodsky, G.M. (1962), Proc. Soc. Exptl. Biol. Med. 109, 451.
Lowry, O.H. and Passonneau, J.V. (1964) Proc. VI Intern. Congr. Biochem., New York, p. 705.
Margreth, A., and Azzone, G.F. (1964), Biochem. J. 92, 73.

Parmeggiani, A., and Bowman, R.H. (1963), Biochem. Biophys. Res. Comm. 12, 268.
Peters, R.A. (1957). Adv. in Enzymology, 18, 113.
Williamson, J.R. and Jones, E.A. (1964), Nature 203, 1171.
Williamson, J.R. (1964a). J. Biol. Chem. 239, 2721.
Williamson, J.R. (1964b). In press.