OBSERVATIONS UPON THE BEHAVIOUR OF SOME PHOSPHATE ESTERS IN BRAIN AT THE START OF CONVULSIONS INDUCED BY FLUOROCITRATE AND FLUOROACETATE

by

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The recent publication by PSCHEIDT, BENITEZ, KIRSCHNER AND STONE[1] has led us to record some experiments made a year ago, and mentioned in lectures in Canada and the U.S.A. by one of us (R.A.P.) in September/October, 1953.

EXPERIMENTAL

Young rats were used; they were mostly litter mates for each experiment and weighed approximately 40 g; hence their metabolism could be rapidly fixed by submersion in liquid oxygen as soon as convulsions appeared. After freezing, the brains were dissected from the skull while the tissue was still at a low temperature in order to prevent post mortem hydrolysis of the phosphate esters, which may take place even at 0° C (ALBAUM, DRURY AND CAYLE[2]). Some observations were also made on other tissues which were dissected out of the frozen carcase.

The inorganic P and phosphate esters present in the nervous tissue samples were estimated by a previously described method (DAWSON AND RICHTER[3]). Inorganic phosphate in heart and kidney tissue was determined by the method of ENNOR AND STOCKEN[4], which enables the ion to be estimated in the presence of phosphocreatine.

Na fluorocitrate (synthetic), for a sample of which we are indebted to Dr. D. E. A. RIVETT[5], was injected intracranially in 0.05 ml isotonic NaCl solution under ether anaesthesia. In two experiments the dose was 100 μg and in the remainder, 50 μg; the equivalent dose for enzymically-made fluorocitrate would be approximately half these amounts. Bearing in mind that as little as 11 μg of enzymic fluorocitrate has produced convulsions when injected intracranially in pigeons, in these small rats the amount used was presumably a large excess. Convulsions occurred in times varying from 16–30 minutes.

Na Fluoroacetate was injected intraperitoneally in doses of 10 mg/kilo in a volume of 0.04 ml approx.; convulsions occurred in 30 to 44 minutes.

RESULTS

The results for the brain tissue are given in Table I. The averages show some fall in Ca insoluble P_{10} (labile nucleotide P) and molybdate-labile P (phosphocreatine), but this is significant for both only in the case of fluoroacetate. With fluorocitrate the drop was significant only in the Ca insoluble P_{10}. The citric acid showed the usual increases.

Table II gives corresponding P_0 and total P_{10} values for kidney and heart tissue. There is clearly no marked change. Citrate values showed the usual increases for fluoroacetate. Intracerebral fluorocitrate caused no change in citric acid content in the heart, but an increase in the kidney, which can be interpreted as due to the passage of some of

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the fluorocitrate from the brain to the kidney tissue, with its consequent block of aconitase.

TABLE I

BRAIN. PHOSPHATE ESTER DETERMINATIONS

Young rats, (a) control, (b) injected with intraperitoneal fluoroacetate, and (c) intracerebral with fluorocitrate. Killed at the start of convulsions.

			Calcium insoluble		*Calcium soluble*			
	Date	*Wt g*	P_a *μg/g*	P_{1a} *μg/g*	*Mol. labile P μg/g*	*Residual P μg/g*	*Citrate μg/g*	*Mins*
(a)	1.2.53	43	205	166	115	191	122	—
Control	24.2.53	35	123	113	91	146	106	—
	25.2.53	40	196	138	92	161	66	—
	3.3.53	34	185	150	100	227	105	—
	4.3.53	34	138	—	89	204	67	—
	10.3.53	32	166	144	86	275	100	—
		mean	169	142	95	201	94	
(b)	1.2.53	38	136	124	82	283	275	38
Fluoroacetate	24.2.53	36	(441)	116	51	117	246	42
Intraperitoneal	25.2.53	40	206	104	72	183	265	39
	3.3.53	34	172	133	67	208	277	38.5
	4.3.53	34	245	101	37	201	294	38.5
	10.3.53	32	175	131	65	239	212	30.5
		mean	187	118	62	205	261	
				$P < 0.05$	$P < 0.01$			
(c)	1.2.53	36	155	117	97	201	286	25
Fluorocitrate	24.2.53	35	248	103	60	185	164	16
Intracerebral	25.2.53	40	206	120	104	171	220	22.5
	3.3.53	36	134	111	74	198	189	26
	4.3.53	36	150	117	79	205	205	26.5
	10.3.53	32	240	132	61	235	126	30
		mean	189	117	79	199	198	
				$P < 0.03$	$P < 0.1$			

In some preliminary results for acetyl choline content, which were done for us by Prof. BURN on acid eserine extracts of the frozen brains by the frog rectus abdominal method, no marked change was found in the cerebral acetylcholine content of 3 convulsing animals as compared with controls.

