MONOFLUOROACETIC ACID AND RELATED COMPOUNDS[1]

MAYNARD B. CHENOWETH

Department of Pharmacology, University of Michigan, Ann Arbor, Michigan

During and since World War II there has been a widening interest in the polyfluorinated hydrocarbons, most of which are very nearly inert biologically. On the other hand, a group of monofluorinated compounds has been found that includes many very toxic substances. Monofluoroacetic acid is the prototype of these compounds, which exhibit pharmacological actions of remarkably different character in different species. It is the purpose of this review to bring together and attempt to explain the available information concerning these potent pharmacological agents. The review has not been undertaken primarily because of interest in the fluoroacetates[2] *per se* but rather because their study contributes greatly to the pharmacological and biochemical understanding of drug actions.

Although first prepared synthetically by Swarts (122) in 1896, monofluoroacetic acid (FCH_2COOH) and its derivatives attracted very little attention from chemists and none from pharmacologists until the early 1940's when Polish chemists (96), escaping to England, brought word of the toxicity of the methyl ester of fluoroacetic acid which they had prepared (60). It was soon established by intensive studies under the auspices of the armed services in England and in this country that the fluoroacetates are exceedingly curious substances. The compounds are so toxic to dogs that 50 micrograms per kilogram cause prolonged convulsions of central nervous system origin and death from respiratory failure; yet they are only 1/200 as toxic to monkeys, in which species cardiac poisoning and ventricular fibrillation are the cause of death. The reasons for these seemingly capricious effects present problems of interest to biologists.

Security precautions during the war prevented prompt publication of research results and it therefore occasionally was difficult to acknowledge properly some of the investigative work done during that period. During this period Marais, in an independent study reported in 1944 (93), succeeded in isolating potassium monofluoroacetate from the South African plant, *Dichapetalum cymosum*. This plant, known locally as "Gifblaar," is a well-recognized hazard to stock and cattle (131). The occurrence of fluoroacetic acid in this plant is believed to be the first example of a naturally occurring organic fluoride; it completes the list of natural organic halogen compounds and presents an interesting problem in biosynthesis.

A considerable body of practical experience with the use of sodium fluoroace-

[1] Original investigations reported in this communication were supported (in part) by research grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

[2] The use of "fluoroacetates" as a loose generic name for this series of compounds is based on the fact that fluoroacetic acid and its simple compounds are the lowest active members of a homologous series.

tate (Compound # 1080, hence the common name of "ten eighty") as a rodenticide and general mammalian pest control agent has accumulated since Treichler and Ward of the United States Fish and Wildlife Agency introduced the compound for this purpose in 1945. The pertinent original literature should be consulted by those interested in this important application of fluoroacetate (5, 6, 66, 74, 92, 119, 128). It will be reviewed here only when information it contains contributes to the understanding of the biological actions of these compounds. The rodenticidal uses of the chemical cannot be ignored for such uses are of prime ecologic importance. The animal population of a poisoned area may be almost entirely destroyed by sodium fluoroacetate and its widespread use in pest control has been the cause of several human fatalities.

I. SPECIAL CHEMISTRY OF THE FLUOROACETATE COMPOUNDS

A. Synthesis

The synthesis of fluoroacetic acid, its derivatives and analogs has been studied intensively (60, 93, 122), particularly with the objective of perfecting large scale processes (7, 24, 72, 110, 111, 112, 113). Jenkins and Koehler have described the process and safeguards employed by the chief manufacturer of "1080", sodium fluoroacetate (73). Germane to the present review is the fact that most commercial procedures for production of "1080" result in some contamination of the product with fluorides (usually KF) unless special effort, usually redistillation of an ester of fluoroacetic acid, is taken to purify the product. This can be a source of error in interpreting the results of studies with fluoroacetate, particularly with high concentrations *in vitro*.

B. Structural Aspects

Certain details concerning the physical chemistry of fluoroacetate are important for an understanding of its pharmacological actions. The chemical behavior of monofluorinated organic acids in general is very little different from that of the corresponding unsubstituted acids; this is in contrast to the other monohalogenated acids. Indeed, the process of fluorination, with the corresponding shortening and strengthening of inter-atomic bonds, appears to confer increasing nonreactivity on all molecules (61).[3]

Attention has been directed particularly to the stability of the carbon-fluorine bond in fluoroacetic acid. There are little or no data upon the nature of this bond in longer-chain acids but their pharmacological behavior suggests that the stability of their C-F bond parallels that in fluoroacetate. It has been confirmed repeatedly that rupture of the C-F bond is very difficult (12, 13, 40, 110). Although some samples of sodium fluoroacetate do contain fluorides as contaminants, it is not reasonable to suppose that the fluoroacetates exert their actions by the liberation of fluoride, the pharmacological actions of which are quite different (108). Indeed, Bergmann and Fruton found that fluoroacetate, even after many

[3] The chemistry of the aliphatic fluorine compounds, as it was known in 1941, has been reviewed by Henne (61). Two additional recent reviews (14, 101) of the expanding field of fluorine chemistry should be consulted by those interested.

hours in solution under physiological conditions of pH and temperature, loses no fluorine detectable as fluoride ion (13). However, boiling with 20 per cent KOH for 20 hours will release 50 per cent of the fluorine as KF (110). Bartlett and Barron have published a large list of biologically important chemical substances and enzymes with which fluoroacetate does *not* react, and have contrasted the four monohalogenated acetic acids with regard to their ability to thioacetylate cysteine (12) (see Table I). The rate of replacement of the fluorine of ethyl fluoroacetate by sulfite at 45° C. is expressed by a bimolecular velocity constant of 4.5×10^{-5}, whereas the bromine of ethyl bromoacetate is replaced at 25°C. at a very much greater rate (constant = 18.3), according to Backer and van Mels (8). This extreme nonreactivity of carbon-attached fluorine reflects the general relationship between the rates of reaction and the bond-energy values of the C-halogen bonds as well as the electro-negative values of the halo-

TABLE I

Physical and Chemical Properties of Acetic Acid and Monohalogenated Acetic Acids

(References to source of data are given in parentheses)

COMPOUND	DISSOCIATION CONSTANT OF ACID $K_a \times 10^{-4}$ (130)	ATOMIC RADIUS (104)	INTERNUCLEAR DISTANCE IN ÅNGSTROMS (118)	BOND ENERGY IN KILO-CALORIES PER MOLE (103)	HALOGEN ELECTRONEGATIVITY (103)	REACTION RATE WITH CYSTEINE. TIME TO HALF REACTION AT 23°C. (12)	LD_{50} MICE Na SALT ORALLY
							mgm./kgm.
HCH_2COOH.....	1.8	(H) 0.29	1.14	87.3	—	—	—
FCH_2COOH.....	210.	(F) 0.64	1.45	107.0 114.(59)	4.0	No reaction	17(125)
$ClCH_2COOH$.....	155.	(Cl) 0.99	1.74	66.5	3.0	125 mins.	165(100)
$BrCH_2COOH$.....	138.	(Br) 1.14	1.90	54.0	2.8	6.2 mins.	100(100)
ICH_2COOH.....	75.	(I) 1.33	2.12	45.5	2.5	4.0 mins.	63(100)

gens (Table I). It may be noted that, although chloroacetic acid is the strongest acid of the Cl, Br, I-monohalogenated acetic acids, iodoacetic is the most toxic and chloroacetic the least toxic. The toxicity is probably a reflection of their relative activity as thiol acetylating agents. But fluoroacetic acid, the strongest monohalogenated acetic acid, is also the most toxic. It is apparent that toxicity in this series is directly related to the reactivity of the halomethyl, rather than the carboxyl, moiety of the molecule. The great difference in chemical character between fluoroacetic acid and the more familiar iodoacetic acid is almost certainly a direct cause of the equally great difference in pharmacological action.

C. Analytical Aspects

There appears to be no chemical reaction given solely by fluoroacetic acid which would distinguish it from other compounds. Reactions of acetic acid which depend upon attack of the methyl group do not proceed at all or only under much more drastic conditions when attempted with fluoroacetic acid,

although there is no appreciable qualitative difference with regard to reactions involving the carboxyl groups. Fluoroacetate solutions give the characteristic blue color given by acetate and propionate with lanthanum salts (Kruger and Tschirch test). Hutchens and Kass (68) have made this test quantitative under very controlled conditions; for example, it is capable of detecting fluoroacetate in culture media in a range of 100 to 400 parts per million. The crystalline forms of the barium salts of the four monohalogenated acetic acids are useful for identification and have been described in detail (46). Characterization of sufficiently large amounts of pure fluoroacetic acid has been accomplished by the formation of conventional and suitable derivatives (60, 73, 110), especially fluoroacetamide (7), a useful intermediate.

Although there appears to be no great difficulty in characterizing fluoroacetic acid in the organic chemist's laboratory, quite the contrary is true when determination of small amounts of the compound in biological material is attempted. To date, nearly all methods depend upon splitting off and detecting the fluoride ion. Quantitative methods for the determination of fluoride with zirconium alizarin sulfonate (57) or as lead chlorofluoride (49) have been employed. Much has been accomplished toward the detection of fluoroacetate in drinking water by drastic measures to release the fluoride ion which are rather convenient for field use (57). Ramsey and Clifford have recently described (106) a method for the accurate and specific determination of fluoroacetate in food and other biological material in concentrations as low as 0.2 part per million, a degree of sensitivity which has very practical importance.

D. Stability of Sodium and Methyl Fluoroacetate Solutions

The extraordinary stability of the fluorine-carbon bond has attracted considerable attention and has led to the impression that compounds of fluoroacetic acid are very stable. This is not entirely true. Although solid sodium fluoroacetate, which is highly hygroscopic, keeps well in a desiccator, aqueous solutions of salt or esters decrease in toxicity with time. Albaum (2) showed that methyl fluoroacetate solutions decreased in toxicity even though refrigerated, while at room temperature the process was accelerated. Thus, a solution injected intraperitoneally in the calculated dose of 5 mgm./kgm. killed 7 of 10 rats when it was fresh but only 2 of 12 rats after it was kept 24 days in the refrigerator (approximately 5°C.). A second solution deteriorated at room temperature so markedly that, although 5 mgm./kgm. killed 19 of 24 rats when injected immediately after being prepared it killed but 8 of 20 rats after it had stood for only 7 days. Although precise assays have not been made, observations in this laboratory with solutions of sodium fluoroacetate and sodium γ-fluorocrotonate have indicated a similar rate of deterioration. As far as toxicity to yeast is concerned, however, fluoroacetate solutions remain unchanged for 1 month at 3° to 5°C. (77).

It is to be expected that hydrolysis of the methyl ester rapidly occurs and Price and Jackson (102) have found a half-life of less than an hour for this reaction at pH 7.0. This is not particularly important as a cause of the decreasing

activity inasmuch as a similar decrease is noted with the sodium salt. As pointed out under section I-B, no fluoride ion is released under these conditions. Woods (133) has suggested the possibility that spontaneous decarboxylation accelerated by the resonance effects of the fluorine atom may occur according to the reaction

$$FCH_2COONa + H_2O \rightarrow NaHCO_3 + CH_3F \uparrow$$

with the liberation of the highly volatile, relatively non-toxic methyl fluoride. Thus, although no fluoride ion would accumulate in the solution, the actual content of fluoroacetate in the solution continually decreases. This type of reaction is often catalyzed by traces of halogen ions. Until more is known about this phenomenon, investigators using fluoroacetates should prepare solutions just prior to use. Deterioration does not seem to be a serious problem when solutions of sodium fluoroacetate are used in routine rat poisoning operations, perhaps because of the generally short (2 to 3 day) period of exposure of the poison (6).

II. TOXICITY

A. Response of Various Species to Toxic Monofluorinated Acids

Very few compounds are known which exert such variable pharmacological actions in different species as does fluoroacetate. Not only does the LD_{50} vary from 0.06 mgm./kgm. in the dog to well over 500 mgm./kgm. in the unique case of the South African clawed toad (*Xenopis laevis*), but the qualitative character of the pharmacodynamic action of the drug is equally varied. The major point of attack may be either the central nervous system or the heart. Both may be affected to varying degrees in some species, but it is usual to find that one organ is primarily concerned while the other is but slightly, or not at all, affected. Death may result from (a) respiratory arrest following severe convulsions, (b) gradual cardiac failure or ventricular fibrillation, or (c) progressive depression of the central nervous system with either respiratory or cardiac failure as the terminal event. All these responses follow a long and essentially irreducible latent period after the administration of the poison by any route. These phenomena are discussed in section III.

In Table II, the main toxic effect, if known, is indicated by 4+ and on the same scale 1+ indicates that this effect is very rarely seen. Although there is some difference between the sodium salt and the methyl ester of fluoroacetic acid when they are applied to frog tissue (18, 23, 38, 39), there appears to be no difference between them in the intact animal (30). No distinction between the two chemicals is made in Table II although most of the data were obtained with sodium fluoroacetate.

Study of the information presented in Table II indicates that, among the warm-blooded species, primates and all types of birds are generally the least susceptible to fluoroacetate poisoning, whereas the carnivora and *wild* rodents appear to be particularly sensitive. The Texan pocket gopher (*Geomys breviceps* sp.) is the most sensitive species so far described, all nine of those studied being killed by an intraperitoneal injection of 0.05 mgm./kgm. It is noteworthy that

TABLE II

Toxicity of Fluoroacetate Compounds

SPECIES	LD_{50}	ACCURACY*	ROUTE	HEART†	BRAIN†	REFERENCE
Sodium or methyl fluoroacetate; fluoroethanol						
	mgm./kgm.					
PRIMATES						
Man (*Homo sapiens*)	2–5	3	Oral	4+VF, F	2–3+C	6
Rhesus Monkey (*Macaca mulatta*)	4.0	2	I.V.	4+VF, F	2–3+C	30
Spider Monkey (*Ateles geoffroyi*)	15.0	2	I.V.	4+VF, F	0	30
UNGULATES AND RUMINANTS						
Goat	0.6	2	I.M.	4+VF	0	30
Sheep	2.0	3	Oral	4+VF	0	105
Horse	1.0	2	Oral I.M.	4+VF	1+C	30, 52, 53
Swine (young)	0.4	1	I.P.	4+VF	4+C	30
Swine (adult)	<1.0	2	Oral	4+VF	4+C	115
CARNIVORES						
Canines:						
Dog (mixed breeds)	0.06	2	I.V.	0	4+C	30
	0.10(LD_{100})	1	I.V.	0	4+C	30, 37
Coyote (*Canis latrans nebracensis*)	0.10	1	I.P.	0	4+C	129
FELINES						
Cat (*Felis domesticus*)	0.20	1	I.V.	2+VF	4+C	99
RODENTS						
Rats:						
Albino Rats	5.0	1	I.M. S.C. I.P.	1+VF 2+F	3+C 4+D	30, 129
	2–3	2	S.C.	—	—	52
	2.5	—	Oral	—	—	74
Cotton Rat (Florida) (*Sigmodon hispidus littoralis*)	0.1	1	Oral	—	—	129
Wood Rat (California) (*Neotoma intermedia*)	1.5	2	Oral	—	—	129
Wood Rat (Arizona) (*Neotoma a. albegula*)	0.8	1	I.P.	—	—	129
Norway (Adult Wild Maryland) (*R. R. Norvegicus*)	0.22	1	Oral	—	—	44
Norway Rat (Florida)	3.0	2	Oral	—	—	129
Alexandrine Rat (*R. r. alexandricus*)	0.5	3	Oral	—	—	129
Black rat (*R. r. subsp.*)	0.1	—	Oral	—	—	74
Mice:						
Albino (Maple Grove)	19.3	1	S.C.	0	4+C	70
(Carworth)	17.0	1	Oral	—	—	125

