increase in the amount of catheptic digestion by increasing quantities of myosin solution. The overall colorimetric change due to cathepsin digestion (from 40% to 50% light absorbed) was comparable to an increase in concentration of a standard tyrosine solution from 0.0025% to 0.005%. A leveling off phase, indicative of equilibrium, was recorded with myosin-pepsin and myosin-free trypsin solution, but not with myosin-cathepsin solutions.

Curves showing the amount of digestion by proteolytic enzymes in 1 cc of muscle protein solution during different time intervals, are shown in Fig. 2. In all instances there was a rapid increase in the amount of digestion during the first 5 minutes. This was followed by a slower, but steady, increase in the rate of digestion.

Summary. 1. An investigation was made to obtain information regarding the presence and localization of proteolytic enzymes in muscle protein fractions from frog leg muscles. 2. Pepsin and cathepsin were localized in the myosin fraction while trypsin was localized in the myosin-free fraction. No proteolytic activity was displayed by the myoalbumin fraction. 3. The myogen content of the frog muscles is considerably lower than that reported for mammals.

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Effect of Sodium Monofluoroacetate on Multiplication of Eastern Equine Encephalomyelitis Virus.* (19754)

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Sodium monofluoroacetate has been shown to affect the citrate metabolism of tissues from animals treated with this compound. Potter (1) has found that the citrate level of rat brain after treatment of the animal with this drug was approximately 6 times that of normal. Tricarboxylic acid fractions isolated from tissues of fluoroacetate-treated animals(2) have shown the presence of a fluorine-containing compound ("inhibitor fraction") that would inhibit the oxidation of citrate. Recently it has been found(3) that aconitase activity is inhibited by this "inhibitor fraction". The inhibitory effect is thought to be produced by a fluorine-containing compound other than fluoroacetate and which has been shown to be chromatographically inseparable from the tricarboxylic acid preparations. It has been suggested that the inhibitory substance is fluorocitrate$_2$(4).

Ackermann(5) has found that fluoroacetate will cause an initial depression in the rate of influenza virus production in experimentally infected mouse lung. However, the virus concentration in the treated lung tissue was found to be similar to that of the non-treated lung tissue at a later period in the virus incubation time.

Ainslie(6) has investigated the effect of this compound on the rate of virus production in Lansing poliomyelitis virus infected mice and has noted a significant delay in the virus growth which he was able to relate to an increased survival time of the infected animals treated with fluoroacetate, but the natural course of the infection was not otherwise altered.

Materials and methods. Swiss albino mice

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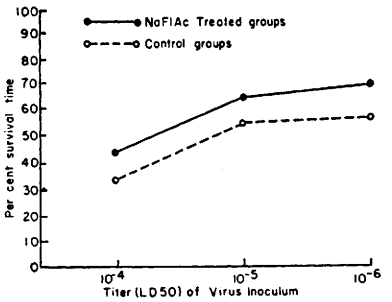

FIG. 1. Effect of sodium monofluoroacetate on the survival time of infected mice.

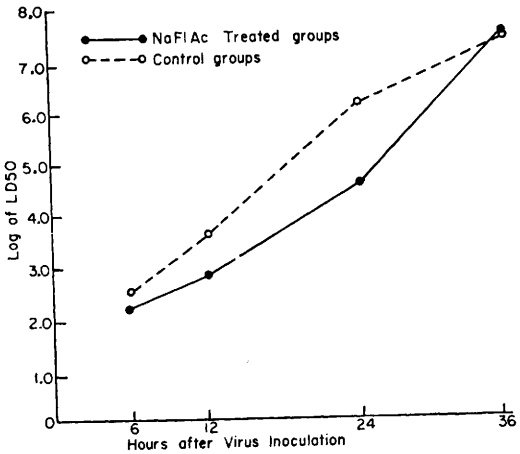

FIG. 2. Effect of sodium monofluoroacetate on the growth curve of virus.

weighing 10 to 15 g (3 to 5 weeks old) were used throughout this work. The mice were matched evenly for weight and age in each experiment.

The fluoroacetate‡ was freshly prepared for intraperitoneal injection by dissolving in isotonic phosphate-NaCl solution (pH 7.4). Mice serving as the "treated" groups were given 5.0 mg/kg of this compound in a volume of 0.15 ml one hour before virus inoculation. This served to eliminate any animals from the experiment that might die from toxic effects of the drug.

