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THE TOXICOLOGY AND PHARMACOLOGY OF METHYL FLUOROACETATE (MFA) IN ANIMALS, WITH SOME NOTES ON EXPERIMENTAL THERAPY

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It has been known for about ten years that methyl fluoroacetate (MFA), $CH_2F.COOCH_3$, was a substance of great toxicity, with very interesting pharmacological properties. In 1942, Briscoe, and Feldberg, Kilby, and Kilby found that it caused death by convulsions and respiratory failure. Later in 1943, Kilby and Kilby studied it and allied substances further and concluded that the toxicity was due to the CH_2F group.

In this paper further work is described on its pharmacological and toxicological properties.

Material and Methods

The sample of MFA employed had b.p. 104° C. and sp.gr. 1.17. It was a clear, colourless liquid with a faint fruity smell; it mixed readily with water, and in dilutions which were toxic, it was tasteless and quite odourless.

Animals.—In addition to mice, rats, guinea-pigs and rabbits, the larger animals, cats, dogs, monkeys and goats were used for this investigation; also a horse.

Toxicity.—Experiments were conducted with each species and the drug was administered by mouth or by injection as freshly made solutions in distilled water or saline, or by inhalation. For the latter the substance was vaporized rapidly by heat in a 10 cu.m. chamber. Chemical analyses showed that there was little variation in concentration. After administration the animals were observed carefully and records were made of their behaviour over the whole period until their death.

As this substance had a convulsive action the effects of other known convulsants were compared in the most favourable species, and for purposes of record and study a colour film was made of a dog and two monkeys after MFA, and also of monkeys after nicotine, strychnine, and metrazol. The effect of MFA on a spinal monkey was also filmed.

Autopsies were made on animals directly after death. The effect of MFA on the blood chemistry was investigated in rabbits, dogs, and goats. Blood samples were collected under paraffin, and serum and plasma were separated shortly after. Samples of blood were taken into oxalate tubes for blood sugar, non-protein nitrogen, potassium, calcium, and inorganic phosphate.

Haemoglobin was estimated by the Haldane carbon monoxide method, plasma proteins and non-protein nitrogen by micro-Kjeldahl digestion and nesslerisation (Wong; *cf.* Peters and Van Slyke, 1932), potassium by the micro-cobaltinitrite method of Kramer and Tisdall (*cf.* Peters and Van Slyke, 1932), calcium by the method of Kramer and Tisdall (*cf.* Harrison, 1930), sugar by Hagedorn and Jensen's method (Peters and Van Slyke, 1932), chloride by Sendroy's method (1937), and inorganic phosphate by the method of Obermer and Milton (1932) adapted for use with a "Spekker" absorptiometer.

Kymographic tracings of carotid blood pressure and respiration were obtained on cats anaesthetized with sodium barbitone, respiration being recorded by Gaddum's technique (1941), which measures the volume of air breathed in litres per minute.

Observations of the clinical effects of MFA were made on both spinal and decerebrated cats, and on a monkey, and kymographic records were obtained of the knee-jerk in spinal, decerebrated and chloralosed cats by means of an electrically operated patellar hammer; in the spinal cat the action of MFA on the threshold stimulus of the flexor reflex (tibialis anticus) was also investigated (Sherrington and Liddell, 1929).

A series of rats were given electrical convulsions on two successive days, and the electrical convulsive threshold was again measured one hour after a subcutaneous injection of MFA. The apparatus used for human electric convulsive therapy was employed (Golla, Walter, and Flemming, 1940).

Finally, various therapeutic measures were attempted, both before and after administration of MFA, and before and after onset of convulsions; dogs were mostly used for these experiments.

Results

Toxicity by injection, by mouth, and by inhalation

The approximate LD50 doses are summarized in Table I. It is fully realized that the figures

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for guinea-pig, cat, dog, goat, and monkey are based on evidence from an inadequate number of animals and do not give an accurate assessment of the LD50 doses; the data, however, are sufficient to indicate a marked species variation in toxicity without the more wholesale sacrifice of experimental animals which would have been involved in arriving at more accurate figures.