TABLE II—*Continued*

SPECIES	LD_{50}	ACCURACY*	ROUTE	HEART†	BRAIN†	REFERENCE
Sodium or methyl fluoroacetate; fluoroethanol—*Continued*						
	mgm./kgm.					
Mice—Cont'd.						
Albino others	10.0	1	I.P.	—	—	129
	16.0	2	S.C.	—	—	105
	5.0	2	S.C.	—	—	52
Meadow Mouse (*Microtus (haydeni)*)	0.5	2	Oral	—	—	129
Deer Mouse (*Peromyscus sp.*)	4.0	1	Oral	—	—	129
House Mouse (*Mus musculus*)	8.0	2	Oral	—	—	129
Hamsters	3.0	2	I.P.	—	3+C 4+D	30
Rabbits:						
New Zealand White	0.25	1	I.V.	4+VF	0	30, 70
Pigmented	0.5	3	I.V.	4+VF	0	37
Dutch & others	0.5–1.0	3	S.C.	—	.	30, 70
Guinea Pigs	0.35	1	I.P.	0	4+C	30, 70
	0.25 (LD_{100})	2	S.C.	—	—	105
Ground Squirrels:						
Apache spotted (*Citellus spilosoma cavescens*)	0.4	3	I.P.	—	—	129
Columbian (*Citellus columbianus columbianus*)	0.9	2	I.P.	3+	4+D	129
Fisher (*Citellus b. Fisheri*)	0.3	—	Oral	—	4+C	129
Pocket Gopher:						
Breviceps—Texas (*Geomys breviceps sp*)	<0.05 (LD_{100} in 9 animals)		I.P.	—	—	129
Tuza—Florida (*Geomys floridanus*)	0.2	2	I.P.	—	—	129
Kangaroo Rats:						
Bannertail (*Dipodmys s. spectabilis*)	0.1	1	I.P.	—	—	129
Merriam (*Dipodomys m. merriami*)	0.15	2	I.P.	—	—	129
BIRDS						
Chickens:						
White Leghorn	7.5	1	Oral	—	3+D	42
Rhode Island Red	5.0	3	Oral I.V.	4+	3+D	30, 129
Plymouth Rock	5.5	2	Oral	—	—	129

TABLE II—*Continued*

SPECIES	LD_{50}	ACCURACY*	ROUTE	HEART†	BRAIN†	REFERENCE
	mgm./kgm.					
Pigeons:						
Florida	9.0	2	Oral	—	—	129
Colorado	2.5	2	Oral	—	—	129
Passerine:						
English Sparrow (*Passer domesticus*)	2.5	1	Oral	—	—	129
Game Birds:						
Gambels Quail (*Lophortyx gambeli*)	20	1	Oral	—	—	129
Carrion Feeding Birds:						
Golden Eagle (*Aquila chrysaetos*)	5.0	3	Oral	—	—	129
Black vulture (*Catharista urubu*)	15.0	3	Oral	—	—	129
POIKILIOTHERMS						
Frog:						
Rana pipiens	150.0	1	S.C.	0	2+C 4+D	30, 99
South African Clawed Toad (*Xenopis laevis*)	>500.0	2	I.P. S.C.	—	—	105
Sodium γ-fluorocrotonate $FCH_2CH{=}CHCOONa$						
Rhesus Monkey (*Macaca mulatta*)	2.5	3	I.V.	4+VF	2+C	37
Dog	0.05–0.07	2	I.V.	0	4+C	37
Rabbit (Albino)	0.15	2	I.V.	4+VF	0	37
Mouse (Albino) (*Rockland Swiss*)	1.0	2	I.V.	0	4+C	37
	2.0 (LD_{100})	1	I.V.	0	4+C	37
Rat (Albino)	1.0	2	I.P.	0	3+C 4+D	37
Rana pipiens	25.0	3	S.C.	—	2+C 4+D	37
Methyl γ-fluorobutyrate $F\text{-}CH_2CH_2CH_2COO\cdot CH_3$						
Rhesus Monkey (*Macaca mulatta*)	3–5	3	I.V.	4+F	3+C	29
Cat	0.2	2	I.V.	0	4+C	29
Rabbit	0.10	1	I.V.	4+VF	1+C	29

TABLE II—*Concluded*

SPECIES	LD_{50}	ACCURACY*	ROUTE	HEART†	BRAIN†	REFERENCE
Ethyl 5-fluorohexanoate $F\text{-}CH_2CH_2CH_2CH_2CH_2COO\cdot C_2H_5$						
	mgm./kgm.					
Rabbits	0.2–0.5	2	I.V.	—	—	109
Rats	2.3	2	I.M.	—	—	109
Mice	4.0	2	S.C.	—	—	109

Key to Scale: — = No information.
0 = Never seen.
1+ = Very rarely seen.
2+ = Occasionally seen. Can be expected.
3+ = Generally seen.
4+ = Characteristic action of drug. Always seen.

* Accuracy: 1—Highly accurate.
2—Accurate enough for practical work.
3—Estimate on few observations.

† VF = Ventricular fibrillation.
F = Cardiac failure (not VF).
C = Convulsion.
D = Depression.

See (96, 109, 129) for data on a number of other species and compounds which were not suitable for inclusion in this table.

laboratory strains of rats and mice are quite resistant to fluoroacetate, but that there is much variation between strains (see also section III-F, d). There is a tendency, to which the guinea pig is a striking exception, for herbivorous animals to manifest cardiac effects and for carnivores to develop *per primum* central nervous system convulsions or depression, whereas in more or less omnivorous species both the heart and central nervous system may be affected. Although a sex difference in respect to sensitivity has been reported for certain wild ducks (129), it has not been noted in other species. As might be expected, elevated environmental temperatures increase the sensitivity of mice to fluoroacetate to an appreciable degree (37).

Cold-blooded vertebrates are generally very insensitive to fluoroacetate, but it is clear that the increased toxicity of fluorocrotonate is much more apparent in *Rana pipiens* than in most mammalia. The sensitivity of frogs to fluoroacetate is not significantly increased when the frogs are kept in water at 32°C. (37), but frogs are more sensitive in the summer than in the winter (18).

Such data as are available indicate that fish are relatively insensitive to fluoroacetate in the water surrounding them (81). *Anopheles larvae* are very sensitive to fluoroacetate (43). Indeed, the insects that have been studied (ants, roaches (5), aphids (65) and moths (72)) are generally very sensitive to fluoroacetate. Fleas are killed by feeding on poisoned rats (92). Microorganisms have not been studied extensively, but Kalnitsky and Barron (77) have made considerable use

of sensitive microorganisms and plant seedlings in elucidating the mechanism of action of fluoroacetate (v.i.). Mold growth (*Physarella Oblonga* Morgan) is inhibited by fluoroacetate in low concentrations (1). As a matter of practical importance Gratch and coworkers (58) have reported that fluoroacetate in concentrations used for rat poisoning (approximately 2%) has no bacteriostatic properties against *P. pestis*. Therefore, it does not interfere with cultivation of these organisms from the tissues of rats obtained by poisoning operations with fluoroacetate.

B. Active and Inactive Fluorinated Compounds

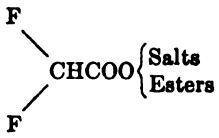
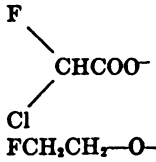
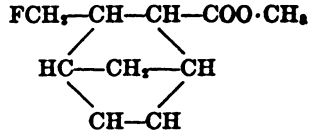
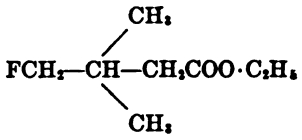
As various monofluorinated compounds, almost exclusively aliphatic, were prepared and tested for toxicity, it became apparent that slight changes in structure were sufficient to abolish completely the dramatic fluoroacetate-like activity. In Table III the structures of a number of key compounds have been arranged under two columns: "active" and "inactive." (An "inactive" compound is one which has little pharmacological activity, or no more than might be expected from the corresponding non-fluorinated compound; for example, 50 mgm./kgm. of 1-fluoropropanol have no effect on rabbits (see footnote 3). Certain conclusions can immediately be made when such a tabulation is inspected. The only active compounds are straight-chain compounds with an *even number* of carbon atoms in which *one fluorine atom* is substituted in a *terminal position.* However, certain other requirements must be met. Thus, FCH_2CH_2Cl is inactive although FCH_2CH_2OH is very active, presumably because little conversion of $—CH_2Cl$ to —COOH can occur in the body, whereas fluoroethanol, analogously to unsubstituted ethanol, can be rapidly oxidized to the corresponding acetate in the body. Fixation of the α and β carbons of γ-fluorobutyrate in a methylene ring or loading the β carbon of γ-fluorobutyrate with one or two methyl groups results in inactivity. On the other hand, γ-fluoro β-hydroxy butyrates are very active. The conclusion is inescapable that compounds in the series which cannot form the fluoroacetate ion directly or through biochemical alteration have no characteristic fluoroacetate activity. This conclusion was understood in its essential features by McCombie and Saunders as early as 1943 (96).

It does not necessarily follow, however, that the toxicity of compounds capable of forming fluoroacetate *in vivo* is due *entirely* to the formation of fluoroacetate. There is evidence that γ-fluorobutyrate, for example, exerts a toxic action *per se*, independently of any action exerted by the fluoroacetic acid which may be formed by the β-oxidation of γ-fluorobutyric acid. Indeed, the toxicity and pharmacodynamics of the two compounds are quite dissimilar, which would appear to be sufficient evidence for a difference in mechanism. For example, progressive cardiac failure without ventricular fibrillation is noted in rhesus monkeys poisoned with fluorobutyrate, and fluorobutyrate-poisoned rabbits manifest signs of parasympathetic stimulation which are not characteristic of fluoroacetate (29). In addition, Kalnitsky and Barron have shown that rabbit kidney cortex, at least, does not convert fluorobutyrate to fluoroacetate and that the effects of the two agents are distinctly different (78).

TABLE III

Active and Inactive Compounds

(Data from 24, 25, 96, 108, 109, 110, 111, 112, 113)

NUMBER OF CARBONS IN LONGEST STRAIGHT FLUORINATED CHAIN	ACTIVE	INACTIVE
1	None	$F\text{-}COO\cdot C_2H_5$ $F\text{-}CH_3$
2	FCH_2COO {Salts / Esters} FCH_2CHO FCH_2CH_2OH FCH_2CN FCH_2CONH_2 FCH_2COF FCH_2COCl	  $FCH_2CH_2—O—CH_2CH_2COOH$ FCH_2CH_2Cl $ClCH_2COF$ $FCH_2CH_2SO_2Cl$
3	None	$FCH_2CH_2CH_2OH$ $CH_3CHFCOO\cdot CH_3$
4	$FCH_2CH_2CH_2COO$ {Salts / Esters} $FCH_2CHOHCHCOO\cdot CH_3$ $FCH_2CH{=}CHCOO$ {Salts / Esters}	$CH_3CH_2CHFCOO\cdot CH_3$  
5	None	$FCH_2CH_2CH_2CH_2COO\cdot R$
6	$FCH_2CH_2CH_2CH_2CH_2COO\cdot R$	
7	—	—
8	$FCH_2(CH_2)_6COO\cdot R$	—
9	—	—
10	$FCH_2(CH_2)_8COO\cdot R$	—
11	None	$FCH_2(CH_2)_9COO\cdot R$
12	$FCH_2(CH_2)_{10}COO\cdot R$	

On the other hand, γ-fluorocrotonic acid does not appear to have any qualitative action in the intact animal different from that of fluoroacetate. This may be related to an increased ease of oxidation at the double bond, so that fluorocrotonic acid perhaps exists as such for a very much shorter time than does fluorobutyric acid. The greater toxicity of fluorocrotonate may be related to the greater ease with which it penetrates to the active area. Fluoroethyl fluoroacetate ($FCH_2COO \cdot CH_2CH_2F$) is about as active as fluoroacetate on an equimolar basis (111), but it acts much more quickly; this suggests an increased rate of cellular penetration (29).

Fluoroacetyl salicylic acid (110) may be cited as another example of a molecule which has an action not to be expected from the sum of actions of its groupings. It is as toxic to mice as fluoroacetate on a weight basis (compare acetylsalicylic acid); yet the characteristic action of fluoroacetyl salicylic acid is depressant rather than convulsant as would be expected if deacetylation with the liberation of fluoroacetate occurs in a manner similar to that described by Smith (120) for acetylsalicylic acid.

It appears that a fluorinated compound must have a structure capable of partially, but *not completely*, mimicking a natural metabolite if it is to be highly active pharmacologically. Factual evidence favoring this attractive interpretation of the data of Table III will be presented in Section IV. The serious consequences of such imperfect mimicry will be discussed in Section III.

III. RESPONSES OF ORGANISMS TO FLUOROACETATES

The majority of studies of a pharmacodynamic character have been made with methyl or sodium fluoroacetate. Some work has been done with fluoroethanol, but little beyond the determination of toxicity has been done with most of the other two-carbon analogs. There has not been a comparable amount of work with the four- and six-carbon compounds and it is possible, although not probable, that some modification of the conclusions concerning these compounds may be necessary in the future.

A. Absorption

The fluoroacetate compounds are all absorbed to some extent from all sites of application, although the lower members of the series are irregularly absorbed through the skin. Thus, application of methyl fluoroacetate in doses of 100 mgm./kgm. to the plucked skin of guinea pigs causes no poisoning according to Foss (52), although Saunders and Stacey (110) report an LD_{50} of 20 mgm./kgm. for methyl fluoroacetate placed on the clipped back of rabbits. The longer chain compounds and higher esters appear to be more readily absorbed; for example, 2-ethyl hexyl fluoroacetate on the shaved ear of a rabbit is lethal in 6 to 10 hours at a dose level of 10 mgm./kgm. (65). It is apparent that these compounds are not readily absorbed through the unbroken skin; but caution should be observed, especially when handling compounds likely to be oil-soluble.

Absorption through the pulmonary epithelium is very efficient in the case of the members of this series studied so far, and absorption of dusts of sodium fluo-

roacetate is equally effective (110). It is sufficient to point out that such data as are available indicate the same degree of toxicity for these compounds when they are inhaled as the esters as when they are injected as sodium salts. For example, Saunders and Stacey (110) have reported an LC_{50} in rabbits for methyl fluoroacetate of 0.1 mgm./l. (10-minute exposure). This corresponds roughly to their intravenous LD_{50} of 0.25 mgm./kgm.

There is no noteworthy difference between the toxicities of orally, subcutaneously, intramuscularly, intraperitoneally or intravenously administered methyl or sodium fluoroacetate (30, 93, 129). Very slight differences observed in the unexpected direction of an increased toxicity after oral administration (30) are probably not significant. Buckle, Pattison and Saunders (25) caution that methyl fluoroacetate is less toxic when injected subcutaneously in propylene glycol than in sodium chloride solution. Because of (1) solubility, (2) stability and (3) the long latent period before symptoms can be produced, which allows time for absorption, it may be concluded that there is no important difference between any non-percutaneous route of administration. This is an uncommon occurrence in pharmacology.

B. Distribution in Body Tissues

Knowledge of the distribution of fluoroacetate is largely inferential in origin. It might be assumed that the readily water-soluble sodium or methyl fluoroacetate would be distributed fairly evenly throughout body water. Recently, Ramsey and Clifford using their method for the determination of fluoroacetate, have presented data on orally poisoned rats (7 mgm./kgm.) which indicate an even distribution of fluoroacetate between the brain, heart, liver and kidney. Now that a method capable of detecting small amounts of fluoroacetate in animal tissues is available, it would be extremely desirable to ascertain whether the distribution of fluoroacetate is different in those species in which the heart or central nervous system is primarily affected. In this connection one experiment might be cited. Two dogs of approximately equal weight ate equal portions of heart muscle or skeletal muscle from a poisoned horse and were apparently capable of differentiating the amount of available fluoroacetate inasmuch as the dog eating the heart muscle died (53). It is common for domestic animals eating poisoned rats to be killed by the fluoroacetate still present in the rat (114).