Mice were infected by injection of virus intracerebrally, in doses of 0.03 ml, using Eastern equine encephalomyelitis virus.§ Mice were observed for a period of 10 days after infection, although symptoms of infection usually developed within 36 to 72 hours.

Results. Fluoroacetate-treated and normal control animals were separated into groups of 10 animals each and inoculated with varying dilutions of virus (Fig. 1). The per cent survival time was obtained by using a possible ten days of survival for each mouse in the group of 10 animals, thus arriving at the 100% survival time for total survival of the group. There was a consistent increase in the survival time of treated over non-treated control animals.

To evaluate the effect of the fluoroacetate on the survival time of the mice, a larger number of animals were inoculated with a dilute suspension of the virus at "zero time", half of which had been previously treated with fluoroacetate. Five mice from both the treated and control groups were sacrificed at the indicated intervals (Fig. 2) and the brains removed to determine the virus titre (or content) at the given period. The virus concentration was determined by the 10-fold serial dilution method, employing 5 mice for each dilution per virus sample. The LD_{50} dose was calculated by the method of Reed and Muench(7). As can be seen, the fluoroacetate definitely depressed the amount of infective virus in the treated animals during the initial 24-hour period, which has also been shown to be the time of a demonstrable effect of this drug on citrate metabolism in brain tissue(1). However, by the 36 hour period the virus concentrations of the 2 groups are comparable.

It was deemed necessary to determine whether the fluoroacetate could interfere with the amount of virus necessary to establish an infection and, accordingly, titrations of a virus sample were carried out using a) a group of normal mice, and b) a group of fluoroacetate-treated mice. No significant effect of the drug on the susceptibility of the mice relative to virus dosage could be noted (Table I).

The possibility of a direct virucidal action of the drug on the virus was investigated by

‡ The sodium fluoroacetate was kindly supplied by the Monsanto Chemical Co. as a fluorine ion-free compound.

§ This virus was kindly supplied by the Laboratory of the United States Public Health Service, Montgomery, Ala.

TABLE I. *In Vivo* Effect of Sodium Monofluoroacetate on Susceptibility of Mice to Virus Infection.

	NaFlAc-treated group	Control group
Virus sample A (EEE)*	LD_{50} $10^{-6.5}$	LD_{50} $10^{-6.5}$
" " B (EEE)	LD_{50} $10^{-4.5}$	LD_{50} $10^{-4.6}$

* EEE—Eastern equine encephalomyelitis virus.

TABLE II. *In Vitro* Effect of Sodium Monofluoroacetate on Infectivity of Virus (Virucidal Effect).

Virus treated with:	Virus LD_{50}
NaFlAc	$10^{-5.4}$
NaAc	$10^{-5.4}$
Control	$10^{-5.4}$

dividing a virus sample into three parts and diluting them as follows: a) 1:20 to a final concentration of 0.001 M sodium monofluoroacetate, b) 1:20 to a final concentration of 0.001 M sodium acetate, and c) 1:20 with distilled water. These virus dilutions were then incubated at 37°C in a water bath for 1 hour and subsequently titrated in young mice. No significant differences could be noted among the titration results from these three virus samples (Table II.)

Discussion. The results presented indicate that the growth of Eastern equine encephalomyelitis virus is inhibited by the administration of sodium monofluoroacetate in a similar fashion to the inhibition of the viruses of influenza and Lansing poliomyelitis in mice by this drug. The present experiments show that fluoroacetate neither inactivates this virus upon direct contact nor alters the susceptibility of the experimental animal to the virus infection. On the other hand, the experiments do indicate that the chemical prolongs the course of the experimental infection.