TABLE I

TOXICITY OF MFA

Animal	Dose mg./kg.	Oral			Subcutaneous		
		Mortality	%	Approx. LD50 mg./kg.	Mortality	%	Approx. LD50 mg./kg.
Mouse	15				9/10	90	
	10				9/10	90	
	8				10/10	100	
	7	5/5	100				
	6	2/5	40	6–7	10/10	100	5
	5	0/5	0		5/10	50	
	4				0/10	0	
	2				0/10	0	
	1				0/10	0	
Rat	6				10/10	100	
	5	5/5	100		20/20	100	
	4	5/5	100	3–4	20/20	100	
	3	1/5	20		10/10	100	2–3
	2	0/5	0		2/10	20	
Guinea-pig	5	1/1	100		1/1	100	
	3	1/1	100				
	2	1/1	100		1/1	100	
	1	1/1	100		2/2	100	
	0.6	0/1	0				
	0.5			0.4	1/1	100	0.2
	0.4	1/1	100				
	0.25				1/1	100	
	0.1				0/2	0	
Rabbit	10	2/2	100		2/2	100	
	5	6/6	100		4/4	100	
	4				2/2	100	
	2	2/2	100	0.5	6/6	100	
	1	2/2	100		4/5	80	0.5–1.0
	0.5	1/2	50		1/4	25	
	0.2				0/1	0	
Cat	10	1/1	100				
	1	1/1	100				
	0.1			<0.3	1/1	100	0.3
	0.3	1/1	100		1/2	50	
Dog	1.0	1/1	100				
	0.3	3/3	100		5/5	100	0.1–0.2
	0.1	1/3	33	0.1–0.2	1/1	100	
	0.05	0/1	0				
	0.02	0/1	0				
Goat	3.0	1/1	100		2/2	100	
	1.0	1/1	100	<1.0	2/2	100	<1.0
Monkey	12	2/2	100		1/1	100	
	10	1/2	50		0/1	0	
	3	0/1	0	10–12			10–12
	1.5	0/2	0				

One horse was injected with 1.5 mg. MFA/kg., which proved fatal.

The toxicity by inhalation was investigated more fully in the rat and the mouse than in other animals; the LD50 for rats was 450 mg./cu.m. for 5 min. and for mice above 1,000 mg./cu.m. for 5 min.; 332 rats and 280 mice were used.

Skin absorption is of little importance as no deaths occurred in a small series of guinea-pigs with doses up to 100 mg./kg. on the plucked skin of the abdomen.

Pharmacological effects

There is some difference in detailed behaviour of the species examined, after poisoning with MFA, just as there is a difference in lethal dose, but for the sake of brevity these will be described in groups.

(*a*) *Mice, rats, and guinea-pigs.*—After a lethal dose by injection there is a delay of about 15 minutes to 2 hours before the onset of symptoms. The animals then become quiet and limp and at the same time rather apprehensive; a stage of hyper-excitability follows, when the animals may jump a foot or more or rush wildly around their cage in circles; tonic convulsions then occur with intervening periods of dyspnoea and flaccidity. Repeated fits are usual and the animal may die either during a fit or in the flaccid interval.

(*b*) *Rabbits.*—These show a similar period of delay, followed by progressive muscular weakness with gasping infrequent respiration. Convulsions start suddenly and death soon occurs.

(*c*) *Cats.*—After a lethal dose (1 mg./kg.) there is a period of about 100 minutes before the onset of symptoms, which are initiated by retching and vomiting, even after injection. The paretic stage follows with acceleration of respiration, incontinence, inco-ordination, and inability to move the limbs. Eye movements are normal, pupils equal but large and they react briskly to light. Pinna and conjunctival reflexes and knee jerks are brisk and there is some knee clonus. Convulsions develop suddenly after about 150 minutes. These tonic fits occur at intervals of about 5 minutes and finally death ensues in about 230 minutes. The heart continues to beat feebly after respiration fails.