C. Detoxication and Excretion

Observations that animals may be killed by the fluoroacetate remaining unaltered in poisoned animals or excreted unchanged in the urine (106) suggest that fluoroacetate is not changed in the body to any important extent. Urinary excretion is the only route so far demonstrated for the removal of the poison.

Although cumulation does occur (52, 105), it is not an outstanding characteristic of this poison. Administration of one half to one fifth of an LD_{100} daily will usually result in acute symptoms in 3 to 10 days; but if 3 days elapse between doses, repeated administration can be carried on indefinitely (52, 99). This suggests that elimination of that portion of the administered dose which was actually

exerting a toxic action is accomplished in about 24 hours. One may infer that at least some enzyme-fluoroacetate combinations are reversible.

D. Development of Tolerance

Tolerance to increasing doses of fluoroacetate has been demonstrated in the mouse and rat (35, 105, 127) and possibly exists in the rhesus monkey (37); but it could not be produced in the dog or rabbit (35, 37). Like cumulation, the development of tolerance is not a characteristic feature of the drug's action. The phenomenon is interesting, however, not only in its species specificity but also in its temporal and quantitative aspects. Rats receiving 0.5 mgm./kgm. (LD_{10}) by any route become largely resistant to the effects of 5.0 mgm./kgm. (LD_{75} for this strain) within more than 4 hours and less than 24 hours; this resistance lasts about 48 hours. It extends only partially to slightly higher challenging doses, and the ratio of doses cannot be extended; animals surviving 5 mgm./kgm. (with symptoms) are as sensitive as controls to 15 mgm./kgm. on the following day.

During the period of protection after 0.5 mgm./kgm., there is an increase in the ability of the rat to acetylate p-aminobenzoic acid. This may reflect partial inhibition of acetate turnover, resulting in the accumulation of a larger amount of acetate available for acetylation of foreign amines. Such an accumulation of acetate may also exert a protective action against a subsequent and larger dose of fluoroacetate (see sections III, IV).

E. Latent Period

All students of the actions of fluoroacetate have been impressed with the unusually long and variable latent period between the administration of the drug and the development of the characteristic response. This latent period occurs in all species so far studied, following intravenous injection. Application of concentrations of the methyl ester or the salts of fluoroacetic acid, equivalent to those existing in an intact animal poisoned with an LD_{50}, to the isolated heart and gut of the rabbit, the exposed brain of dogs, rabbits and monkey, or *in vitro* to enzyme preparations usually produces no immediate changes in the behavior of the organ or system. There is ordinarily no difference between these two agents except in the case of the isolated frog nerve preparation which Boyarski, Postel, Rosenblatt and Gerard (18) have found utterly refractory to the sodium salt but sensitive to the methyl ester. Although isolated intact frog muscle is more sensitive to the ester, the sodium salt is not inactive on this preparation (39).

To illustrate the latent period, data taken from current research in this laboratory may be used. An LD_{95} of sodium fluoroacetate (0.5 mgm./kgm.) injected intravenously in white rabbits requires 125 minutes (S.E. $\pm$ 12.7) to cause ventricular fibrillation and death. Symptoms of poisoning are not detectable for at least one-half hour after administration of the fluoroacetate. Increasing the dose to 25 or 250 mgm./kgm. will shorten the latent period to 20 minutes but cannot provoke the immediate responses characteristic of many drugs. Doses of the order of an LD_{20} may cause symptoms of poisoning and death of an occasional animal 48 hours or longer after administration.

The four-carbon compounds, γ-fluorocrotonate or the fluoroethyl ester of fluoroacetic acid, are more toxic than fluoroacetate and have a distinctly shorter latent period. For example, intravenous injection of an LD_{90-100} of sodium fluorocrotonate (0.3 mgm./kgm.) kills rabbits in about 60 minutes. Incomplete data indicate that dogs are very little more sensitive to fluorocrotonate in terms of the dose requirement but that the latent period for an LD_{100} (0.1 mgm./kgm.) is markedly shorter (approximately 90 minutes) than the 273 (S.E. ± 73) minutes for a corresponding LD_{100} of fluoroacetate (0.1 mgm./kgm.). Experiences of this nature suggest that the latent period can be shortened by increasing the chain length, thereby increasing the lipid solubility as well as decreasing dissociation, and thus facilitating cell penetration. No information is yet available on the latent period associated with the long chain compounds studied by Saunders (25, 109), although they are much more toxic than fluoroacetate. It has been shown (15) that the effectiveness of 2×10^{-6} M sodium fluoroacetate in decreasing the oxygen consumption of yeast is greatly increased by a low pH of the medium, a condition which increases the number of undissociated fluoroacetic acid molecules. This has also been found to be true of iodoacetic acid (3). In fact, undissociated molecules of acids are generally credited with being the form which actually penetrates cells (63).

The latent period in rabbits between the administration of sodium fluoroacetate and the onset of ventricular fibrillation or convulsions can be appreciably shortened by the prior administration of large amounts (approximately 1 gram/kgm.) of sodium bicarbonate, fumarate or chloride, but it cannot be eliminated entirely (36). The recent work of Hyde, Beckett and Gellhorn (71) has shown that certain agents facilitating cholinergic transmission potentiate many convulsant drugs. In mice, small asymptomatic doses of neostigmine (0.25 mgm./kgm.) greatly shorten the latent period of fluoroacetate (20 mgm./kgm.) induced convulsions, but do not eliminate it entirely. The decrease is to about one fifth of the control period (37). Neostigmine produced no change in the latent period when administered with fluoroacetate to dogs or rabbits. There does not seem to be any satisfactory explanation of these observations at present.

The latent period associated with fluoroacetates can probably be considered the result of at least two major factors: (1) the ability of the various fluorinated compounds to penetrate the cell, and (2) the time required for disruption of intracellular processes to become manifest as gross organ dysfunction; in principle this second factor is very similar to that which accounts for the latent period of insulin-induced convulsions. The future will decide which of the two factors is the more important.

F. Effect upon Intact Animals (30, 52, 53, 93, 105, 129)

The directly observable effects of an injection of sodium or methyl fluoroacetate, or fluoroethanol, in unanesthetized animals differ in many respects depending upon the species of animal employed (see Table II). They have been most frequently observed in rabbits, dogs, monkeys and rats, and these species seem to encompass the major variations in types of response.

a) RABBIT (*Cardiac*): After an intravenous injection of sodium fluoroacetate (0.5 mgm./kgm.) in white rabbits, no change in the animal is discernible for about one-half hour. The first effect noted is usually a weakness of the neck and front legs and a decrease in activity. This state may progress to a marked extent but usually remains moderate until the occurrence of a sudden, violent convulsion of a clonic nature, typically associated with a cry. Opisthotonus, mydriasis and blanching of the retina rapidly develop, followed by progressive relaxation, a few gasping respirations and death. If the thorax be opened immediately, the auricles are found to be beating and the ventricles usually fibrillating. Occasional repeated convulsive bouts are always found to be the result of cardiac syncope, when the animals are carefully followed electrocardiographically (30). Fluorobutyrate causes considerable peristalsis and defecation in rabbits, a response not notable with the fluoroacetates. Except for the greater speed of action, fluorocrotonate is qualitatively indistinguishable from fluoroacetate.

b) DOGS (*Central nervous system*): The onset of fluoroacetate-induced effects (usually 4 to 5 hours after 0.1 mgm./kgm.) in the dog is heralded by a few minutes of barking and howling, "absence" (non-recognition of human presence), actions suggestive of fearful hallucinations, hyperactivity and finally a tonic spasm followed quickly by running movements. Tonic spasms and running movements may alternate or even completely cease, and the dog may appear normal at times; but ultimately the repeated anoxic assaults on the respiratory center during convulsions result in respiratory paralysis. The heart is often markedly slowed during convulsive seizures but rarely ceases activity until some time after the respiration has ceased. Death is typically the result of the effects of repeated and prolonged convulsions on the respiratory center, and never primarily cardiac in origin.

c) MAN AND RHESUS MONKEY (*Mixed response*): Although cats and pigs are perhaps more typical of the "mixed response" type of species (see 30), the rhesus monkey is of greater interest because such data as have been accumulated by the reviewer (through the most diverse channels, see also (52)) indicate that the response of adult man to fluoroacetate may be identical with that of the rhesus monkey. Children appear to be more prone to myocardial failure than to ventricular fibrillation as the terminal event; but, in general, they are very similar to the rhesus monkey in their responses to the poison.

As in man, in whom it may prove a diagnostic problem, the convulsive seizures due to fluoroacetate poisoning in the rhesus monkey are strikingly epileptiform. One or two hours after administration of the poison the animal may vomit and becomes apprehensive and seclusive. ("The first indication of poisoning in man is the onset of epileptiform convulsions after an initial period of nausea and mental apprehension" (52).) A few minutes later, actions suggestive of auditory hallucinations are followed immediately by nystagmus. Twitching of the facial muscles, often unilateral, heralds the onset of the convulsive seizure. It quickly spreads to involve the pinnae and the masseter muscles. Spread of the convulsive activity over the rest of the body is then very rapid, ending in a jerking, symmetrical convulsion in which the spasmodic, violent jerks may occur at a rate of

3 per second. Tonic components are seen but do not dominate the pattern as they do in the dog. The animal is apparently unconscious during this period; but, as the seizure passes off, it will gradually attempt to regain its feet and ultimately does so about 30 minutes after the onset of the attack. The monkeys appear depressed for some time but often recover entirely from the convulsion. A complete second seizure is infrequently seen. Generally, the animal becomes weaker over the period of the next few hours (see cardiac status), but is often standing or otherwise exerting himself when suddenly stricken by ventricular fibrillation and death. Spontaneous recovery from ventricular fibrillation in the monkey is uncommon.

d) RATS (*Depression*): Although convulsions of a tonic nature, preceded by one- or two-hour period of decreasing activity associated with hypersensitivity to external stimuli, are the usual result of the injection of 5 mgm./kgm. of sodium fluoroacetate in rats of unspecified ancestry, death is the result of respiratory depression which gradually occurs long after convulsive activity has decreased or entirely ceased. Very large rats (over 400 grams) occasionally develop ventricular fibrillation; but, as might be expected from the general experience with fibrillation in small animals, this is uncommon. Some of the confusion in reports from different laboratories may be explained by a recent observation of the effects of the same dose of fluoroacetate in two strains of albino rats. Male Wistar rats received directly from the Wistar Institute convulse only occasionally and die after a period of respiratory depression lasting 5 to 24 hours. Comparable male rats of the Sprague-Dawley strain received directly from their stocks uniformly develop convulsions within 1 or 2 hours after injection and continue to convulse in a manner very similar to the dog. Death occurs in 4 or 5 hours (37). Although superficially these rats are identical, Anker (4) has demonstrated a very striking metabolic difference which will be discussed later.

Rats which have survived an LD_{50} of fluoroacetate for 24 hours differ from most species (which are usually completely recovered in this time if they are to survive at all) in that they are still markedly affected. Disinclination to move is immediately apparent and is probably caused by a gross intention tremor which appears when the animal is forced to move. An extreme bradycardia can be detected by palpation or electrocardiographically (31). Complete recovery, if it is to occur, usually results within 48 to 72 hours after poisoning. Thiamine and atropine have no effect on these phenomena, but the tremor is temporarily quite exaggerated by diphenhydramine in doses that do not affect normal rats (37).

e) *Special responses in various species:* Ward and Spencer (129) noted emesis as a strikingly characteristic and early symptom of fluoroacetate poisoning in several species of carrion-feeding hawks and owls. Judged from the nature of the animals this is not surprising; nor does the profound sweating noted in poisoned horses by Frick and Boebel (53) appear unusual. Watt and Breyer (131) record that symptoms of poisoning in cattle which have eaten "Gifblaar" can be delayed by withholding water from the animal, a point of some theoretical and practical interest, and animals which recover are exceedingly thirsty. In this connection it is known that frogs allowed to imbibe water through their skin after

poisoning, which they do to the extent of a 50% weight gain, die more quickly than those kept dry (99). Mice are often anuric despite large amounts of parenteral fluids associated with therapeutic measures (70).

G. Effect on Specific Systems

a) *Cardiovascular:* The actions of fluoroacetate upon the cardiovascular system have been studied most extensively by Chenoweth and Gilman (39) who published a number of plates illustrating some of the phenomena described below In general, most if not all the changes in the circulatory system produced by fluoroacetate can be explained by the action of the poison upon the heart itself; extracardiac effects, if present, are masked by the magnitude of the cardiac events. There is frequently a slight and transitory rise in mean arterial pressure which does not long persist. It is blocked by atropine and has been related by Foss (52) to the nicotine-like action of large doses of fluoroacetate. However, the general pattern is one of declining blood pressure (31, 105). Failure of the myocardial contractile power is steady and has been demonstrated in isolated heart-lung, perfused heart and papillary muscle preparations (31) as well as by direct inspection (105).

Constriction of the coronary arteries does not appear to occur and is certainly not the prime cause of the cardiac irregularities. During the course of poisoning no changes in capillary permeability to protein-bound dyes or in hemoconcentration, as indicated by hematocrit readings, have been observed (31). An elevation of hemoglobin levels in the goat has been ascribed to splenic contraction (52). As the heart decreases in contractile power it loses the ability to elevate the blood pressure in response to epinephrine or to compression of the descending aorta.

The development of numerous arrhythmias is apparent even on routine kymography, but the bizarre changes which occur can only be appreciated by electrocardiography, preferably by some technic permitting continuous visualization of the rapidly fluctuating changes. There appear to be differences even among cardiac-sensitive species in regard to the types of changes noted in the heart. Common to all, however, is a notable elevation in the amplitude of the T wave, although this perhaps most marked in the monkeys. Progressive downward shifting of the pacemaker was seen in the horse, goat (31) and sheep (105) and the electrical signs of activity in the auricle often disappeared. Prolongation of the P-R interval in the progressive fashion described by Wenckebach was especially marked in the goat, but was also seen in the cat. In direct opposition to this, the rhesus and spider monkeys showed no changes in auricular activity nor in auriculo-ventricular conduction.

Ventricular premature contractions are seen in nearly all species and are especially prominent in the rabbit and monkeys where they occur at first in a peculiarly systematic fashion (1:2, 1:4, etc.). Shifting alternation of the cycle length, QRS voltage, T wave height, shape, direction or take-off is very marked in the monkey and is often not predictably related to a *pulsus alternans*. The alternation of the pulse extends ultimately to a uniform 50% pulse deficit. This has also been reported in at least one fatal human poisoning. Ventricular fibril-

lation may occur at any time, but usually when the heterotopic ventricular arrhythmias are prominent; it appears to be initiated by mechanisms similar to those described by Wiggers for electrically-induced ventricular fibrillation. The occurrence of auricular fibrillation has not been noted in any species.

The actions of fluoroacetate upon the sensitive mammalian hearts are directed toward decreasing contractile power and disorganizing conduction and excitation. Failure or fibrillation as the end result is a manifestation of the relative importance of these effects in different species. It is not known that these effects are all the result of the same action of fluoroacetate, but it may be recalled that the specialized conduction tissue of the heart is contractile muscle tissue as well, suggesting that only a single action may be involved. There appears to be no relation between the gross amount of Purkinje tissue in the various species (56) and the development of conduction defects.

b) *Nervous system:* The magnitude of the direct effects of fluoroacetate upon the nervous system of sensitive species is apparent upon inspection of a poisoned animal. It must be emphasized that anoxic convulsions arising from cardiac syncope, such as ventricular fibrillation (in the rabbit, for instance), should not be confused with convulsions resulting from a *per primum* action of fluoroacetate upon the central nervous system, such as occurs in the dog. As indicated in Table II, both types of convulsions may occur in some species. This section will deal exclusively with seizures arising from a direct effect of fluoroacetate upon the nervous tissue. The pattern of these convulsions in intact animals has been described in section III F. With the exception of the remarkable similarity of fluoroacetate-induced convulsions in rhesus monkeys and in man to a *grand mal* epileptic seizure, the gross character of the convulsions in other species is not of great interest.

