effects of such an induced hypothermia upon personnel exposed to a stressful situation in the cold.

Summary. Healthy adult male and female cats were restrained in essentially normal positions by binding of legs or head to stationary supports. The effect of exposure on the body temperature of these animals in a cold room (−15°C ± 2°C) was compared with that of dead animals and with controls which were housed individually in large cages. Temperatures were taken with indwelling rectal thermometers in the dead and restrained animals and at the beginning and end of the tests in the case of the control cats. Body temperatures of the restrained animals over a 2-hour exposure fell markedly (5.6°C) as compared to the control group (1.2°C). The temperature fall of the dead animals was the greatest (10.2°C). Since the animals were not caged, the hypothermia which resulted must be attributed to factors other than restricted breathing, perhaps partially to emotional stress.

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Effect of Fluoroacetate Upon Poliomyelitis in Monkeys.* (20793)

THOMAS FRANCIS, JR., GORDON C. BROWN, AND ALEXANDER KANDEL.
From the Department of Epidemiology, School of Public Health University of Michigan, Ann Arbor.

Recent studies from this laboratory have demonstrated that the intraperitoneal administration of sodium fluoroacetate inhibits the growth of influenza virus in the lungs of mice (1) and that it suppresses the growth of the Lansing strain of poliomyelitis virus in the brains of mice(2). In the latter report it was shown that this compound also effected an increase in the incubation period of disease. These experiments as well as others employing tissue cultures(3) demonstrated that fluoroacetate does not destroy the virus upon simple contact but rather that its effect is obtained by interrupting the Krebs cycle so as to interfere with the utilization of citric acid. The accumulation of citric acid in large quantities in animal tissues following fluoroacetate administration has been adequately demonstrated(4-7) and its continued synthesis without further metabolism appears to be due to inactivation of the enzyme system necessary for the oxidation of citrate. The present report describes the effect of intravenously inoculated fluoroacetate upon cynomolgus monkeys and their infection with poliomyelitis virus.

Methods. Cynomolgus monkeys (*Macaca irus*) weighing between 1.5 and 3.5 kg were used throughout this study. A solution of sodium fluoroacetate was freshly prepared in distilled water and inoculated intravenously in measured quantities to give exactly the desired mg per kilo of body weight. Toxicity tests determined that a dosage of 5 mg per kilo was very near the maximum tolerated dose but was well tolerated by the animals except for occasional and temporary weakness, irritability, and anorexia. The citrate content of the brain tissue in 3 monkeys following administration of this dose of the drug was determined by the pentabromoacetone method(7). The values in γ/g wet tissue were found to be 33, 29, and 36, respectively. Normal values of 40, 41, and 42 obtained with untreated monkeys demonstrate that the brain is not the site of citrate accumulation. The significance of this finding will be considered in the discussion. In the experiments to be

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TABLE I. Effect of Fluoroacetate upon Infection of Cynomolgus Monkeys with 100 PD_{50} Poliomyelitis Virus.

	Par	Not par	P	Onset paralysis (days)	Avg onset
			Treated (0 day)		
	3	2	.0952	8, 9, 13, 0, 0	10
	4	1	.0522	9, 11, 14, 17, 0	12.75
	3	1	.0216	8, 11, 18, 0	12.3
	7	1	.0192	5, 9, 10, 12, 17, 17, 19, 0	12.7
	8	2	.0043	8, 8, 8, 9, 10, 10, 12, 14, 0, 0	9.9
	8*	2	.0010	9, 9, 9, 11, 11, 12, 14, 14, 0, 0	11.1
Total	33	9			11.3
%	78.5	21.5			
			Treated (+3 day)		
	5	0		9, 11, 11, 16, 16	12.6
	5	0		7, 9, 11, 11, 11	9.8
	2	1	.4063	12, 12, 0	12
	6	4	.0129	6, 7, 7, 8, 9, 10, 0, 0, 0, 0	7.8
Total	18	5			10.1
%	78.2	21.8			
			Controls		
	10	0		5, 7, 7, 7, 8, 9, 9, 9, 11, 11	8.3
	5	0		7, 7, 8, 11, 11	8.8
	5	0		6, 6, 9, 11, 21	10.6
	4	0		4, 13, 16, 17	12.5
	10	0		7, 9, 9, 9, 9, 9, 10, 10, 10, 12	9.4
	10	0		7, 8, 8, 8, 10, 10, 14, 14, 17, 19	11.5
Total	44	0			9.9
%	100	0			

* 10 PD_{50}.