(*d*) *Dogs.*—The latent interval is only about 30 minutes after 0.3 mg./kg. A quiet period is followed by hyper-excitability with loud barking and wild, inco-ordinate, impulsive activity, associated with incontinence of urine and faeces. Retching and vomiting may occur before this stage. The excitable stage suddenly merges into

one of convulsions. At first tonic and extensor, with dilated pupils and brisk reflexes, later the fits are clonic, with champing of the jaws and inco-ordinate arrhythmic running movements of all four limbs. After a brief respite with heavy panting, the whole pattern is repeated at intervals of 1–2 minutes until death occurs, once again from respiratory failure.

(*e*) *Goats and horses.*—These animals show a latent period followed by weakness, collapse, tachypnoea, cold sweating in the horse, a marked fall in temperature, rapid pulse and death from respiratory failure. In neither animal were convulsions seen.

(*f*) *Monkeys.*—The monkeys (*Macacus Rhesus*), though less susceptible, are most interesting. After a latent interval of about one hour, with a dose of 10 mg./kg. by mouth, they become quieter, not taking much notice of their surroundings. Retching and then vomiting, even after injection of the poison, are usual. In about an hour the pupils are dilated. After 70 minutes one such animal was sitting up with pallid face, its head turned looking over the right shoulder. It defaecated incontinently and after a few minutes its head was rotated again to the side and there were coarse jerky tremors of the head. These symptoms followed at intervals of a few minutes and conjugate deviation of the eyes was seen in conjunction with the head rotation. It appeared very dazed and suddenly fell over and started convulsions, which were tonic at first and then clonic. It looked strangely round and made no attempt to escape after this convulsion. A similar seizure was seen in a few minutes, initiated once again by head rotation and conjugate deviation of the eyes to the right side. An asymmetrical spread of the convulsion to the right side was followed by a generalized tonic fit, succeeded by clonic spasms. During the asymmetrical stage there was a one-sided risus sardonicus, slight head retraction, glabellar spasm and the knee jerks were accentuated. This spasm of the frontalis muscle was a prominent symptom accompanying the upward and outward eye deviation. The tonic stage usually lasted 30–40 sec. and the clonic 2–3 min. and these were succeeded by a relaxed phase when the animal appeared semi-conscious and exhausted, even prostrate. Repeated convulsions may progress to full unconsciousness and death, or there may be a cessation of fits followed by a slow recovery, the animal finally sitting up in an inco-ordinate and dazed manner. After some hours it will seem little the worse, having regained full activity and appetite. During the whole of this period monkeys are silent, but their expression conveys firstly an apparent headache, then fear, anxiety, and distress, and finally loss of appreciation of their plight.

Comparison of MFA with other convulsant drugs

A detailed comparison was made of the effects of MFA with those of nicotine, picrotoxin, strychnine, and leptazol in intact cats, monkeys, and rats and it was quite clear that MFA resembles leptazol more closely in action than it does nicotine or strychnine, but its effects develop much later and last much longer. Electrically induced convulsions in the rat were identical in appearance with the MFA tonic fit.

Cumulative action

MFA is apparently not entirely excreted or detoxicated within 24 hours, and if doses below the convulsant threshold are given daily to dogs some cumulative effect is seen.

Dog 28, wt. 5.9 kg., was given 0.025 mg./kg. (1/4 of the lethal dose) by mouth for five days. It was completely unaffected until after the fifth dose, when convulsions started and death ensued.

Administration of bigger sub-convulsant doses, however, can continue with impunity on alternate days or less frequently, and either the animal is unaffected or death occurs if the dose is raised to the lethal. This again suggests that there is little tolerance to repeated and increasing doses.

Dog 33, wt. 9.1 kg., was given 0.025 mg./kg. every third day by mouth in 30 days without any symptoms at all.

Dog 63, wt. 12 kg., was dosed as shown in Table II and death occurred after the usual lethal dose of 0.1 mg./kg. had been reached.