The regions of the central nervous system affected by fluoroacetate do not appear to be very sharply circumscribed. Studies on peripheral nerve have been carried out for the purpose of elucidating the mechanism of action of fluoroacetate, and will be discussed in section IV. The spinal cord can be shown to be sensitive: (a) by local application of fluoroacetate to the cord (34), with convulsive activity developing in a discrete area; (b) by the occurrence of convulsions below the level of a cord transection following an intravenous injection (34); and (c) by experiments in spinal and decerebrate cats (52). It usually requires supralethal doses to demonstrate involvement of the cord and it is probably of no importance in the intact animal. Curiously, spinal rhesus (?) monkeys twitch after 100 mgm./kgm. of methyl fluoroacetate but do not convulse (52). Reflexes mediated through the spinal cord of the cat are accentuated in the first few minutes of fluoroacetate-induced activity, but convulsions soon intervene and make further study impossible (52).

Recordings of the electrical activity of the brain, either directly from the brain surface or from the calvarium, of curarized or anesthetized animals have been made by Ward (126) and by Chenoweth and St. John (34). Using cats, Ward found that large doses of sodium fluoroacetate (2.0 mgm./kgm., 10 times the LD_{100}) injected intravenously or into the lateral ventricles produced marked

increases in electrical activity of subcortical areas, particularly the thalamus and hypothalamus. A high frequency, rather low amplitude activity was found to be particularly characteristic of the thalamus, while bursts of slow waves of a 3 to 8/sec. frequency in the cortex were noted which were synchronous with the envelope of the fast spikes of the thalamus. Slow wave activity of the hypothalamus was not regularly reflected in the cortex.

When smaller intravenous doses of fluoroacetate ($\frac{1}{2}$ to 2 times the LD_{100}) were administered to dogs under similar conditions, Chenoweth and St. John found an increased frequency and amplitude of waves recorded from temporo-parietal and occipital regions of the cortex but relatively little change in activity of frontal areas or cerebellum. Local application of fluoroacetate, either as the sodium salt or the methyl ester, resulted in relatively local changes in activity which spread so slowly as to suggest the probability that diffusion of the poison rather than primary radiation of the electrical activity was the cause of the increased involvement. They have stressed the apparent similarity of the spike and dome pattern often seen during the action of fluoroacetate to the electroencephalographic pattern of clinical *petit mal* epilepsy.

Electrical activity of the cortex reaches very high potentials during fluoroacetate poisoning but it can be obliterated by barbiturates and anticonvulsants (34) as well as by narcotic concentrations (9) of carbon dioxide (126). The sensitivity of rats to electrically-induced convulsions is increased about ten times by fluoroacetate (52). The apparent potentiation by neostigmine has been mentioned.

Rabbits poisoned by intravenous injections of fluoroacetate never reveal any electroencephalographic abnormalities until ventricular fibrillation occurs; but their brain tissue is not entirely resistant to fluoroacetate for convulsions typical for the dog can be induced in rabbits by the administration of sodium or methyl fluoroacetate directly into the cerebrum. This is, in a sense, a corollary to the observation that dogs prepared for electroencephalographic recordings under curare and artificial respiration occasionally develop ventricular fibrillation many hours after large doses of fluoroacetate, a fact indicating that the dog heart is not completely resistant to fluoroacetate (34).

c) *Other systems:* Because death from acute fluoroacetate poisoning is the result of cardiac or respiratory arrest in a relatively short and unpredictable period, there is little opportunity to observe changes in systems other than the heart or nervous system. The actions of fluoroacetate are probably exerted upon nearly all actively metabolizing tissues of the body, but the effects are difficult to demonstrate *in vivo*.

Skeletal muscle may be directly affected, notably in rabbits in which there is early head-drop and fore-limb weakness; these effects do not seem to be primarily the result of the lowered blood pressure, although the point has not been proven. Frog skeletal muscle is affected by fluoroacetate *in vitro* (38, 39). Contractions of isolated rabbit intestine are depressed by concentrations of sodium fluoroacetate of the magnitude presumed to exist in animals poisoned with an LD_{100} (50, 132).

Although kidney tissue is obviously very sensitive *in vitro* (see Section IV), renal dynamics do not seem to be much affected *in vivo*. Himwich *et al.* (62) noted an insignificant decrease in glucose Tm in chronically poisoned dogs, but cumulation of the poison occurred and convulsive death precluded the development of serious kidney malfunction. In 5 chronically poisoned cats (0.1 mgm./kgm. per day), with deaths at 3, 8, and 10 days and two survivors, high blood non-protein nitrogen levels of 134 and 120 mgm. % were noted in two cats at 8 and 10 days, respectively; this suggests definite renal damage (99).

Overall hepatic status is difficult to assess under any circumstances. A currently popular test of at least one function is the duration of anesthesia induced by short-acting barbiturates in laboratory animals. It has become apparent in recent studies in this laboratory that in fluoroacetate-poisoned animals there is a very great prolongation of the anesthetic effects of sodium thiopental, sodium pentobarbital, sodium phenobarbital and possibly even sodium barbital. The effect is not permanent, and the return to a normal duration of anesthesia is complete in less than a week. This has been observed in mice, rabbits, dogs and a rhesus monkey. It appears likely that it is the result of failure of the fluoroacetate-inhibited liver to detoxify these barbiturates, since the response to them is prolonged in proportion to the degree to which hepatic detoxication is believed to be important in their elimination. Studies on this and several related phenomena are currently in progress.

H. Pathological Changes

a) *Anatomical:* The histopathologic changes in fluoroacetate poisoning have not been described at length, perhaps because they do not contribute much to an understanding of its action. They appear to be largely the result of progressive cardiac failure with congestion of the abdominal viscera and lungs (52, 105). In the chicken there may be generalized petechial hemorrhages, especially noteworthy in lungs and ovaries (42), but this phenomenon appears quite specific for this species. Ordinary pathological studies do not seem to have been especially helpful in this field, nor are they likely to be so in the future.

b) *Biochemical:* Changes in the blood and tissue levels of various metabolites have been studied in a number of species of animals poisoned with fluoroacetate. A consistent increase in blood glucose levels, occasionally to 400 mgm./100 cc., has been reported in rabbits (94) and goats (52). Excretion of glucose in the urine may be expected to follow such a rise in blood sugar and does commonly occur in poisoned animals. Possibly some of the extra glucose in the blood is derived from liver glycogen since it has been found that poisoned rabbits have a marked reduction in liver glycogen (94). Lactic acid blood levels are elevated in poisoned rabbits (94, 95, 99), but it is exceedingly difficult to define the exact significance of such a change. Pyruvic acid blood levels rise and the lactate: pyruvate ratio is also increased (94). Preliminary experiments have indicated recently that blood and urine levels of acetic acid are elevated in dogs (37).

A considerable and rapid rise in serum inorganic phosphate has been reported in goats (52) and rabbits (95). Some of this phosphate may come from muscle

because rabbit heart muscle shows a marked decrease in total acid-soluble phosphorus and organic phosphorus (95). Plasma levels of other important electrolytes are found to be elevated, particularly just before death occurs. Foss has reported changes in plasma potassium from control levels of 17 mgm./100 cc. to 25 mgm./100 cc. after poisoning has progressed (52). Others report minor increases in potassium and calcium (95). These electrolyte changes are probably nonspecific and reflect the morbidity of cells weakened by an attack at some other point; similar electrolyte alterations occur under many conditions.

IV. THE INTIMATE MECHANISM OF ACTION

A great deal of illumination has been cast upon the manner in which a dose of a fluoroacetate compound brings about the death of an animal. Having been assured that the heart or central nervous system is ultimately disabled by events familiar to pharmacologists, one may properly inquire, "How do these events come about"? Indeed, this is currently the most interesting aspect of the fluoroacetate problem.

There is no longer any reason to believe that fluoroacetate is in any way pharmacologically analogous to iodoacetate, although this was a natural *a priori* assumption. The behavior of poisoned animals, the chemical character unrelated to iodoacetate and the failure of low concentrations to inhibit significantly any system sensitive to iodoacetate seem sufficient reasons to discard this concept entirely. In addition, even though the chemical nature of fluoroacetate suggests no basis for the assumption that fluoride ion might be split off and be the cause of the toxic action of fluoroacetate, the actions of the fluoride ion are entirely different from those of fluoroacetate. One must of necessity conclude that the fluoroacetate ion exerts its action as such and not because of any obvious chemical reactions with it or because of toxic breakdown products.

British workers have succinctly summarized their earlier efforts to identify the systems attacked by fluoroacetate as follows: "No enzyme system has been found which is inhibited to any extent by methyl fluoroacetate" (96). In a most important communication in 1947 describing work carried on in 1944, Bartlett and Barron (12) have reported experiments on the metabolism of animal tissues from which they conclude that, "Fluoroacetate probably acts by inhibiting the formation of "active" acetate (the so-called C_2 compound, which may be an acetyl derivative or an acetate radical)". Because of the importance of this provocative conclusion, it is appropriate to review the observations upon which it is based as well as some experiments which do not justify this conclusion.

A. Tissue Slices, Homogenates and Microorganisms

It was found that 0.001 to 0.2 M fluoroacetate decreases the oxygen uptake of tissues. Tissue slices from rats moribund after an LD_{50} of fluoroacetate were found to oxidize acetate 20 to 30% less vigorously than control slices. The degree of inhibition of acetate oxidation could be increased by adding fluoroacetate to normal tissues *in vitro*; inhibitions as great as 90% occurred in heart slices after 0.005 M fluoroacetate was added. This inhibition might be interpreted as

occurring *pari passu* with cellular morbidity except for the fact that the inhibitions of O_2 uptake of guinea pig brain (which does oxidize acetate and does convulse) and of rabbit brain (which does not oxidize acetate and does not ordinarily convulse) were found to be 53% and 11%, respectively.

Experiments were then performed to ascertain the mechanism of acetate blockade and several known routes of pyruvate and acetate metabolism were tested. (1) *Pyruvate→ acetate:* The oxygen consumption of kidney slices in the presence or absence of pyruvate was decreased to about the same extent (60%) by 0.01 M fluoroacetate; yet in the presence of pyruvate, which apparently was oxidized, acetate accumulated. It is apparent that the further oxidation of this acetate had been inhibited. (2) *Acetoacetate* $\rightleftharpoons$ *acetate:* The utilization of acetoacetate by rat kidney slices was completely inhibited by 0.02 M fluoroacetate. Conversely, the formation of acetoacetate from acetate was accelerated by fluoroacetate and this was still further increased as the proportion of oxygen in the gas phase was increased, reaching 233% of normal in pure oxygen. One may infer from this that acetoacetate is converted *in toto* to acetate, the further oxidation of which is inhibited so that acetate accumulates as the end-product of the reactions: glucose → pyruvate → acetate and forces the reaction: acetate → acetoacetate. (3) *Pyruvate → acetate → succinate:* The formation of succinate from pyruvate was 77% inhibited by 0.02 M fluoroacetate. Since none of the individual reactions of the Krebs' tricarboxylic acid cycle was found to be affected by fluoroacetate (see also (84) for a statement of disagreement), this may be interpreted to indicate that pyruvate enters the Krebs' cycle in large part through acetate and not directly. (4) *Other reactions:* Pyruvate and lactate formed by deamination of alanine accumulate in rat kidney slices in the presence of fluoroacetate, a fact that might be readily predicted if it were established that oxidation of pyruvate through acetate is inhibited. Glucose metabolism is directed toward lactate (aerobic glycolysis) to the extent of nearly one half, but the anaerobic utilization of glucose is completely unaffected in kidney slices. Aerobic oxidative synthesis of carbohydrate from pyruvate and acetate is markedly inhibited by fluoroacetate; this fact suggests that, as the entry of acetate into Krebs' cycle is inhibited, this cycle may be a pathway for the synthesis of carbohydrate from pyruvate and acetate. Anaerobic conversion of pyruvate to lactate and acetate was not affected (see also 26). (5) *Acetylation of foreign amines:* The acetylation of sulfanilamide and of p-aminobenzoic acid by rabbit liver slices was increased by 0.02 M fluoroacetate. (This has been confirmed *in vivo* in rats and rabbits (37, 123).) The chemical reaction of acetylation is not affected; formation of acetylcholine from choline in the presence of glucose or pyruvate is not affected by 0.02 M fluoroacetate (see also 85). Therefore, it may be assumed that the increased acetylation of foreign amines is the result of an inhibition of acetate metabolism in consequence of which more acetate becomes available for acetylations.

Having been thus provided with a basis for the concept that fluoroacetate interferes with the oxidation of acetate in various animal tissues, Kalnitsky and Barron (77) studied the details of the phenomenon in baker's yeast and bacteria.

The oxidation of acetate by baker's yeast was 95% inhibited by 0.001 M fluoroacetate (30% by 0.00001 M), only 5% by 0.001 M bromoacetate and not at all by 0.001 M chloro- and iodoacetate; the specificity of the reactions involved are thus demonstrated. Fluorobutyrate and fluorocrotonate (0.001 and 0.003 M) had no inhibitory action at all. The oxygen uptake of yeast suspensions was nearly completely inhibited by fluoroacetate added 15 minutes before acetate, and practically unaffected when the two were added together. Appreciable reversal of such fluoroacetate-induced inhibition could be obtained by adding higher concentrations of acetate (0.1 M). Additional evidence for the specificity of the inhibition was obtained when it was found that acetate oxidation of specially washed yeast was completely inhibited by 0.00075 M fluoroacetate while pyruvate oxidation was only 79% inhibited.

By the use of a different approach, it was reasoned that if fluoroacetate is a specific inhibitor of acetate oxidation there should be no immediate inhibition of the O_2 uptake associated with the oxidation of ethanol to acetate through acetaldehyde. This was found to be the case; oxidation of ethanol by baker's yeast in the presence of 0.01 M fluoroacetate progressed exactly as in the absence of fluoroacetate until the accumulation of unoxidized acetate affected the rate of oxidation of ethanol to acetate. Less complete inhibition of acetate oxidation produces less block of ethanol oxidation; Black and Hutchens (15) found that 0.001 M fluoroacetate did not completely prevent the continuation of ethanol oxidation through acetate.

The anaerobic dissimilation of pyruvate to acetate and formate by *Escherichia coli* was unaffected by 0.01 M fluoroacetate although the oxidation of pyruvate to acetate by this organism was definitely inhibited. *Neisseria gonorrheae* does not dissimilate pyruvate but does oxidize it directly to acetate. This reaction is 40% inhibited by 0.01 M fluoroacetate, 26% by 0.02 M acetate and 52% by both together. These experiments add emphasis to the view that inhibition of pyruvate oxidation by fluoroacetate is due to the accumulation of acetate, since even acetate alone is slightly inhibitory.

Quite different results were obtained when another microorganism, *Corynebacterium creatinovorans*, was studied. An *increase* in the endogenous respiration of this organism was produced by both fluoroacetate and fluorobutyrate. It was suggested that this is the result of diversion of cellular metabolism by fluoroacetate toward oxidative pathways in a manner similar to that produced by low concentrations of cyanide and azide. Although acetate oxidation by this organism was completely inhibited by fluoroacetate, fluorobutyrate had no effect at all.