described, a dose of 5 mg/kilo was administered once either on the day of virus inoculation (0 days) or 3 days after virus (+3 days). The virus was the original Mahoney strain which had been isolated in this laboratory and adapted to subcutaneous invasiveness by repeated passage via this route. It was preserved as titrated pools in glass sealed ampoules in the frozen state until used. With the exception of one experiment which employed a 10^{-3} dilution, all inoculations consisted of one ml of a 10^{-2} dilution which represented 100 PD_{50} of virus when given subcutaneously between the scapulae. Appropriate numbers of control monkeys were inoculated with virus alone and the animals were observed daily for clinical signs of illness. All questionable diagnoses were clarified by histological examination.

Results. The results of 6 experiments are summarized in Table I. All monkeys inoculated with virus alone developed paralysis. This result among the control animals emphasizes the adequacy of the viral dosage employed in these tests. In contrast, 9 of 42 monkeys (21.5%) which received 5 mg of fluoroacetate per kilo on the day of virus resisted the clinical infection as did 5 of 23 animals (21.8%) receiving the drug on the third day after virus.

The probabilities of these results occurring by chance were calculated by use of exact probability for a specific combination.† These probabilities for the 0 day treated groups are listed in Table I. From these it will be seen that the likelihood of chance occurrence of the results after 2 experiments was 5 in 100, and after 3 experiments as 2 in 100. Although the survival rate for all 6 experiments was calculated as being very significant (1 chance in 1000) the last 3 experiments were actually unnecessary since significance had been reached much earlier. This illustrates the advantage of calculating significance more frequently during the course of such experimental work. In contrast to the "0 day" results, significance was not reached until the fourth and final experiment when drug was administered on the third day after virus, although the per cent of survival was remarkably similar.

The average incubation period in drug-treated monkeys which developed paralysis was not significantly different from that of the control group.

Discussion. The suppression of viral growth in isolated tissues cultured outside the intact host is readily accomplished with a wide variety of metabolic inhibitors. Although these experiments provide valuable information concerning the growth requirements of a given virus such compounds are

† The equation is $P = (r_1 ! r_2 ! C_1 ! C_2 !)/(a! b! c! d! N!)$ where the letters are taken from the form:

a	b	r_1
c	d	r_2
c_1	c_2	N

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rarely effective in the intact animal where chemical interference with virus is usually accompanied by the inhibition of some metabolic process essential to the well being of the host. Although efforts to affect the infection of monkeys with Brunhilde type poliomyelitis have been discouraging, some success has been achieved against the Lansing strain of poliomyelitis virus in mice(2,8-11). These results suggest that where survival is the criterion of effect, some advantage is gained in experimental chemotherapy with inhibitors which act upon the essential cellular metabolism. While the primary effect of fluoroacetate is a blockage of the oxidation of citric acid, the secondary effect is probably a temporary depletion of certain free amino acids of the cells which are diverted to citrate formation(12). It is through these secondary effects that one would expect viral development to be influenced(13). Since the duration of the effect of fluoroacetate in animals is short, and since in the present studies it was administered several days before virus could reach the central nervous system, its influence was probably directed upon some extraneural tissue which was important in the initiation of the infection. Furthermore, the data regarding citrate accumulation indicate that the levels of fluoroacetate employed in these experiments did not affect the brain. This finding concurs with more extensive studies(5,6) in other laboratories which have shown that the primary and secondary effects of fluoroacetate are not uniform in all tissues of the same species or in different species(14). This is in direct opposition to the conclusions on this subject reached by Mogabgab and Horsfall(15) who assumed that all tissues respond uniformly to the drug and concluded that the oxidation of citrate was a reaction unimportant to the development of influenza virus. Additional evidence of an extraneural effect of fluoroacetate is seen in the finding that the period of viremia is markedly reduced in monkeys treated with this compound(16). More extensive examination of tissues affected by fluoroacetate should reveal vital information of the pathways and sites of viral multiplication.

Summary. 1. Cynomolgus monkeys were treated with 5 mg/kilo of sodium monofluoroacetate on the day of, or 3 days following, subcutaneous inoculation of Type 1 poliomyelitis virus. 2. Nine of 42 monkeys which received drug on the day of virus resisted clinical infection as did 5 of 23 animals receiving drug on the third day after virus. All control animals became paralyzed. 3. Citrate did not accumulate in brain tissue of drug-treated monkeys suggesting that the action of fluoroacetate was at some site other than the brain. 4. The significance of these findings is discussed.

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