TABLE II

Date	Dose, mg./kg.	Dose, mg.
22 June	0.05	0.6
24 ,, ...	0.05	0.6
26 ,, ...	0.05	0.6
28 ,, ...	0.05	0.6
30 ,, ...	0.08	1.0
2 July	0.08	1.0
5 ,, ...	0.10	1.2
Total ..	0.46	5.6

Post-mortem appearances

In the animals dying after a long series of severe convulsions, signs of asphyxia were found. Often the animal died in the position of a tonic extensor fit with rigid limbs and tail, bulging livid eyes and cyanosed tongue. The blood was thick and dark in colour and the veins and the

TABLE III

Time	Condition	Hb%	g./100 ml.	mg./100 ml. plasma				
			Plasma protein	Non-protein N	K	Ca	Cl	Inorg. PO_4
Before	anaesthetized	64.5	6.0	25	18.7	10.2	387	3.6
90′	preconvulsive	89.0	6.8	25	22.6	8.4	406	2.45
125′	after convulsions ..	103.0	6.9	30	47.8	12.8	414	6.40

right side of the heart were distended. The ventricles were usually contracted. All the organs were congested and dark, especially the liver, but the spleen was small, dark, and contracted. The lungs were bluish, showing congestion, areas of collapse, and emphysema, and the bronchi often contained frothy fluid, but there was no oedema. Sometimes petechial haemorrhages were seen in pleura and endocardium. The brain and meninges were usually congested. Goats did not show convulsions and there was little to find except congestion. The kidneys and other organs did not reveal anything of interest.

Effect on the blood chemistry

Blood sugar.—It was suggested that MFA might cause hypoglycaemic convulsions, but investigation of two rabbits and a dog after large doses, many times lethal, revealed if anything a rise in blood sugar, as one might expect in a condition associated with convulsions and hypoxia.

Table III shows the effect of MFA (2 mg./kg.) by subcutaneous injection in a dog under light nembutal anaesthesia.

It was thought that the considerable rise in serum potassium might be due to the convulsions and asphyxia, and so similar investigations were made on two goats without an anaesthetic (Table IV).

The rise in non-protein nitrogen and serum potassium was significant in each experiment, but only as a terminal event. In the dog the greater rise was probably due to the severe muscular convulsions. Calcium showed a slight fall in the goats, but this was not sufficient to cause tetany. The considerable haemoconcentration noted in all animals was not accompanied by an alteration in plasma proteins, and it might be explained by an outpouring of erythrocytes from the spleen, which at autopsy was always empty. Again, in the goats there was a considerable rise in inorganic phosphate.

Effect of MFA on decerebrated and spinal animals

Decerebrated cats after 5 mg./kg. I.V.—Extensor rigidity was enhanced; the forelimbs were hyperextended and the head retracted. Respiration was stimulated in rate and depth. Convulsions began within 25 min. of injection, continuing at intervals. Swallowing movements were seen after 25 min., followed later by retching. Respiration began to fail, but by applying artificial respiration the heart was kept beating for several minutes.

Spinal cats after 4 mg./kg. I.V.—After 60 min. all reflexes were more brisk and the knee jerk was followed by irradiation of stimulus until finally convulsions started. Pinching of the foot-pad stimulated a mass reflex and then convulsions, and these continued, clonic and occasionally tonic, intermittently

TABLE IV

Time	Condition	Hb%	g./100 ml.	mg./100 ml. plasma				
			Plasma protein	Non-protein N	K	Ca	Cl	Inorg. PO_4
GOAT 506								
Before	normal	56.5	6.8	27	17.2	9.9	309	3.72
40′	collapsed and dyspnoeic	66.0	6.1	31	19.1	9.4	338	6.75
110′	distressed and tachypnoeic	68.0	6.4	31	18.8	9.1	328	7.65
160′	moribund	72.3	6.5	36	25.6	9.0	328	8.75
GOAT 507								
Before	normal	66.0	6.8	35	14.1	9.8	364	4.80
40′	collapsed and dyspnoeic	77.5	6.2	35	17.3	9.1	356	6.55
110′	moribund	82.0	6.5	39	22.0	8.6	366	8.30

until the heart stopped three hours after injection.

In another cat given 2 mg./kg., spontaneous movements of the forelimbs and twitching of the dartos muscle and penile retraction were seen.

Spinal monkey.—After an initial phase of spinal shock lasting 2½ hours, MFA was given, and during the course of 4½ hours a total dose of 100 mg./kg. (10 times the normal intact lethal dose) was injected.

