of liver tissue and containing 70–80 mg of proteins, NADP (1·5 μmoles) glucose-6-phosphate (50 μmoles), glucose-6-phosphate dehydrogenase (0·5 unit), $MgCl_2$ (25 μmoles), nicotinamide (50 μmoles), potassium phosphate buffer pH 7·0 (280 μmoles) and Nitrazepam (0·2–4·0 μmoles) in a total volume of 5 ml. The samples were incubated in nitrogen atmosphere at 37° while shaking for various periods but usually 1 hr.[1]

The metabolites of Nitrazepam were followed by thin layer chromatography on luminescent silicagel (Kiesegel CAMAG) using the solvent system toluene: acetone: ammonia (50:50:1).[5] Nitrazepam was determined spectrophotometrically[6] at 260 and 310 nm after elution from the thin layer its total recovery was 83% of the incubated quantity. The 7-amino derivative was determined either by direct spectrophotometry at 250 nm or after Bratton–Marshall reaction[5] at 555 nm. Protein determination was performed according to Lowry.[7]

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Observations upon the effect of fluoroacetate and pyruvate upon the isolated atria from rat heart

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THE EXPERIMENTS reported here were designed in the hope of confirming with rat heart atria the results of Williamson, Jones and Azzoni;[1] with whole perfused hearts poisoned by fluoroacetate, they found a reversal by addition of pyruvate. This, they interpreted, was due to partial reversal of the fluorocitrate block by increasing amounts of pyruvate, the glycolytic formation of pyruvate being blocked at the phosphofructokinase stage by the increasing citrate formation.[2,3]

Methods and materials. The atria were cut from normal beating hearts of beheaded Sprague–Dawley male rats (190g) fed with a normal balanced diet. They were suspended at 30° in oxygenated Ringer of composition in 1 l., NaCl 9g, KCl 0·42g, $CaCl_2$ 0·24g, $MgCl_2$ 0·005g, $NaHCO_3$ 0·6g, pH 7·3, gas mixture O_2 95% and CO_2 5%. Forty-five min was required for equilibration; it is best to wash three times during this period. For recording the beats a light isotonic lever was used with a load of 0·5g, giving four times magnification and with an ink polyethylene pen.

Citric acid estimations were made in Cambridge (Taylor's method[4]) on the weighed atria after quick drying and immersion in 10% trichloracetic acid for transport. The compounds used for test were prepared as stock solutions which were kept frozen, except when in use; dilutions were made with the modified Ringer and heated to 30° before adding to the bath.

Chemicals. Na pyruvate of high quality was from Fluka, A. G. Buchs; Na fumarate and l-noradrenaline bitartrate were respectively from Recordati, Milan. Na fluoroacetate (Monsanto) was 95% pure (personal communication from Mrs. Shorthouse). Synthetic Na fluorocitrate was kindly provided by Mr. Brown.

Results. Normal behaviour of spontaneously beating atria. The rate of the beats and the normal height (under our conditions) is given in Table 1. We have taken as a qualitative reference for evaluating the relaxation, the time of disappearance of that part of the wave falling below the base line. (Fig. 1) The citrate content found after 2 min work was 83μg/g and after 90 min work 102μg/g. (Table 2) Untreated atria continue to beat with insignificant variation for 7 hr.

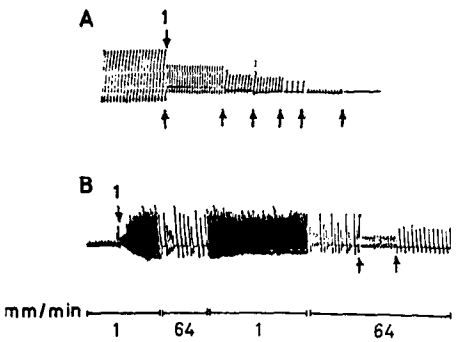

FIG 1. Condensed graph showing sections of the mechanogram tracings of isolated atria of rats. Arrows beneath each break.
(A) Mechanogram (64 mm/min) of fluoroacetate (3mM) poisoned atria from prior to treatment until the block. Arrow number 1 administration time of 3mM fluoroacetate.
(B) Noradrenaline improvement of fluoroacetate poisoned atria. Arrow 1 administration of the last dose of noradrenaline.

TABLE 1. BEHAVIOUR OF RAT ATRIA IN PRESENCE OF F-ACETATE AND AFTER REMOVAL OF GLUCOSE

F-acetate 3 mM contact time (min)	Disappearance repolarization wave (min)	Beating block time (min)	At 180 min % Chronotrop.	At 180 min % Inotrop.
5	none	none	91·2	66·7
25	none	none	68·5	41·7
50	50 (45 – 60)*	none	68·5	41·7
150	50 (45 – 60)*	194	—	40·0
Ringer without glucoxe	155 (130 – 180)*	200 (160 – 240)	80·0	6·7

A group of three atria were used in each experiment.
*Extreme figures.
Normal chronotropism 169·6 ± 2·1 S.E. beats/min.