As was mentioned, the greatest inhibition of acetate oxidations (as measured by decreased O_2 uptake) by fluoroacetate occurred when fluoroacetate was added to the yeast some minutes prior to the addition of the acetate substrate. After two hours the inhibition apparently decreased considerably (as measured by increased O_2 uptake), a fact which Kalnitsky and Barron interpreted as the indirect result of the slow accumulation of citrate and its increasing movement into the carboxylic acid cycle. The same characteristic decrease of inhibition was noted

in the growth curves of *Tetrahymena geleii* in a glucose but not in an acetate medium (48). A further study of the effect of fluoroacetate upon citrate formation was later made by Kalnitsky (75, 76). Using rabbit kidney cortex homogenate, he found that not only fluoroacetate but barium and magnesium salts as well appear to increase the formation of citrate from oxaloacetate. The effect of barium and magnesium is the result of inhibition of citrate utilization. On the other hand, the slight inhibition of citrate utilization (at fumarate? (84); *v.i.*) produced by high concentrations of fluoroacetate can only account for about 60% of the accumulation of citrate in the presence of fluoroacetate. It was concluded that the increased citrate content might be the result of the inhibition of acetate oxidation reflected through a series of reversible reactions in a manner analogous to the effect of malonate on pyruvate oxidation.

Another approach to the problem of the greater inhibition of fluoroacetate on acetate oxidation by yeast when the fluoroacetate was added before the acetate was made by Black and Hutchens (15). Working in different laboratories, they confirmed the general fact that a more prolonged inhibition of acetate oxidation is obtained by allowing a longer period to elapse between the addition of the inhibitor and the substrate. They suggest that this is similar to the delay noted by Lynen (91) in starved yeast before acetate oxidation becomes vigorous and that it is the result of cellular depletion of certain substances found by Lynen to be essential for acetate oxidation in yeast. Ethanol was found to be particularly efficacious in accelerating the oxidation of acetate by yeast either untreated or pretreated with fluoroacetate.

When calculations of the oxygen consumption of yeast were made using the final rate after equilibrium has been reached, Black and Hutchens found that pyruvate oxidation is more sensitive to fluoroacetate than is acetate oxidation. Because the delay in oxidizing pyruvate is less than for acetate, these workers decided that the earlier conclusion of Kalnitsky and Barron that acetate oxidation is more sensitive than pyruvate was the result of an error in technic. Hutchens, McMahon and Podolsky (69) have recently reported that the inhibition by fluoroacetate salts of pyruvate-induced oxidations in yeast depends on the pH of the medium. However, inhibition of acetate-induced oxidations in yeast and *Chilomonas paramecium* is independent of pH. While this may have been one of the actual causes of differences in opinion concerning the specificity of fluoroacetate-induced inhibitions in yeast metabolism, Hutchens *et al.* find pyruvate oxidation much more sensitive than acetate oxidation in the case of *Chilomonas*. Although it is very hazardous to change species in the middle of an argument about fluoroacetate, it can be pointed out in support of this that Bueding (26) proved that in the filarial worm, *Litomosoides carinii*, pyruvate oxidation is very much more sensitive than acetate. In addition, he stated, "No evidence has been obtained that fluoroacetate inhibits the respiration of the filariae because of a competitive inhibition of acetate oxidation." Fluoroacetate (0.001–0.004 M), while producing a decrease in the total respiration and motility of the organism, actually produced an accumulation of pyruvate and a decrease in the formation of acetate from glucose. Although in this study he has rigorously tested these

conclusions, Bueding also demonstrated that the metabolic characteristics of *L. carinii* are unique in that they differ from those of other helminths and, indeed, from those of most other invertebrates.

The anthropocentric may draw more comfort, therefore, from the results of a later study by Kalnitsky and Barron (78) on the effects of fluoroacetate and fluorobutyrate on fatty acid and glucose oxidation in kidney homogenates. Homogenates of rabbit kidney cortex oxidize acetic and many other fatty acids vigorously. The oxidation of acetate was immediately, and practically completely, inhibited by 0.001 M fluoroacetate and fluorobutyrate. This is in contrast to the total lack of effect of fluorobutyrate on acetate metabolism in yeast. This discrepancy was shown *not* to be the result of conversion of fluorobutyrate to fluoroacetate by kidney. Two other sharp differences between yeast and mammalian tissue were noted. The apparent release of the fluoroacetate-induced inhibition of yeast metabolism with time does not occur in kidney suspensions nor does ethanol have the least effect on the degree of acetate oxidation. Fluorobutyrate proved to be a more potent inhibitor of butyrate oxidation than did fluoroacetate; 0.00005 M fluorobutyrate inhibited butyrate oxidation 86% while at the same concentration fluoroacetate produced only 32% inhibition. Oxidation of higher fatty acids was also inhibited to varying extents by both fluoroacids.

In contradistinction to the case in yeast where it developed slowly, glucose oxidation was rapidly inhibited by fluoroacetate in kidney homogenates, as was also that of acetate. However, as in their previous studies, they found that pyruvate oxidation was not inhibited until a considerable portion (20%) of the added pyruvate had been oxidized. It appears that the specificity of fluoroacetate inhibition described for yeast is not to be found in mammalian tissue, in this case, rabbit kidney. It is interesting that fluorobutyrate is a more effective inhibitor of butyrate oxidation than is fluoroacetate. One can only speculate at this time about the effects of appropriately fluorinated higher fatty acids.

By actual analysis for acetate, Colowick, Berger, Slein and Cori (41) found that rabbit kidney cortex homogenates removed about 35% less acetate after addition of 0.005 M fluoroacetate. They add the new information that the extra oxygen consumption caused in dialyzed extracts of rabbit kidney cortex by addition of various metabolites is inhibited by high concentrations of fluoroacetate (0.05 M) to varying degrees. Glucose is most sensitive, the extra oxygen uptake being inhibited 68%, and phospho-enol pyruvate is approximately the same, the inhibition being 65%. Unfortunately, acetate does not appear to have been tested. The effect of fumarate was found to be inhibited 53% but other metabolites and components of the tricarboxylic acid cycle were relatively little affected. The curious sensitivity of fumarate-induced extra oxygen consumption does not appear to be entirely coincidental for Boyarski, Postel, Rosenblatt and Gerard (18) found it to be specifically effective in preventing the decrease in the action potential of methyl fluoroacetate-poisoned frog nerve. On the other hand, it actually increased the toxicity of sodium fluoroacetate to intact rabbits (36). These matters require considerable clarification. Liébecq and Peters (84) have reported recently that studies with centrifuged, homogenized guinea pig kidney

and pigeon brain preparations indicate the possibility that a "fluoro-C_2 active fragment" may be formed which enters the tricarboxylic acid cycle and becomes an inhibitor of this cycle. They found, as did Kalnitsky (76), that citrate accumulates during poisoning *in vitro* while acetate does not. The relation of results of many *in vitro* studies to the events occurring *in vivo* is difficult to establish. It is possible that more physiological preparations could prove more useful.

B. Working Muscles

The use of an actively functioning, normally organized preparation such as the frog sartorius muscle has led to some additional and slightly different information. Colowick, Berger, Slein and Cori (41) found that the oxidative resynthesis of phosphocreatine by frog sartorius following one minute of tetanic stimulation was depressed as much as 40% by previously soaking the muscle for 1 hour in 0.005 M methyl fluoroacetate. These findings were explained as the indirect result of depressed tissue respiration, for the simultaneous oxygen uptake of these muscles following stimulation was decreased from an average 150% increase to only 35% increase. Essentially the same results were obtained when dinitrophenol was used to increase basal oxygen consumption, for 0.005 M sodium fluoroacetate inhibited the increase about 70%.

Similar results were obtained when caffeine was used by Clarke and Riker (39) to stimulate the frog sartorius, the excess oxygen consumption of muscles contracting under such circumstances being decreased by methyl fluoroacetate. The respiration of resting muscles is not affected significantly at similar concentrations of methyl fluoroacetate. The oxidative recovery heat which normally follows a single maximal twitch was abolished by 0.001 M methyl fluoroacetate or 0.01 M sodium fluoroacetate, yet the muscles continued to contract, exactly the reverse of the situation with iodoacetate where an increasing oxidative recovery heat production may accompany contractile failure. The depression of the activity oxygen consumption of these muscles induced by fluoroacetate can be abolished completely by 0.01 M acetate, glycerol monoacetate, pyruvate and oxalacetate. Ketoglutarate, fumarate, malate and succinate were less effective, and glucose and lactate were without any significant effect.

In a continuation of this study, Clarke and Riker have recently found that, unlike iodoacetate, fluoroacetate does not inhibit anaerobic glycolysis and, under the conditions of their experiments with frog muscle, the rate of glycolysis is actually increased. Because the aerobic accumulation of lactate that normally results from muscle activity is significantly less in the presence of fluoroacetate and since lactate formation is not inhibited by fluoroacetate, it appears that lactate must be metabolized in the poisoned muscle. The explanation of these results is to be found in the fact that, in this preparation at least, the action of fluoroacetate appears to be to inhibit oxidative carbohydrate breakdown; as a consequence, the anaerobic carbohydrate breakdown which occurs in activity is enhanced, and this results in a rapid turnover of lactate without its accumulation. It is evident that this will provide sufficient energy to permit muscular contraction.

Three independent studies of the effect of fluoroacetate upon the spontaneous contractility of isolated upper segments of rabbit small intestine have been made. The effectiveness of acetate as a source of energy for the contraction of otherwise substrate-free preparations of the type described by Furchgott and Shorr (55) led Furchgott to study the interrelations of fluoroacetate and acetate on this preparation. Experiments performed during 1946 by Furchgott (54) indicated that glucose could supply energy for contraction of intestinal smooth muscle in the presence of fluoroacetate under either aerobic or anaerobic conditions, although acetate could not. Fluoroacetate poisoning was irreversible when carried out under aerobic conditions. However, if fluoroacetate was added during a period of anoxia, allowed to remain in the muscle chamber for over 30 minutes, and then washed out before the restoration of oxygen to the muscle, there was no toxic effect.

Later, Farah, West and Angel (50), upon examining this system found that both glucose and acetate were effective antagonists to fluoroacetate, and that there are considerable differences in the character of the response of the gut to fluoroacetate in the presence of these substrates. They have shown that although fluoroacetate depresses contractility more rapidly when glucose is the sole substrate than when acetate alone is present, the percentile decrease in amplitude of contraction at a given concentration of fluoroacetate is greater in the presence of acetate than when glucose is the substrate. There is a direct relation between (a) the time required for 0.0008 M sodium fluoroacetate to produce 95 to 100% inhibition of contraction and (b) the concentration of sodium acetate present in the bath, the higher the concentration of acetate the longer the time required for this inhibition to result. Butyrate and pyruvate, when they are the sole substrates, are not detectably different from acetate with respect to the percentile reduction in contraction amplitude produced by a given concentration of fluoroacetate.

Although regular contractions in glucose are stopped by high concentrations of fluoroacetate, there remains an irregular, high amplitude "fluoroacetate resistant" contraction when glucose is present with or without other substrates; this phenomenon is seldom or never seen when acetate alone is the substrate. It was noted by all groups which investigated the problem. Farah *et al.* (50) have found that only mannose acts like glucose whereas galactose, fructose, pyruvate, butyrate, caproate, caprylate, succinate, fumarate and α-keto-glutarate are, like acetate, unable to support these contractions. These contractions are not abolished by anaerobiosis (N_2, cyanide) or by malonate, although azide and iodoacetate are effective in abolishing them. It has been known for some time, as Farah *et al.* have pointed out, that intestinal smooth muscle can utilize glucose and mannose as a source of contraction energy during anaerobiosis. They feel that it is possible that energy for the fluoroacetate resistant contractions may be obtained from anaerobic glycolytic pathways.

That glucose is more effective than acetate in maintaining motility of gut segments in the presence of high concentrations of fluoroacetate was confirmed by Weeks and Chenoweth (132). When intestinal strips are allowed to contract

to exhaustion in Krebs-Henseleit solution in the absence of substrate, the normal stimulatory action of added 0.005 M sodium acetate is prevented by addition of 0.00032 M sodium fluoroacetate 5 minutes before the sodium acetate, whereas subsequent addition of 0.005 M glucose is still effective. Although they found that glycerol monoacetate is a very effective antagonist to fluoroacetate *in vivo* and sodium acetate is definitely not, in the isolated intestinal segment preparation sodium acetate is nearly five times as effective as glycerol monoacetate against fluoroacetate. Other experiments demonstrated that glucose-induced contractions, after fluoroacetate inhibition (0.01 M) in acetate, were of the same amplitude (approximately 30% of control values) as the contractions which persisted when the same concentration of fluoroacetate was added to muscles with a glucose substrate. These various observations can probably be explained by assuming, and there appears to be good reason to do so, that intestinal muscle under these circumstances obtains energy for contraction from at least two sources: (1) the breakdown of glucose which can occur anaerobically and (2) reactions of the tricarboxylic acid cycle into which acetate enters. When acetate, as the sole available substrate, is blocked from entry into the cycle by high concentrations of fluoroacetate, contraction ceases; in contrast, low concentrations of fluoroacetate are unable to produce a block in the presence of excess acetate. In the presence of glucose, energy for contraction is still available despite the blockade of acetate produced by fluoroacetate. This blockade may be increased until only the fluoroacetate, anaerobiosis-resistant contractions remain.

C. Working Nervous Tissue

According to Shanes and Brown (117), the preservation of the resting potential of frog nerve depends upon formation of pyruvate by the glycolytic cycle and the subsequent aerobic metabolism of this substrate. Because 0.01 M methyl fluoroacetate interferes with the redevelopment of the resting potential of nerve in oxygen following a period of anoxia, Shanes (116) felt that this was sufficient to suggest an interference with pyruvate metabolism. Conversely, the simultaneous addition of methyl fluoroacetate and sodium pyruvate to nerve in oxygen maintains a higher resting potential than when pyruvate is omitted, a fact which suggests a beneficial effect of added pyruvate not noted with acetate. During poisoning, he found that the threshold of excitability of frog sciatic nerve to condenser discharges steadily increased.

A series of studies has recently been published which has revealed several new facts about the action of fluoroacetate (17, 18, 22, 23, 45, 98). The sodium salt of fluoroacetic acid is nearly without action on frog nerve or brain *in vitro* although methyl fluoroacetate has several actions. Thus, the ester decreases the action potential of frog sciatic nerve and reduces conduction velocity by a process of blocking conduction in fibers; the threshold of the larger fibers is raised before that of the smaller fibers at concentrations of 0.005 M. The respiration of such nerves is decreased to 20% of normal. Sodium fumarate added before, or up to 15 minutes after, the methyl fluoroacetate protects against the action

potential changes in a 2:1 molar ratio, but the respiration of the nerve may still be 50% decreased. Succinate is equally effective in a 5:1 molar ratio, but ethanol, acetate, pyruvate, α-keto-glutarate and glucose are ineffective (18). Although their data have not yet been fully reported, Doty and Gerard (45) have found that methyl fluoroacetate will depress the resting oxygen consumption of frog nerve as described, but that the increased oxygen consumption which occurs on stimulation is not affected. Thus, with 0.001 M methyl fluoroacetate the resting Q_{O_2} is decreased by 25% whereas the activity increase is unaffected and the action potential is undisturbed. They caution, however, that the presence of respiring, non-conducting elements in a nerve trunk must be considered when interpreting these results.

In Gerard's isolated frog brain preparation, sodium fluoroacetate is ineffective whereas methyl fluoroacetate inhibits respiration 45% at 0.012 M (23). At this level the brain potentials are decreased about 50%. At 0.01 M methyl fluoroacetate there may also be a 50% decrease in cholinesterase activity. On this preparation the beneficial effects of fumarate are again seen, but they are rendered somewhat less specific by observations that fumarate protects to some extent against di-isopropyl fluorophosphate and that under certain circumstances as little as 0.000,001 M sodium fumarate can itself induce bizarre electrical changes in the brain.