Irradiation of excitor stimuli was more pronounced. Chest stimulation by tickling evoked movements of the limbs and trunk, and gentle upward pressure in the lumbar region with two fingers caused active contraction of abdominal muscles to take the weight of the lower half of the body. The tail twitched continuously. Later there were spontaneous twitching movements of the rear toes, but no actual convulsions occurred in this preparation as in the spinal cat.

Blood pressure and respiration of anaesthetized cats

Doses of MFA lethal in 18 hours, i.e., 0.3 mg./kg., had no effect on the B.P. or respiration, but when the dose was increased to 2.9 mg./kg., there was a small but gradual rise in pressure. Respiration was stimulated and the volume in litres per minute increased, but after a total dose of 9 mg./kg. it became gasping in type, getting slower and more spasmodic.

Comparison was also made with acetylcholine (Fig. 1) before and after atropine (both muscarine and nicotine effects). After atropine 5 mg./kg. MFA had no immediate effect on either B.P. or respiration, but a very large dose (50 mg./kg.) produced an immediate stimulation of respiration and a rise in B.P. similar to that produced by a large dose of acetylcholine. The animal soon began to vomit and respiration ceased; the heart continued beating for about 5 min. more, while the blood pressure fell rapidly.

Knee jerk

Chloralosed cat (Fig. 2).—There was increased extensor tone and the jerk was brisker and the lever did not return to its original base line. Later the reflexes diminished for a while and the animal started generalized convulsions and respiration failed.

Decerebrated cat (Fig. 3).—Patellar stimuli evoked irradiation of reflex responses, but the knee jerk was not apparently increased. Extensor rigidity was accentuated. Convulsions started and respiration failed.

Spinal cat.—The knee jerk suddenly increased for three or four patellar stimuli, and then further records were impossible as generalized convulsions supervened.

Effect on flexor reflex of the spinal cat (Tibialis anticus contraction via reflex arc of popliteal and peroneal nerves) (Fig. 4).—After injection of 2 mg./kg. MFA, the threshold shock was found at regular intervals. The results are plotted graphically (the distance in cm. of the coil against the time in minutes) and show that there is a progressive reduction in threshold, until finally with the coil moved away to 33 cm. (normal 24 cm.) the tibialis anticus goes into myoclonus. Further records were impossible as the preparation developed generalized tonic and clonic convulsions.

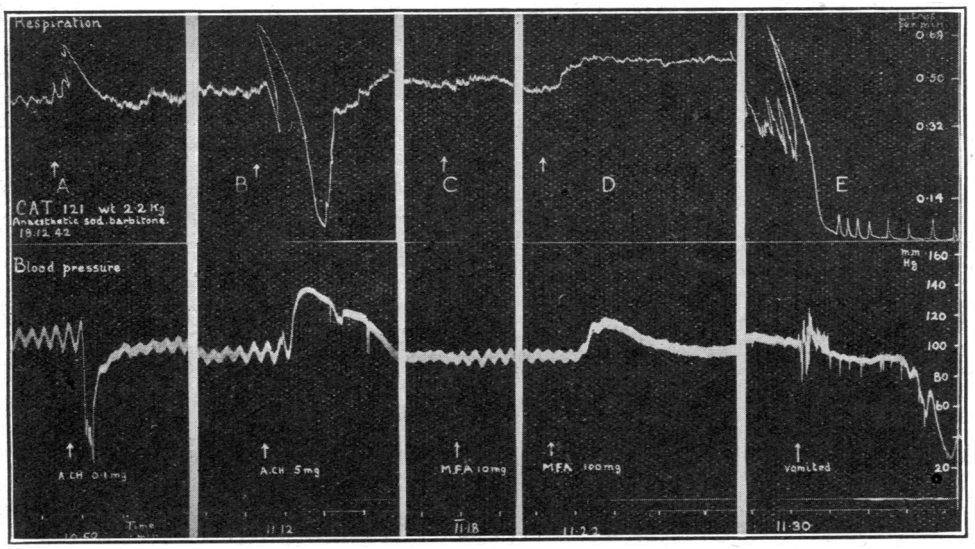

FIG. 1.—Comparison of acetylcholine and MFA on B.P. and respiration. At A, 0.1 mg. Ach; at B, 5 mg. Ach; between A and B, 10 mg. atropine sulphate; at C, 10 mg. MFA; and at D, 100 mg. MFA.