The current theory of virus multiplication is that the virus utilizes some of the enzyme systems of the host cell for its reduplication and that this process requires energy. Ackermann(8) claims that for the synthesis of influenza virus, the proper functioning of the Krebs cycle is essential as this cycle seems to offer the energy necessary for the synthesis of some essential component of the virus particle, such as nucleoprotein. As fluoroacetate has been shown to inhibit the oxidation of citrate and consequently to reduce the amount of energy to be gained from the proper functioning of the Kreb's cycle, Ackermann's hypothesis seems to offer a reasonable explanation not only for the reduced synthesis of influenza virus in fluoroacetate-treated tissues, and for that of Lansing poliomyelitis but also for Eastern equine encephalomyelitis virus. Doty and Gerard(9) have shown that fluoroacetate will significantly depress the resting Qo_2 of excised nerve without altering the active Qo_2 or spike height of the stimulated nerve for a period of seven hours. However, the possibility that changes in the cellular metabolism (other than the blocking of energy sources) might produce conditions unsuitable for virus synthesis has not been excluded.

As the rate of infective virus production in the treated tissues was lower than in the control tissues, but not completely inhibited, it is suggested that the reduced production of virus may be due to a) a longer period of virus incubation per infected cell or b) a smaller burst size of virus per infected cell. As the cell population of the brain is by no means homogeneous, it might also be suggested that the virus-sensitive cells may differ in their sensitivities to fluoroacetate, thus limiting the efficiency of the inoculated virus in producing a new generation of infective particles. As the fluoroacetate effect decreases, the efficiency of the virus should increase until the point of saturation of susceptible cells is approached(10).

Summary. The reduplication of Eastern equine encephalomyelitis virus in mouse brain is delayed in mice previously treated with sodium monofluoroacetate. This chemical seems to prolong the survival time of the infected animals, although it does not otherwise alter the course of infection. In high concentration, the chemical has no direct effect *in vitro* on the infectivity of the virus, nor does it show an *in vivo* effect on the susceptibility of the host to the infection with this virus.

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Inhibition of Aminotripeptidase.* (19755)

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An aminoexotripeptidase which hydrolyzes one peptide bond of a tripeptide such as l-leucylglycylglycine and diglycylglycine (GGG) has been demonstrated in a number of tissues by Ellis and Fruton(1). This enzyme, referred to by these authors as tripeptidase, was inhibited by a number of metal ions and by relatively large concentrations of cysteine, but not appreciably by cyanide, sulfide or citrate ions. Although lymphoid cells are said to be especially rich in tripeptidase(2), it is distributed in other cell types (3), and has been found in several microorganisms(4). Stern and co-workers(5), have shown that human white blood cells have a high tripeptidase content, and that the activity of leucocytes was about 40 times greater than that of erythrocytes. The tripeptidase activity of serum is increased in certain pathological conditions such as bone fracture(6), burns(7) and a number of diseases(8). It has been observed in this laboratory that a tripeptidase is present in synovial effusions of a number of arthritic diseases, and is especially rich in the synovial fluid of patients with rheumatoid arthritis(9).

We have observed, in the present investigation, that the activity of tripeptidase from calf thymus, rabbit skeletal muscle and human synovial fluid, with respect to the hydrolysis of GGG, is inhibited by a group of compounds possessing anti-histamine, local anesthetic and anti-cholinesterase activity in concentrations as low as 0.0001 M.

Methods. Peptidase activity was determined using the titrimetric method of Grassman and Heyde(10). The reaction mixture contained 0.5 or 1.0 ml of the enzyme solution, 0.5 ml of 0.25 M GGG, previously neutralized to pH 7.8, 0.1 ml of inhibitor solution neutralized to pH 7.8 just prior to use, and sufficient 0.02 M veronal buffer at pH 7.8 to bring the total volume to 2.5 ml. The reaction was carried out in stoppered flasks and in a shaking water bath at 37.5°C. In general, reaction was allowed to proceed to 60% hydrolysis of one peptide bond, and 4 or 5 analyses in duplicate were obtained by titration of 0.2 ml samples, removed from the incubation mixture, with 0.015 N KOH in 90% ethanol. The hydrolysis of GGG was zero order to the extent of approximately 60% splitting of one peptide bond. Results are expressed as per cent hydrolysis per hour.

Calf thymus peptidase extracts were prepared according to Fruton(2), and rabbit skeletal muscle extracts according to Smith (11). The extracts obtained were frozen and thawed twice to remove extraneous protein without diminution in total activity. When inhibitor compounds were added, the reaction was followed for a variable time given below, dependent on the extent of inhibition. In the case of benadryl and procaine, the hydrolysis curves were not linear because of decomposi-

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