Toxic action of fluoroacetate. Treatment with Na fluorocitrate produced no effect, which we interpret as a failure in penetration. Addition of Na fluoroacetate (3·0 mM) for a time of contact of 150 min produced a negative chronotropic effect, and a more pronounced negative inotropic effect. In most cases episodes of arrhythmia occurred followed by low chronotropism (24 beats/min); there

TABLE 2. CITRIC ACID CONTENT OF RAT HEART ATRIA, μg/g, WET WEIGHT

Exp.		Treatment	Citric acid
1.	(a)	2 min work	83
	(b)	90 min work	102
	(c)	Fluoroacetate for 90 min then 90 min work	959
2.	(a)	None	71
	(b)	Fluoroacetate 25 min.	833
	(c)	Fluoroacetate 25 min followed by pyruvate + fumarate every 30 min until 290 min	893

In each group of each experiment four atria were used; Weights Exp. 1 240 – 245 mg; Exp. 2 224 – 270 mg; Na fluoroacetate 300 μg/ml; Na pyruvate 100 μg/ml Na fumarate 10 μg/ml. In each experiment male rats formed a comparable group.

followed a prolonged refractory period (3 spikes/min). This picture ended with a complete block of atrial activity in 208 ± 13 min after starting the fluoroacetate treatment. A lesser time of contact (25 min) decreased the beats/min to 78·1 ± 7·2 per cent of the maximum and the strength of the beat to 41·7 per cent after about 180 min five minutes exposure gave a transitory block of the atria after 20 min, with diminution of the beat to 58·8 per cent and of the inotropism to 66·7 per cent of the normal. From this the atria recovered in 170 ± 3 min. We also found an decrease in the diastolic relaxation at 55 (45–65) min from giving the fluoroacetate. When analysed at the end of the experiment, citrate contents were increased to 800–900 μg/g approx. (Table 2).

The effect of pyruvate + fumarate. In order to test the hypothesis of Williamson *et al.*,[1] addition of pyruvate and fumarate was used to make certain that enough 4-carbon acid was present to form extra citrate. The best working doses were found to be 100 μg/ml pyruvate and 10 μg/ml fumarate. (Several experiments had to be discarded because of the use of an impure specimen of pyruvate.) When each compound was added alone, the inotropic activity was reduced to 94·4 per cent and the beats to 97 per cent of the initial activity; but this was rectified by washing the "organ" vessel. The two compounds, when added together, gave a non-significant increase of approximately 10 per cent in beats. When pyruvate + fumarate was added to atria poisoned with fluoroacetate for more than 50 min, the atria regained their normal resting potential, with recovery of the beat and of inotropic activity to 41 per cent at 320 min. There was, therefore, some effect; but in the continued presence of fluoroacetate, the atria did not overcome the actual block, even though the dose of the compounds was repeated every 15 min, a treatment which should have given any displaced fluorocitrate ample chance to be removed. Hence in our experiments with atria, most of the block was not influenced by the treatment with with pyruvate + fumarate. This fact was also reflected in the citrate estimations, (Table 2). In two experiments quoted of which the last was especially trustworthy, there was virtually no change in the citrate content as a result of adding the acids. With these atria, therefore, we did not see reversal.

Further points. During the course of these experiments, some other observations were made upon the atria.

Glucose. It was found that omission of the energy (glucose) supply gave a picture overlapping that of fluoroacetate except that the beats retained 80 per cent of the normal activity until death. After 266 ± 21 min, there was always a decrease in diastolic relaxation (disappearance of the normal repolarization wave) followed in 50 min by a complete block of activity.

Noradrenaline given at a definite stage had a remarkable effect. After 25 min contact with fluoroacetate, atria did not respond to a normal dose of 0·1 μg/ml noradrenaline. But when 15 μg/ml of noradrenaline was given three times at intervals of 20 min inotropic activity was completely restored; at the same time the atria regained the normal response to 0·1 μg/ml of noradrenaline. In one complete experiment, no difference was detected in the citrate content of the atria exposed to noradrenaline. It was further found that if noradrenaline was given immediately before the fluoroacetate block, the time of survival of the atria is prolonged to (perhaps) 335 min. In these circumstances the normal

resting potential did not recover; the rate was (approx.) 12 beats/min, the spikes showing a normal height.

Discussion and summary. In these experiments with atria there was no support for the attractive idea[1] that pyruvate reverses the action of fluorocitrate in causing accumulation of citrate; but it should be stressed that their observations were upon the whole heart. The failure to reverse is, however, consistent with earlier experiments (Peters,[5]). It appears, however, as if there can be some recovery by washing after a low dose of fluoroacetate, which is enough to restore a return of the beat and improved inotropism. This may be due to washing out citrate. Nevertheless, the general toxicity of fluoroacetate could account in part for the asthenic state observed by Peters and Morselli.[6]

Regarding the interesting results with noradrenaline, these may be due to reactivation of phosphokinase on the lines described by Murad *et al.*[7]

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Effect of cysteine on 5-aminolaevulinate hydrolyase from liver in two cases of experimental intoxication

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THE PRESENT investigation was undertaken to study the effect of -SH groups of cysteine on the activity of 5-aminolaevulinate hydrolyase (δALA dehydratase) (adding 5-aminolaevulinate and cyclizing, EC 4.2.1.24) extracted from the liver of animals receiving chemical compounds known to disturb the porphyrin metabolic pathway.

The ability to induce experimental porphyria by allylisopropylacetamide (AIA) in rats and rabbits has been fully demonstrated.[1–3] In most cases the *in vitro* assay of δALA dehydratase activity in rat liver was carried out using -SH compounds (glutathione, cysteine, etc.).