Effect on electric convulsive threshold in rats

By varying the voltage or time in 1/10 sec. the convulsive threshold was determined on two succes-

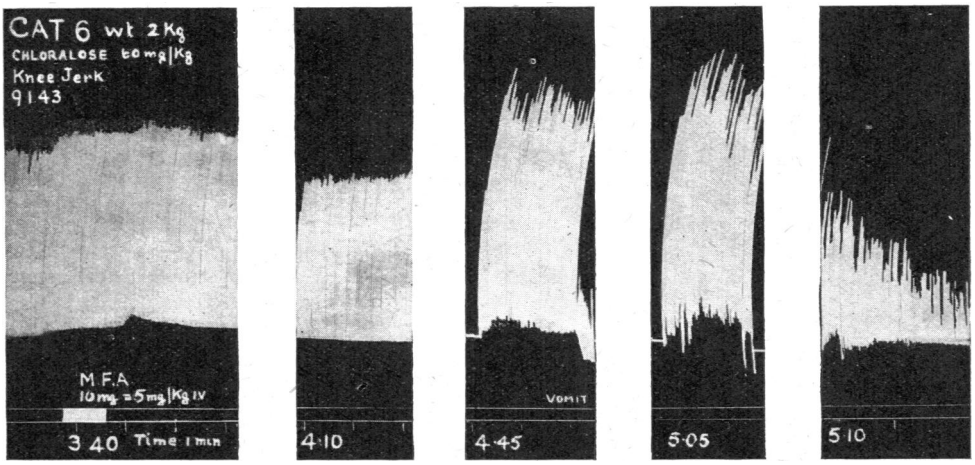

FIG. 2.—Effect of MFA on knee jerk of chloralosed cat.

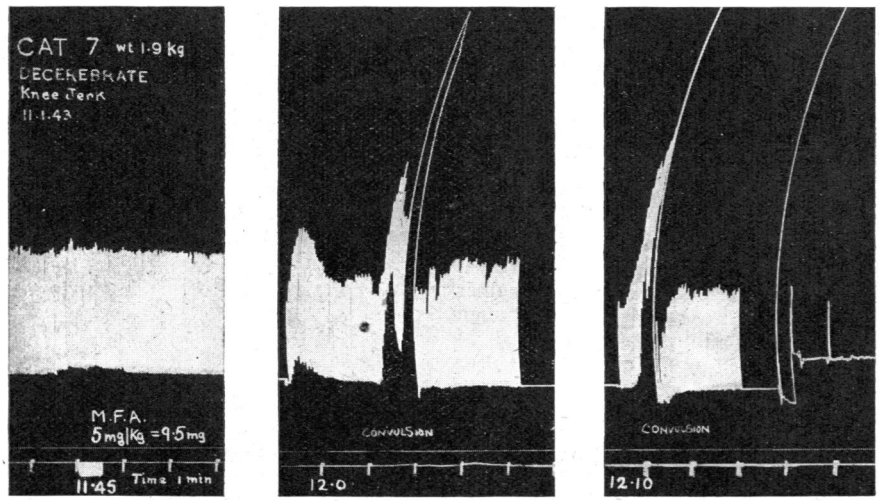

FIG. 3.—Effect of MFA on knee jerk of decerebrate cat.

sive days in 20 normal rats, and on the third day the threshold was determined again 3/4–1 hour after injection of 5 mg./kg. MFA.

The results demonstrated quite clearly that the electric convulsive threshold is reduced by at least ten times. The convulsions obtained by electric shock and MFA were identical, but the electrically induced convulsions produced in the injected rats were more severe and of longer duration.

Experimental therapy

Convulsive phase.—Dog 19, given 1 mg./kg. MFA by mouth, suddenly started convulsions after 87 min. Attempts were made to inject sodium phenobarbitone, but these failed and chloroform was administered on an open mask. Although the severity of the tonic and clonic spasms was reduced the convulsions continued and the dog died before full anaesthesia was obtained.