The inactivity of sodium fluoroacetate does not appear to be solely the result of inability to penetrate cells, for it is inactive on rat brain or dog nerve homogenates in which cells are disrupted. Malic dehydrogenase of rat brain is sensitive to methyl fluoroacetate (20% inhibited at 0.001 M), but in general dehydrogenases are not much affected by fluoroacetate (98).

D. Isolated Perfused Hearts

By the use of a recirculating system for perfusing isolated hearts through the coronary arteries over long periods with bacteria-free Ringer's solutions (32), it has been found that concentrations of methyl or sodium fluoroacetate comparable to those calculated to exist in rabbits or rhesus monkeys poisoned with an LD_{100} cause a gradual decline in the amplitude of contraction, occasional alternans and rarely, if ever, fibrillation (28). The relative sensitivity of rabbits and monkeys to fluoroacetate is also manifested in their isolated hearts. Essentially the same degree of cardiac incompetence was produced by methyl fluoroacetate acting over a two-hour period in a concentration of 0.00001 m (0.5 mgm./L) on the isolated rabbit heart as by an intravenous dose of 0.5 mgm./kgm. in the intact animal (equivalent to about 0.6 mgm./L of body water). In the case of the monkey heart, 0.001 M fluoroacetate in the perfusion fluid produces effects which correspond roughly to those of an intravenous dose of 5 to 10 mgm./kgm.

The substitution of sodium acetate for the glucose of the perfusate in both rabbit and monkey hearts effectively maintained contraction and exerted an extensive protection against the effect of added fluoroacetate (33). In some instances protection was definite when the molar ratio of acetate to fluoroacetate

approximated unity, but usually a higher ratio was necessary. Pyruvate was somewhat less effective in maintaining contractions and in protecting against fluoroacetate, perhaps only because of toxic impurities (20).

Because the presence of acetate prevented the action of practical concentrations of fluoroacetate, it was impossible to demonstrate any change in the utilization of acetate by the heart in the presence of fluoroacetate. However, when failure was produced by the addition of 0.01 M fluoroacetate to monkey hearts with either glucose or pyruvate as the substrate, there was an acetate accumulation of as much as 3 grams/kgm. dry weight/hour over the three-hour exposure period. No other change in the utilization or production of acetate, pyruvate, lactate, α-keto-glutarate or glucose was detected.

Despite the protection exerted *in vitro*, sodium acetate by itself exerts no protective effect whatever against the toxic action of fluoroacetate in the intact rabbit (36, 37).

E. Mechanism Studies in Intact Animals; Antidotes

Because humans accidentally or wilfully ingest rat poisons, there can be no doubt of the desirability of an effective antidote to fluoroacetate. The studies so far described do not offer much hope that any highly effective treatment of well-established fluoroacetate poisoning will be found. Indeed, most investigators have been content if their results with antidotal therapy contribute something to an understanding of the mechanism of action of the poison.

It has been mentioned that sodium acetate, although it is the most likely candidate, is not an effective antidote or prophylactic in rabbits poisoned with fluoroacetate. However, Tourtelotte and Coon (125) have found that in mice, at least, sodium acetate (2 to 3 gram/kgm.) will protect against sodium fluoroacetate. Ethanol, which may be considered simply a source of acetate *in vivo* or, more complexly, a catalyst of the Krebs' cycle, is also effective (1.6 gram/kgm.). Ethanol and acetate together are distinctly more than twice as effective as either alone, a fact which suggests a synergistic effect.

Hutchens *et al.* (70) have reported more fully on the effectiveness of ethanol alone or with sodium acetate in protecting mice against fluoroacetate. Ethanol is also effective in rabbits, and to a lesser extent in guinea pigs, but not at all in dogs. It was noted that barbiturates were somewhat effective in protecting dogs but not mice. Although there is no doubt that the judicious administration of barbiturates (bearing in mind the prolongation of sleeping time described in III-G, c.) will control convulsions induced by fluoroacetate in dogs (52, 70, 125), animals which survived in this laboratory manifested changes characteristic of cortical damage. It would thus appear that, although overt convulsions are prevented, the pathological pattern of fluoroacetate poisoning has been unaffected.

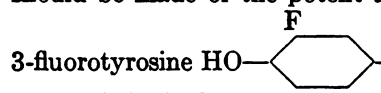
The effectiveness of sodium acetate *in vitro*, despite a generally unfavorable response to it *in vivo*, led to a search for other sources of C_2 moieties. One of the most promising has been glycerol monoacetate which has protected rats, rabbits, dogs and rhesus monkeys against fluoroacetate (36, 37). Equimolar doses of

glycerol monoacetate and ethanol, given to rhesus monkeys after fluoroacetate poisoning had progressed, were strikingly different in effectiveness, ethanol apparently being of no value although its effectiveness in mice and rabbits has been confirmed. Several other such compounds have been found which exert a protective action against either fluoroacetate or fluorocrotonate.

The simultaneous administration of large amounts of insulin and glucose is often effective in dogs and rabbits, although neither substance is effective alone (37). (The forcing of glucose is also protective against anoxic anoxia (21).) Anoxia caused by methemoglobinemia (approximately 70%) produced either by sodium nitrite or p-aminoacetophenone is a very effective antidote or prophylaxis for fluoroacetate poisoning in those species in which it can be induced, *e.g.*, mice and dogs (37). McNamara (97) has recently found that physiological sodium chloride solution exerts a definite protective effect against fluoroacetate poisoning in rabbits, a fact which may account, in part at least, for the heterogenous character of some of the substances that appear to exert a moderate protective action. It serves no useful purpose to list in detail all those substances which have proven ineffective; but, in general, salts of fatty acids, anticonvulsants, vitamins and most metabolic intermediates are without effect. Potent antifibrillatory, autonomic and cardiac drugs are generally of no therapeutic value; they may act differently after fluoroacetate (e.g., 50).

V. MISCELLANEOUS FLUORINATED COMPOUNDS

Several familiar compounds in which fluorine has replaced hydrogen or chlorine have been prepared and are of some interest here. Although 2,3-difluoro succinic acid (80), HOOC—CHF—CHF—COOH, appears to inhibit succinic dehydrogenase completely in very low concentrations, it is of singularly low toxicity, the LD_{50} in mice and dogs (salt or dimethyl ester) being above 200 mgm./kgm. (37). When fluorine is substituted for chlorine in sesqui-H, a very potent analog of mustard gas, the resulting compound F—CH_2CH_2—S—CH_2CH_2—S—CH_2CH_2—F, is nontoxic with neither vesicant nor fluoroacetate-like activity (96). This suggests that the body is unable to rupture the C-S link in this compound to obtain fluoroacetate and adds emphasis to the fact that the vesicant action of the mustards is dependent upon reactive halogens. Other compounds in which fluorine has been substituted for an hydrogen atom, such as di- (2-fluoroethyl) fluorophosphate or triethyl lead fluoroacetate, combine some of the characteristic activity of the parent compound with that of fluoroacetate (109). Mention should be made of the potent antithyroid activity reported by Litzka (87) for



3-fluorotyrosine. This activity may be the cause of the high toxicity (LD_{50}, 12.5 mgm./kgm.), since 1- or 4-fluorobenzoic acids are virtually nontoxic (79, 86). Boyer, Evans and Phillips (19) were unable to demonstrate an effect on the basal metabolic rate of rats, although they confirmed the toxicity of the compound. These compounds have been included because they give some indication of the way in which fluorine atoms in place of

hydrogen atoms may be used to obtain information concerning biological reactions.

VI. DISCUSSION

Nearly all the original data on monofluorinated fatty acids so far available in the open literature and much of that in the classified literature have been presented in this review. There appears to be a plethora of information on less important points but a dearth of incontrovertible data on the more important questions. What interpretation can be given the facts now available?

It is clear that the substitution of one fluorine atom for one hydrogen atom of a compound which is known, or suspected, to be of biological importance confers unusual pharmacological properties upon that compound provided that the substitution be made in the proper place. This proper place is always in the terminal position of a straight chain fatty acid containing an even number of carbon atoms, to cite the best-defined series. Thus 2-monofluoroacetic acid is active, 3-monofluoropropionic acid is inactive and 4-monofluorobutyric acid is very active, although 2-monofluorobutyric acid is inactive, and so on. Substitution of any atoms or groups other than one fluorine atom on the terminal carbon atom does not result in characteristic activity. More complex compounds containing a suitable grouping, for example, esters of 2-monofluoroacetic acid or 2-fluoroethanol, exert a typical pharmacological action if they can be broken down in the organism to yield fluoroacetic acid. This principle has been applied in a few less simple cases to indicate whether or not the body can rupture certain linkages, the C-S bonds in the chain F—C—C—S—C—C—S—C—C—F being an example. It appears to be a technic of some value, although open to criticism on the grounds that such a compound might be a specific inhibitor *per se*, should it *not* prove to be inert (for instance, fluoroacetyl salicylic acid).

The toxicity of suitably substituted even-numbered fatty acids in contrast to the inertness of odd-numbered homologs is superficially further evidence for β-oxidation of fatty acids, the assumption being that the even-numbered chains are broken down to the toxic fluoroacetic acid. Then, in order to account for the much greater toxicity (and the relative differences in various species) of the longer chains as compared to fluoroacetic acid on a mole per kilogram basis, it is necessary to *assume* that (a) they penetrate cells much more efficiently and/or (b) that release of fluoroacetate as an "active" F-C_2 radical occurs, preferably directly in the area where it could do the most harm. To the contrary, the quantitative and qualitative differences between the actions of the fluorinated higher fatty acids and the shortest active acid suggest most strongly that they act at separate sites. Evidence has been presented that fluorobutyrate, for example, is a much more active inhibitor of butyrate oxidation in mammalian tissue than is fluoroacetate, while in yeast fluorobutyrate does not inhibit acetate oxidation at all although fluoroacetate actively does so. Under at least one set of circumstances it has been shown that fluorobutyrate does not break down to fluoroacetate. In addition, the gross pharmacological effects of fluorobutyrate differ qualitatively from those of fluoroacetate, although admittedly the difference requires

sharper definition. While it is impossible on the basis of available information to exclude the possibility of β-oxidation of fluorinated higher fatty acids to fluoroacetate, it appears probable that they exert their toxic action in part, at least, by interfering with the metabolism of the corresponding non-fluorinated fatty acid in the cell. It is thus equally easy to dispose of the inactivity of odd-numbered fluoroacids, for it is generally uncommon to find the non-fluorinated homolog important in the main line of fatty acid oxidation.[4]

Certain inactive compounds shown in Table III might be used to add weight to the hypothesis of β-oxidation to fluoroacetate. For example, F—CH_2—

CH_3
|
C—CH_2COOH is pharmacologically inactive, and on structural grounds could
|
CH_3

not be expected to undergo β-oxidation to fluoroacetic acid. Similarly, derivatives of fluorobutyric acid in which carbon atoms 2 and 3 are part of a cyclic structure are inactive, and are not susceptible to β-oxidation. Although the inactivity of these compounds adds support to the idea that β-oxidation of higher ω-fluoro-acids is necessary for activity, it is equally possible to argue that because of steric factors these biologically abnormal compounds never have an opportunity to inhibit butyrate oxidation inasmuch as they are kept from entering a reactive center by their structural deformity and for that reason are pharmacologically inert.

In this case the assumption of "entrance into reactive centers" is based entirely upon the remarkable similarity (see Table I) of the FCH_2- and HCH_2- radicals, the chief differences being that the fluorine atom is about twice the size of the hydrogen atom and is bound more securely to the carbon atom. (Were there no physical-chemical differences at all there would seem to be no reason to expect any pharmacological differences.) If it be assumed that in the process of metabolism fatty acids fit some sort of matrix which requires entry of the terminal methyl group, it is easy to visualize the arrival of a fluorinated methyl group which fits effectively; but, perhaps because of the greater hold the carbon and fluorine atoms have upon each other and because of fluorine's propensity for hydrogen bonding, it cannot readily be dislodged. In such a case as fluoroacetic acid the prior administration of substances which release large amounts of acetic acid in the cell could be expected to influence this phenomenon by competing for the fixation site. Poisoning by fluoroacetate can indeed by prevented or even reversed by such substances in intact animals and in some isolated systems. Unfortunately, a different interpretation can be placed upon this observation. There is no way of knowing with certainty whether added acetate competes with fluoroacetate or simply by-passes a blockade. To clarify this it is necessary

[4] Inactivity may be a relative matter because the doses of odd-numbered compounds tested do not seem to have exceeded 200 mgm./kgm. and have been examined only in a few species. It is possible that some organisms may be very sensitive if odd-numbered fatty acids are actively metabolized by them.

to marshal the data concerning the various possible blockades which have been suggested.

Although it would be extremely helpful to have more data on other compounds, nearly all the detailed investigations concerning inhibition of metabolic processes by the fluoroacids have been performed with fluoroacetic or fluorobutyric acid. The specificity (or even the existence) of the inhibition of the oxidation of acetate and pyruvate by fluoroacetate has been the chief subject of study, the results of which have been vastly complicated by differences in technic, species or organs employed. (One searches the literature in vain for mention of the existence of fluoropyruvic acid, $F—CH_2—\underset{\underset{O}{\|}}{C}—COOH$, which might be utilized to settle some of these questions.)

It is fairly certain that fluoroacetic acid does not inhibit acetylation of foreign or natural amines by mammals. The formation of acetoacetate from acetate is not inhibited and in general—*Litomosoides carinii* being a clear exception—the oxidation of pyruvate to acetate may be directly affected while the disposition of the acetate so formed is certainly affected. Acetate probably exerts an inhibitory effect upon pyruvate oxidation after a sufficient amount of it has accumulated. There appears to be no way at this time of choosing between findings which suggest inhibition of fumarate oxidation and those which do not. In this connection it is possible to argue obliquely that, because fluorosuccinate and other potent competitive inhibitors of succinic dehydrogenase are relatively feeble animal poisons, inhibition of other neighboring steps in the Krebs' cycle is not likely to be the cause of death in mammals poisoned with the usual minute doses of fluoroacetate. The effects on citrate and glucose metabolism may best be explained as the indirect result of inhibition at other points.

It is well established that the breakdown of glucose to triose phosphate can occur anaerobically and aerobically, and is a source of much energy. Pyruvate has long been known as the main end-product of this metabolic chain, lactate being a simple conversion product of pyruvate. However, in the last decade the C_2 fragment, a much discussed entity having many but not all of the characteristics of acetate, has been definitely added as a stage of carbohydrate oxidation beyond pyruvate and as the end-product of fatty acid breakdown (16, 83, 121).