DISCUSSION

The small but significant decreases with fluoroacetate injections in these experiments in the levels of phosphocreatine and labile nucleotide P in the brain are in agreement with PSCHEIDT *et al.*[1], who only found large changes when convulsions were prolonged. Similar small changes were observed here with fluorocitrate in the labile nucleotide values. The magnitude of these changes in the labile phosphate esters does not appear to be nearly as great as those occurring in the cat brain during severe hypoglycaemia (OLSEN AND KLEIN[6]) nor those in the rabbit brain during prolonged anoxia (ALBAUM, NOELL AND CHINN[7]). This suggests at first sight that the metabolic block induced by the fluorocitrate poisoning is insufficient to induce convulsive activity by starving the

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TABLE II

RATS KIDNEY AND HEART, DETERMINATIONS OF PHOSPHATE ESTER AFTER FREEZING

(see Table I)

	Date	Wt. g	Kidney			Heart		
			P_0 µg/g	P_{10} µg/g	Citrate µg/g	P_0 µg/g	P_{10} µg/g	Citrate µg/g
Control	7.3.53	34	164	181	194	244	157	122
	8.3.53	34	138	186	157	190	224	128
	11.3.53	32	140	242	139	160	261	156
	13.3.53	33	175	190	63	255	231	low
	13.3.53	29	152	196	59	245	236	low
		mean	154	199	122	219	222	135
Fluoroacetate	7.3.53	34	134	150	1185	183	153	566
Intraperitoneal	8.3.53	34	166	170	1340	(297)	(126)	554
	11.3.53	32	153	197	683	158	248	358
	13.3.53	29	211	224	1245	265	189	657
	13.3.53	25	133	223	955	195	160	372
		mean	159	193	1082	200	187	501
Fluorocitrate	7.3.53	36	171	154	512	215	188	131
Intracerebral	8.3.53	36	176	149	229	255	181	116
	11.3.53	32	209	151	686	209	221	123
	13.3.53	36	180	205	95	282	219	55
		mean	184	165	380	241	202	106

energy requirements of the nervous tissue. It should, however, be remembered that the result is not decisive, because the fluorocitrate may be both synthesised and also acting at specific points in the tissue. In this case, big decreases in the labile phosphate esters in these regions would be masked by the larger quantities of esters in the whole tissue. It is indeed possible, if unlikely, that the energy derived from respiration may be passed from the phosphorylation chain to essential neurological processes, without water-soluble A.T.P. acting as a direct intermediary, so that changes in the A.T.P. level only represent passive reflections of the energy equilibrium. Nevertheless, the small changes found are reminiscent of work on muscle tissue and suggest that they are more concerned with re-establishing the normal condition of the nerve cell following neuronal discharge. There is a definite dilemma; the convulsive state seems to be initiated by an interference by fluorocitrate with metabolism at the aconitase stage of the citric acid cycle; yet there is no appreciable change in energy-rich phosphate esters. The question arises therefore whether the lowered metabolism causes a hyperexcitable state of the tissue by increased ammonia content as suggested by BENITEZ, PSCHEIDT AND STONE[8], or even perhaps by a shift in pH.

It is interesting also that the level of labile P present in the kidney and heart after fluoroacetate administration also changes very little from the control value at the beginning of convulsive activity, although the citric content shows a relatively enormous increase. Here again it will have to be decided whether the large concentration of citrate in the kidney arises in connection with the excretory function of the kidney or whether the tissue's own metabolism is active enough to cause the rise. The divergence of our results obtained for heart from those of BUFFA[9], may be explained by the different

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times of killing used, and in their magnitude by the technique used for terminating enzyme activity (see FAWAZ AND HAWA[10]) and by the method we use of measuring labile P so that this includes phosphocreatine as well as labile nucleotide P.

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SUMMARY

1. The concentrations of some phosphate esters and citric acid in the brain, kidney and heart of the rat have been measured at the start of convulsions induced by fluoroacetate injected intraperitoneally and fluorocitrate intracerebrally.

2. No marked changes were observed in the levels of 10 min-labile P in these tissues, although there were small but significant decreases of the cerebral levels of phosphocreatine and labile nucleotide P after fluoroacetate administration.

3. The experiments provide no evidence that the convulsive discharge in fluoroacetate poisoning can be initiated by a lack of water-soluble energy-rich P esters in the brain, although the possibility of localized depletions was not excluded.

RÉSUMÉ

1. Les concentrations du cerveau, du rein et du coeur de rat en esters phosphoriques et en acide citrique ont été déterminées au début des convulsions provoquées par le fluoroacétate injecté dans le péritoine et le fluorocitrate injecté dans le cerveau.

2. Aucune modification des teneurs en P labile de ces tissus n'a été observée, si ce n'est une diminution faible mais significative des teneurs du cerveau en phosphocréatine et en P nucléotidique labile après injection de fluoroacétate.

3. Les expériences réalisées n'ont pu apporter la preuve que la décharge convulsive due à l'empoisonnement par le fluoroacétate puisse être provoquée par un manque d'esters phosphoriques riches en énergie et hydrosolubles dans le cerveau, quoique la possibilité de diminutions localisées ne soit pas exclue.

ZUSAMMENFASSUNG

1. Die Konzentration einiger Phosphatester und der Zitronensäure wurde im Gehirn, in der Niere und im Herzen der Ratte gemessen bei Beginn von Krämpfen, die durch intraperitonale Fluoroacetat- und durch intracerebrale Fluorocitrat-Injektion herbeigeführt waren.

2. Es wurde keine deutliche Veränderung des labilen 10 Minuten Phosphatgehaltes beobachtet, obwohl der cerebrale Gehalt an Phosphokreatin und an labilem Nukleotid-Phosphat gering aber signifikant abfällt nach Anwendung von Fluoroacetat.

3. Die Experimente geben keine Anhaltspunkte dafür, dass die Krampfentladung bei Fluoroacetat-Vergiftung durch einen Verlust der wasserlöslichen energiereichen Phosphatester des Gehirns herbeigeführt werden kann, obgleich die Möglichkeit einer lokalen Erschöpfung nicht ausgeschlossen wurde.

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