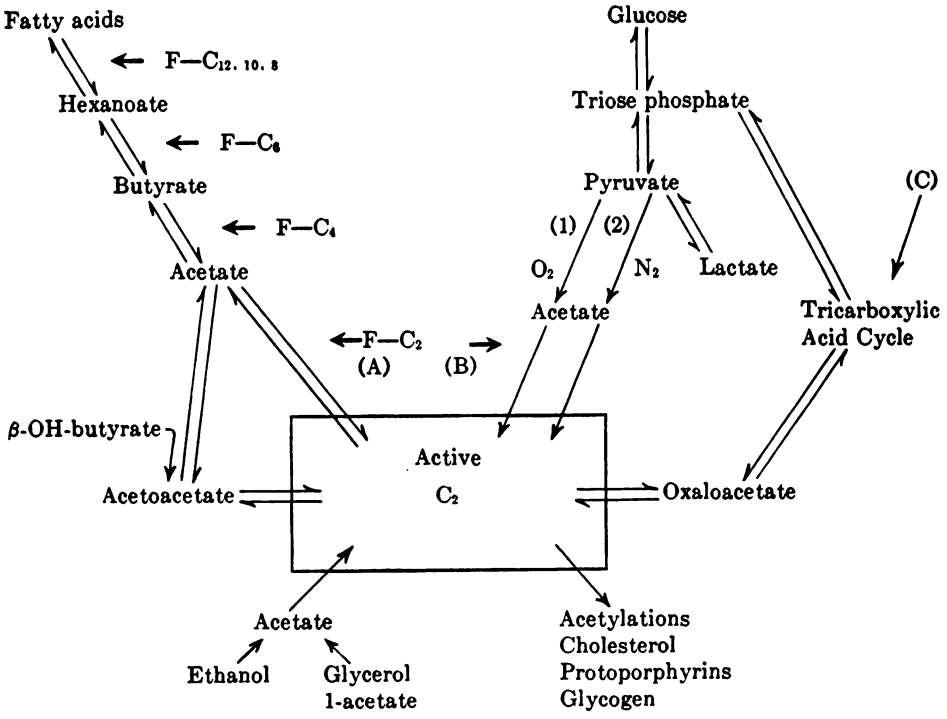
Before presenting the schema depicted in Figure 1, it is necessary to bring out certain less well known observations which relate to the possibility of its general applicability and to the action of fluoroacetate. Differences among species with regard to the action of fluoroacetate must be more than a matter of vagary. If the inhibitions of various metabolic systems and pathways and the responses of intact animals which have been reviewed here are considered in the light of the importance of these systems and pathways to the economy of the organism being studied, some interesting relations are obtained. Thus, although there are no qualitative differences between Wistar and Sprague-Dawley strains of rats in the way they handle the acetate in their acetate pool, Wistar rats turn over less acetate (12 to 15 mM/100 gram/24 hr.) than do Sprague-Dawley rats (20

to 25 mM/100 gram/24 hr.). In addition Wistar rats are unable to convert pyruvate to acetate although Sprague-Dawley rats do so freely (4). The difference in response to fluoroacetate is clear; the strain to which acetate is more important is more sensitive to fluoroacetate (although more resistant to pyridoxine deficiency (27) or alloxan (82)). It has recently been shown that intact rabbits metabolize formate actively, but that dogs and man are nearly unable to do so; incidentally, this accounts for the relative toxicity of methanol to these two latter species (88, 89, 90). Dog muscle, on the other hand, can oxidize β-hydroxybutryate and acetoacetate whereas rabbit muscle can only use acetoacetate (124). The differences between dogs and rabbits in response to fluoroacids are striking. Perhaps a similar relation exists when still other species are considered, for isolated mammae of the goat, a fluoroacetate-sensitive animal, utilize acetate whereas those of the relatively resistant rat and still more resistant mouse do not appear to do so (51). Mention has been made of the ability of guinea pig brain to oxidize added acetate and the relative inability of rabbit brain to do so, the fluoroacetate-induced inhibition of acetate oxidation being greater in brain tissue from the guinea pig than from the rabbit. The convulsive pattern of fluoroacetate poisoning in the guinea pig and the effects on the heart of the rabbit, which oxidizes acetate vigorously (10, 11, 33), intimate that this is no mere coincidence.

When one views these scattered observations on the relation between fluoroacetate action and metabolic pathways in the light of the protective effect of acetate and its donors, it is not unreasonable to suspect that the degree of sensitivity of an animal or its organs to fluoroacetate is an indication of a certain characteristic of its acetate metabolism. To establish this suspicion as a fact will require much effort and it is probable that the problem is even more complicated than appears at first. Reference has been made to studies with isolated frog muscle, rabbit intestine and intact animals which show that there are fluoroacetate-insensitive metabolic pathways giving rise to sufficient energy to maintain function. Evidence has been adduced to indicate that the breakdown of glucose is not sensitive to fluoroacetate and that some such system can be enhanced *in vivo* by partial anoxia (methemoglobinemia) or by accelerating the breakdown with an excess of glucose and insulin. It is highly probable that in addition to individual peculiarities of the metabolic systems for handling C_1–C_4 molecules in a given species a second factor, the glycolytic rate, is important in controlling the sensitivity of the organ or organism. Few comparative data have been published, although Wu and Chang (134) have recently pointed out that the glycolytic rates of isolated eel, toad, turtle and rat hearts decreased (in that order) with their resistance to anoxia. Grossly, the sensitivity to fluoroacetate likewise decreases in the direction of greater glycolytic activity. It is also well known that very young mammals are very much more resistant to anoxia than are older ones. Nine 24-hour old dogs recently tested in this laboratory were markedly resistant to fluoroacetate. Further, the relative sensitivity of various vertebrates to tissue anoxia induced by potassium cyanide (67) is strikingly similar to their order of sensitivity to fluoroacetate. Farah (50) has called attention to the similarity

between the sensitivities to fluoroacetate, cyanide and anoxia of various portions of the gut, which follow Alvarez's concept of a metabolic gradient. Although it is surprisingly difficult to find accurate comparative data (64), such adult mammals as have been found very resistant to anoxia (21) are also resistant to fluoroacetate.

FIG. 1. Tentative Localization of Fluoroacid Blockades



Reaction (1): Oxidative decarboxylation of pyruvate to acetate.
Reaction (2): Anaerobic degradations of pyruvate to acetate and lactate. Enhanced by partial anaerobiosis *in vivo* (methemoglobinemia) and by large amounts of glucose, thus minimizing effect of block (B).
Blockade (A), (B): Competitively antagonized by C_2 donors.
Blockade (C) and others not shown: Minor blockades caused by imperfect introduction of F—C—C—R in high concentrations into a matrix designed for other structures, *e.g.*, malic dehydrogenase.

In Figure 1, the anaerobic degradation of pyruvate to acetate by coupled oxidation-reduction reactions ("dismutation") to lactate and acetate, or by fragmentation ("dissimilation") to acetate and formate, or by still other processes, is shown as unaffected by fluoroacetate, although oxidation of pyruvate or fatty acids through acetate to active acetate is shown as blocked. If allowance is made for variations among species and strains of organisms, such a blockade can account for many of the pharmacological responses so far described. One

phenomenon, however, is outstandingly unexplained. If blockades by the higher fluoroacids actually occur as indicated, why are these acids so much more toxic? Perhaps this will become clear as the understanding of fatty acid metabolism increases. On the whole, it seems possible to explain the pharmacological actions of fluoroacetate itself, together with some of the specific variations noted, as a function of the magnitude and character of the acetate and glycolytic metabolic pathways. The characteristic response of an organism or tissue to fluoroacetate may be determined by the relative importance of these two pathways.

SUMMARY

Conversion of a metabolic intermediate into a very highly toxic compound by the introduction of a single fluorine atom in a strategic position in the molecule has been described for a number of compounds. It appears to be a useful method for producing agents with which metabolic pathways can be differentiated in a large number of species with a minimum of effort, for it is evident that these agents act by virtue of their close resemblance to natural metabolites. As an example, the variation among species in response to monofluoroacetic acid has been related to certain definite differences in metabolism in the species studied.

ACKNOWLEDGMENTS

The author wishes to thank the Office of the Chief, Chemical Corps, for permission to use hitherto unpublished information, and Mrs. Pauline Koepnick and the Editors for aid in the preparation of the manuscript. Dr. Walter Kirner aided greatly in obtaining declassification.

ADDENDUM

The gracious permission of the British Ministry of Supply to refer to information in certain of their classified reports was received too late to permit inclusion of this information in the body of the review. Mention should be made, however, of the high resistance of the *Cercopithecus*, or "green" monkey to methyl fluoroacetate. The LD_{50} appears to lie above 50 mgm./kgm. according to K. J. Carpenter and B. A. Kilby (1944). These workers also found that although fluoroethanol is as active as fluoroacetate in intact animals, it is without action on the isolated perfused heart, presumably because this organ can not convert fluoroethanol to fluoroacetate.

Definite histologic abnormalities in the myocardium, but no unequivocal changes in the central nervous system, were reported by A. M. Barrett (1944) in guinea pigs and rabbits poisoned repeatedly with methyl fluoroacetate. The beating of heart muscle cells in culture is rapidly inhibited by methyl fluoroacetate but the growth of the cell masses is not affected, according to C. B. Allsop and H. B. Fell (1944). The action is specific, for methyl chloroacetate in equivalent concentrations merely kills the cells.

The view that fluoroacetic acid is metabolized to fluorocitric acid, accounting for inhibition of citrate oxidation and the subsequent accumulation of citric acid, has been advanced by Martius (Ann. d. Chem. *561*: 227–232, 1949). This would seem referable to Blockade (C) of Figure 1.

REFERENCES

1. ABBOTT, C. E.: The effects of DDT and of sodium monofluoroacetate upon *Physarella Oblonga* Morgan. Science 102: 71, 1945.
2. ALBAUM, H.: Medical Division, Army Chemical Center. Private Communication.
3. ALDOUS, J. G.: The effect of pH upon the toxicity of iodoacetic acid to yeast cells. J. Biol. Chem., 176: 83–88, 1948.
4. ANKER, H. S.: Metabolism of pyruvic acid in the intact animal. J. Biol. Chem., 176: 1337–1352, 1948.
5. Anon.: Compound 1080 as a rodenticide. Brochure prepared by U. S. Fish and Wildlife Service, 546 Custom House, Denver 2, Colo. (Observation by H. J. Spencer.)
6. Anon.: Instructions for using sodium fluoroacetate (compound 1080) as a rodent poison. Subcommittee on Mammalogy, National Research Council, Chemical-Biological Coordination Center, Washington, D. C., Revised October, 1948.
7. BACON, J. C., BRADLEY, C. W., HOEGBURG, E. I., TARRANT, P. AND CASSIDY, J. T.: Some amides and esters of fluoroacetic acid. J. Am. Chem. Soc., 70: 2653–2655, 1948.
8. BACKER, H. J. AND VAN MELS, W. H.: Vitesse de la réaction des acides halogéno-carboxyliques es des sulfites. II. Rec. trav. chim., 49: 363–380, 1930.
9. BARBOUR, J. H. AND SEEVERS, M. H.: A comparison of the acute and chronic toxicity of carbon dioxide with especial reference to its narcotic action. J. Pharmacol. and Exper. Therap., 78: 11–21, 1943.
10. BARCROFT, J., MCANALLY, R. AND PHILLIPSON, A.: Physiological action of acetic acid in living tissues. Nature, 143: 304, 1943.
11. BARRON, E.S. G. AND MEYER, J.: Oxidation of acetate by heart muscle. Proc. Central Soc. Clin. Res., 18: 59, 1945.
12. BARTLETT, G. R. AND BARRON, E. S. G.: The effect of fluoroacetate on enzymes and on tissue metabolism. Its use for the study of the oxidative pathway of pyruvate metabolism. J. Biol. Chem., 170: 67–82, 1947.
13. BERGMANN, M. AND FRUTON, J. S.: Rockefeller Institute for Medical Research. Private Communication.
14. BIGELOW, L. A.: The action of elementary fluorine upon organic compounds. Chem. Rev., 40: 51–115, 1947.
15. BLACK, S. AND HUTCHENS, J. O.: The oxidation of acetate by yeast in the presence of fluoroacetate. Arch. Biochem., 17: 211–223, 1948.
16. BLOCH, K.: The metabolism of acetic acid in animal tissues. Physiol. Rev., 27: 574–620, 1947.
17. BOYARSKI, L. L., POSTEL, S. AND ROSENBLATT, A.: Enzyme inhibitors on conduction and respiration of frog nerve. Fed. Proc., 7: 11, 1948.
18. BOYARSKY, L. L., ROSENBLATT, A. D., POSTEL, S. AND GERARD, R. W.: Action of methyl fluoroacetate on respiration and potential of nerve. Am. J. Physiol., 157: 291–298, 1949.
19. BOYER, P. D., EVANS, R. J. AND PHILLIPS, P. H.: The toxicity and effect of certain fluorinated derivatives of phenylalanine and tyrosine on the basal metabolic rate of the rat. J. Pharmacol. and Exper. Therap., 73: 176–185, 1941.
20. BRAUN-MENENDEZ, E., CHUTE, A. L. AND GREGORY, R. A.: The usage of pyrivic acid by the dog heart. Quart. J. Exper. Physiol., 29: 91–104, 1939.
21. BRITTON, S. W. AND KLINE, R. F.: Age, sex, carbohydrate, adrenal cortex and other factors in anoxia. Am. J. Physiol., 145: 190–202, 1945.
22. BROOKS, V. B. AND RANSMEIER, R. E.: Enzyme inhibitors on electric activity of frog brain. Fed. Proc., 7: 15, 1948.
23. BROOKS, V. B., RANSMEIER, R. E. AND GERARD, R. W.: Action of anticholinesterase drugs and intermediates on respiration and electrical activity of the isolated frog brain. Am. J. Physiol., 157: 299–316, 1949.
24. BUCKLE, F. J., HEAP, R. AND SAUNDERS, B. C.: Toxic fluorine compounds containing the C-F link. Part III. Fluoroacetamide and related compounds. J. Chem. Soc., 1949: 912–916.
25. BUCKLE, F. J., PATTISON, F. L. M. AND SAUNDERS, B. C.: Toxic fluorine compounds containing the C-F link. Part VI. ω-fluorocarboxylic acids and derivatives. J. Chem. Soc., 1949: 1471–1479.
26. BUEDING, E.: Studies on the metabolism of the filarial worm, *Litomosoides carinii*. J. Exper. Med., 89: 107–130, 1949.
27. CERECEDO, L. R.: Strain differences in the resistance of rats to pyridoxine deficiency. Fed. Proc., 5: 229, 1946.
28. CHENOWETH, M. B AND GILMAN, A.: Pharmacology of fluoroacetate. Fed. Proc., 5: 171, 1946.
29. CHENOWETH, M. B. AND GILMAN, A.: Medical Division, Army Chemical Center. Private Communication.
30. CHENOWETH, M. B. AND GILMAN, A.: Studies on the pharmacology of fluoroacetate. I. Species response to fluoroacetate. J. Pharmacol. and Exper. Therap., 87: 90–103, 1946.
31. CHENOWETH, M. B. AND GILMAN, A.: Studies on the pharmacology of fluoroacetate. II. Action on Heart. Bull. U. S. Army Med. Dept., 7: 687–706, 1947.
32. CHENOWETH M. B AND KOELLE, E. S.: An isolated heart perfusion system adapted to the determination of non-gaseous metabolites. J. Lab. and Clin. Med., 31: 600–608, 1946.
33. CHENOWETH, M. B., KOELLE, E. S., GILMAN, A. AND PHILIPS, F. S.: Unpublished studies, 1945.
34. CHENOWETH, M. B. AND ST. JOHN, E. F.: Studies on the pharmacology of fluoroacetate. III. Effects on the central nervous system of dogs and rabbits. J. Pharmacol. and Exper. Therap., 89: 76–82, 1947.
35. CHENOWETH, M. B. AND ST. JOHN, E. F.: Medical Division, Army Chemical Center. Private Communication.
36. CHENOWETH, M. B., SCOTT, E. B. AND SEKI, S. L.: Prevention of fluoroacetate poisoning by acetate donors. Fed. Proc., 8: 280, 1949.
37. CHENOWETH, M. B., JOHNSON, L. B., KANDEL, A., SCOTT, E. B. AND WEEKS, J. R.: Work in progress.
38. CLARKE, D. A., TOOLE, J. F. AND RIKER, W. F., JR.: Effect of fluoroacetate on oxidative processes in frog muscle. Fed. Proc., 8: 282, 1949.
39. CLARKE, D. A. AND RIKER, W. F., JR.: To be published.
40. COHEN, B., Johns Hopkins University. Private Communication.

41. COLOWICK, S. P., BERGER, L., SLEIN, M. W. AND CORI, C. F.: Washington University, St. Louis. Private Communication.
42. COTTRAL, C. E., DIBBLE, G. D. AND WINTON, B.: Effect of sodium fluoroacetate ("1080" rodenticide) on White Leghorn chickens. Poultry Sci., 26: 610–613, 1947.
43. DEONIER, C. C., JONES, H. A. AND INCHO, H.H.: Organic compounds effective against *Anopheles quadrimaculatus*. Laboratory tests. J. Econ. Entomol., 39: 459–462, 1946.
44. DIEKE, S. H. AND RICHTER, C. P.: Comparative assays of rodenticides on wild Norway rats. I. Toxicity. U. S. Pub. Health Repts., 61: 672–679, 1946.
45. DOTY, R. W. AND GERARD, R. W.: Separate inhibition of resting and active oxygen consumption of functioning nerves. Fed. Proc., 8: 35, 1949.
46. EISENBERG, W. V. AND WILSON. J. B.: Identification of the monohalogen substituted acetic acids as barium salts. J. Assoc. Offic. Agricul. Chemists, 30: 563–567, 1947.
48. ELLIOTT, A. M.: Personal Communication.
49. ELMORE, J. W.: Report on the analysis of rodenticides. J. Assoc. Offic. Agricul. Chemists, 31: 366–368, 1948.
50. FARAH, A., ANGEL, R. AND WEST, T. C.: Action of some enzyme inhibitors on the activity of the isolated intestine. Fed. Proc. 8: 290, 1949. J. Pharmacol. and Exper. Therap. In press.
51. FOLLEY, S. J. AND FRENCH, T. H.: Acetate as a possible precursor of ruminant milk fat, particularly the short chain fatty acids. Nature, 163: 174–175, 1949.
52. FOSS, G. L.: The toxicology and pharmacology of methyl fluoroacetate (MFA) in animals, with some notes on experimental therapy. Brit. J. Pharm. and Chemother., 3: 118–127, 1948.
53. FRICK, E. J. AND BOEBEL, F. W.: Clinical observation of "1080" poisoning. Veter. Med., 41: 196–197, 1946.
54. FURCHGOTT, R. F.: To be published.
55. FURCHGOTT, R. F. AND SHORR, E.: Sources of energy for intestinal smooth muscle contraction. Proc. Soc. Exper. Biol. and Med., 61: 280–286, 1946.
56. GLOMSET, D. J. AND GLOMSET, A. T. A.: A morphologic study of the cardiac conduction system in ungulates, dog and man. Am. Heart J., 20: 677–701, 1940.
57. GOLDMAN, I. S., FLANNERY, M. C., ARENT, L. J., HOAG, J. B. AND BUSWELL, A. M.: Medical Division, Army Chemical Center. Private Communication.
58. GRATCH, I., PURLIA, P. L. AND MARTIN, M. L.: Effect of sodium fluoroacetate (1080) in poisoned rats on plague diagnosis procedures. Preliminary report. U. S. Pub. Health Repts., 64: 339–342, 1949.
59. GRIMM, H. G. AND WOLF, H. in GEIGER, H. AND SCHEEL, K.: Handbuch der Physik, 2nd Edition, 24: 1003, 1933.
60. GRYSZKIEWICZ-TROCHIMOWSKI, E., SPORZYŃSKI, A. AND WNUK, J.: Recherches sur les composés organiques fluorés dans la série aliphatique I–IV. Rec. trav. chim., 66: 413–431, 1947.
61. HENNE, A. L.: Aliphatic fluorides. Chapter XI, in Gilman, H., Organic Chemistry, An Advanced Treatise, Vol. I, 2nd Ed., Wiley, New York, 1943.
62. HIMWICH, H. E.: Medical Division, Army Chemical Center. Private Communication.
63. HÖBER, R.: Physical Chemistry of Cells and Tissues, Blakiston, Philadelphia, 1945. Chapter 12.
64. HOFF, E. C. AND FULTON, J. F., A bibliography of aviation medicine, C. C. Thomas and Co., 1942 and Supplement, G. Banta Publ. Co., Menasha, Wisc., 1944.
65. HORSFALL, J. L.: 2-ethyl hexyl fluoroacetate as aphid poison. U. S. Patent 2,409,859, October 22, 1946.
66. HUGHES, J. H.: Deratization of surface vessels by means of 1080 (sodium fluoroacetate). U. S. Pub. Health Repts., 62: 933–940, 1947.
67. HUNT, R.: Cyanwasserstoff, in Heffter's Handbuch der Experimentellen Pharmakologie I, pp. 772–773, 1923.
68. HUTCHENS, J. O. AND KASS, B. M.: A colorimetric microanalytical method for acetate and fluoroacetate. J. Biol. Chem., 177: 571–575, 1949.
69. HUTCHENS, J. O., MCMAHON, T. AND PODOLSKY, B.: University of Chicago Toxicity Laboratory. Private Communication.
70. HUTCHENS, J. O., WAGNER, H., PODOLSKY, B. AND MCMAHON, T. M.: The effect of ethanol and various metabolites on fluoroacetate poisoning. J. Pharmacol. and Exper. Therap., 95: 62–70, 1949.
71. HYDE, J., BECKETT, S. AND GELLHORN, E.: Acetylcholine and convulsive activity. J. Neurophysiol., 12: 17–27, 1949.
72. I. G. Farbenindustrie, A. G.: German patent 504,886, September 14, 1927, confirmed August 8, 1930. (Through BOCKEMÜLLER, W.: Organische Fluorverbindungen in Sammlung chemischer und chemischtechnischer Vorträge, No. 28, 89 Stuttgart, 1936.)
73. JENKINS, R. L. AND KOEHLER, H. C.: Making 1080 safe. A case study in the safe manufacture and distribution of a hazardous chemical. Chem. Ind., 62: 232–235, 1948.
74. KALMBACH, E. R.: "Ten-Eighty," A war-produced rodenticide. Science, 102: 232–233, 1945.
75. KALNITSKY G.: The effect of $BaCl_2$, $MgCl_2$ and fluoroacetate on the formation and utilization of citrate. Arch. Biochem., 17: 403–411, 1948.
76. KALNITSKY G.: The formation of citrate by extracts of rabbit kidney cortex. J. Biol. Chem., 179: 1015–1025, 1949.
77. KALNITSKY, G. AND BARRON, E. S. G.: The effect of fluoroacetate on the metabolism of yeast and bacteria. J. Biol. Chem., 170: 83–95, 1947.
78. KALNITSKY, G. AND BARRON, E. S. G.: The inhibition by fluoroacetate and fluorobutyrate of fatty acid and glucose oxidation produced by kidney homogenates. Arch. Biochem., 19: 75–87, 1948.
79. KEMPFE, C. A., GREENWOOD, D. A. AND NELSON, V. E.: Studies relating to the toxicity of fluorine compounds. J. Lab. and Clin. Med., 22: 1133–1137, 1947.
80. KHARASCH, M. S.: University of Chicago. Private Communication.
81. KING, J. E. AND PENFOUND, W. T.: Effects of new herbicides on fish. Letter to the Editor. Science, 103: 487, 1946.

82 KRAHL, M. E. AND CORI, C. F.: The uptake of glucose by the isolated diaphragm of normal, diabetic and adrenalectomized rats. J. Biol. Chem., 170: 607–618, 1947.
83. LELOIR, L. F.: The mechanism of fatty acid oxidation. Enzymologia, 12: 263–276, 1948.
84. LIÉBECQ, C. AND PETERS, R. A.: The toxicity of fluoroacetate and the tricarboxylic cycle. Biochemica et Biophysica Acta, 3: 15–230, 1949.
85. LIPTON, M. A.: Mechanism of the enzymatic synthesis of acetylcholine. Fed. Proc., 5: 145, 1946.
86. LITZKA G.: Allegemeine biologische Wirkungen einer kernfluorierten Aminosäure (Fluortyrosin). Arch. f. exper. Path. u. Pharm., 183: 427–435, 1936.
87. LITZKA, G.: Die antithyreotoxische Wirkung des Fluortyrosin. Arch. f. exper. Path. u. Pharm., 183: 436–458, 1936.
88. LUND, A.: Metabolism of methanol and formic acid in rabbits. Acta Pharmacol. et Toxicol., 4: 99–107, 1948.
89. LUND, A.: Metabolism of methanol and formic acid in dogs. Acta Pharmacol. et Toxicol., 4: 108–121, 1948.
90. LUND, A.: Excretion of methanol and formic acid in man after methanol consumption. Acta Pharmacol. et Toxicol., 4: 205–212, 1948.
91. LYNEN, F.: Zum biologischen Abbau der Essigsäure I. Über die "Induktionszeit" bei verarmter Hefe. Ann. d. Chem., 552: 270–306, 1942.
92. MACCHIAVELLO, A.: Plague control with DDT and "1080": Results achieved in plague epidemic at Tumbes, Peru, 1945. Am. J. Pub. Health, 36: 842–854, 1946.
93. MARAIS, J. S. C.: Monofluoroacetic acid, the toxic principle of "Giftblaar," *Dichapetalum cymosum* (Hook) Engl. Onderstepoort J. Vet. Sci. Animal Ind., 20: 67–73, 1944.
94. MAZUR, A., BODANSKY, O. AND ALBAUM, H.: Medical Division, Army Chemical Center. Private Communication
95. MAZUR, A., BODANSKY, O AND ALBAUM, H.: Ibid.
96. MCCOMBIE, H. AND SAUNDERS, B. C.: Fluoroacetates and allied compounds. Nature, 158: 382–385, 1946.
97. MCNAMARA. B. P.: Medical Division, Army Chemcal Center. Private Communcation.
98. MICHAELIS, M., ARANGO, N. I. AND GERARD, R. W.: Inhibition of brain dehydrogenases by anticholinesterases. Am. J. Physiol., 157: 463–467, 1949.
99. MODELL, W., KOSTER, R. AND RIKER, W. F., JR.: Cornell University Medical College. Private Communication.
100. MORRISON, J. L.: Toxicity of certain halogen substituted aliphatic acids for white mice. J. Pharmacol. and Exper. Therap., 86: 336–338, 1946.
101. Numerous Authors: A fluorine symposium. Ind. Eng. Chem., 39: 235–436, 1947.
102. PRICE, C. C. AND JACKSON, W. G.: Some properties of methyl fluoroacetate and fluoroethanol. J. Am. Chem. Soc., 69: 1065–1068, 1947.
103. PAULING, L.: The Nature of the Chemical Bond. Cornell University Press, Ithaca, N. Y., 1939.
104. PAULING, L. AND HUGGINS, M. L.: Covalent radii of atoms and interatomic distances in crystals containing electron-pair bonds. Ztschr. f. Krist., 87: 205–238, 1934.
105. QUIN, J. I. AND CLARK, R.: Studies on the action of potassium monofluoroacetate (CH_2FCOOK) *Dichapetalum cymosum* (Hook) Engl. toxin) on animals. Onderstepoort J. Vet. Sci. Animal Ind., 22: 77–90, 1947.
106. RAMSEY, L. L. AND CLIFFORD, P. A.: Determination of monofluoroacetic acid in foods and biological materials. Presented at the May 12, 1949 meeting of the Washington Section of the American Chemical Society.
107. RENSHAW, B. AND GATES, M.: National Defense Research Council. Private Communication.
108. ROHOLM, K.: Fluor und Fluorverbindungen, in Heffter's Handbuch der Experimentellen Pharmakologie VII 1938.
109. SAUNDERS, B. C.: Toxic properties of ω-fluorocarboxylic acids and derivatives. Nature, 160: 179–181, 1947.
110. SAUNDERS, B. C. AND STACEY, G. J.: Toxic fluorine compounds containing the C-F link. Part I. Methyl fluoroacetate and related compounds. J. Chem. Soc., 1948: 1773–1779..
111. SAUNDERS, B. C. AND STACEY, G. J.: Toxic fluorine compounds containing the C-F link. Part IV. (a) 2-fluoroethyl fluoroacetate and allied compounds. (b) 2:2′ difluorodiethyl ethylene dithioglycol. J. Chem. Soc., 1949: 916–919.
112. SAUNDERS, B. C., STACEY, G. J. AND WILDING, I. G. E.: Toxic fluorine compounds containing the C-F link. Part II. 2-Fluoroethanol and its derivatives. J. Chem. Soc., 1949: 773–777.
113. SAUNDERS, B. C. AND WILDING, I. G. E.: Toxic fluorine compounds containing the C-F link. Part V. (a) Fluorine-containing ammonium salts. (b) Relationship betwen physiological action and chemical constitution. J. Chem. Soc., 1949: 1279–1282.
114. SCALES, J. W.: "1080" rat poison very effective but dangerous later. Farm Research, 8: No. 12, p. 8, 1945.
115. SCHWARTE, L. H.: Toxicity of sodium monofluoroacetate (1080) for swine and chickens. J. Am. Vet. Med. Assoc., 111: 301–303, 1947.
116. SHANES, A.: Action of methyl fluoroacetate on metabolic processes sustaining resting potential of frog nerve. Rockefeller Institute for Medical Research. Private Communication.
117. SHANES, A. AND BROWN, D. E. J.: The effect of metabolic inhibitors on the resting potential of frog nerve. J. Cell. and Comp. Physiol., 19: 1–13, 1942.
118. SIDGWICK, N. V.: The Covalent Link in Chemistry. Cornell University Press, Ithaca, New York, 1933.
119. SIMMONS, S. W. AND NICHOLSON, H. P.: Use of the rodenticides 1080 and ANTU. Pests, 15: Nos. 5, 8, 10, 1947.
120. SMITH, P. K., GLEASON, H. L., STOLL, C. G. AND OGORZALEK, S.: Studies on the pharmacology of salicylates. J. Pharmacol. and Exper. Therap., 87: 237–255, 1946.
121. STADIE, W. C.: The intermediary metabolism of fatty acids. Physiol. Rev., 25: 395–441, 1945.
122. SWARTS, F.: Sur l'acide fluoroacétique. Bull. Soc. Chim. de Paris I, Série 3, 15: 1134, 1896.
123. TEPPERMAN, J. AND MAZUR, A.: Personal communication, 1945.
124. TOENIESSEN, E. AND BRINKMANN, E.: Über den Abbau der niederen Fettsäuren insbesondere der Essigsäure und Ameisensäure im Säugetier und über die Frage der Zuckerbildung aus Fett. Z. physiol. Chem., 252: 169–195, 1938.

125. TOURTELLOTTE, W. W. AND COON, J. M.: Synergistic effect of sodium acetate and ethanol in antagonising sodium fluoroacetate (1080) poisoning in mice. Fed. Proc., 8: 339, 1949.
126. WARD, A. A.: Convulsive activity induced by fluoroacetate. J. Neurophysiol., 10: 105-112, 1947.
127. WARD, J. C.: Personal communication, 1944.
128. WARD, J. C.: Rodent control with 1080, ANTU and other war-developed toxic agents. Am. J. Pub. Health, 36: 1427-1431, 1946.
129. WARD, J. C. AND SPENCER, D. A.: Notes on the pharmacology of sodium fluoroacetate-Compound 1080. J. Am. Pharm Assoc., 36: 59-62, 1947.
130. WATSON, H. B.: Modern Theories of Organic Chemistry, Oxford University Press, London, 1947, p. 36.
131. WATT, J. M. AND BREYER-BRANDWIJK, M. G.: The Medicinal and Poisonous Plants of South Africa. Livingston Co., Edinburgh, 1932, pp. 97-98.
132. WEEKS, J. R. AND CHENOWETH, M. B.: Separation by fluoroacetate of energy sources for smooth muscle contraction. Fed. Proc., 8: 345, 1949.
133. WOODS, L. A.: Personal communication, 1949.
134. WU, J. J. AND CHANG, I.: The glycolytc activity of the hearts of vertebrates. Quart. J. Exper. Physiol., 34: 91-95, 1948.