Preconvulsive stage.—(1) Dog 53 was given 0.3 mg./kg. MFA s.c., and 45 min. later 4 mg./kg. dilantin (sodium diphenyl hydantoin) was given by mouth. As the excitable stage had started after 110 min. 40 mg./kg. sodium phenobarbitone was injected intramuscularly. Convulsions of modified nature began in 120 min., and death followed 10 min. later.

(2) Dog 57 was injected with 0.3 mg./kg. MFA s.c., and this was followed by 40 mg./kg. sodium phenobarbitone after 115 min. at the beginning of the excitable stage. Fits started after 138 min., and another 40 mg./kg. sodium phenobarbitone was injected intramuscularly; very mild convulsions rather like gross tremors continued without any

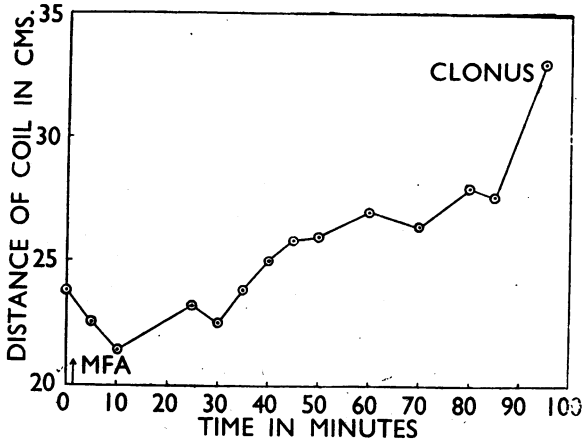

FIG. 4.—Effect of MFA on flexor reflex (tibialis anticus) of spinal cat. MFA: 2 mg./kg. s.c. Ordinates: distance of coil in cm. Abscissae: time in minutes.

tonic rigidity of the chest or restriction of respiration, but death occurred at 195 min.

(3) Dog 58, 0.3 mg./kg. MFA s.c. After 120 min. it was quiet and was given dilantin 10 mg./kg. by mouth, but convulsions started at 150 min. Then 2 mg./kg. omnopon (equivalent to 1 mg./kg. morphine) was injected intramuscularly. There was no benefit, and it died at 165 min.

(4) Dog 54, 0.3 mg./kg. MFA s.c. The first 105 min. were symptom free; 2 mg./kg. omnopon was then injected. The dog was partly narcotized at 140 min., when the excitable stage started and another dose of omnopon (2 mg./kg.) was given. Although the dog became quieter, convulsions occurred at 180 min., but were modified by the morphia. It died at 225 min.

(5) Dog 55, 0.3 mg./kg. MFA s.c. After no symptoms for 120 min., hyoscine hydrobromide (0.02 mg./kg.) was injected and repeated again at 195 min. as the excitable stage had begun. Controlled and modified convulsions started at 230 min., lasting until 275 min., when death occurred.

(6) Dog 53, 0.3 mg./kg. MFA by mouth. Thirty min. later sodium phenobarbitone (20 mg./kg.) was given by mouth, and another 20 mg./kg. again by mouth at 90 min. This treatment had effectively delayed symptoms by 6½ hours, and the dog, although sleepy, was sitting up and ate a big meal. At 7½ hours his pupils dilated and the excitable stage had begun. Another 20 mg./kg. sodium phenobarbitone was injected intramuscularly and he became quieter. The same dose was repeated again at 8½ hours although he was sleeping. No further anxiety was felt, and the dog remained semicomatose for 30 hours and gradually made a full recovery.

Prophylaxis

(1) Dog 34 was given 84 mg./kg. methylphenobarbitone (Rutonal M. and B.) by mouth on one day and 56 mg./kg. on the next day, and this was followed by 0.4 mg./kg. MFA when the dog was yawning and sleepy.

All symptoms were postponed until 6¼ hours, when the hyperexcitable stage began. He was then given 0.35 mg./kg. tubocurarine chloride intramuscularly. Modified fits followed, and continued at intervals of about 3 min. The knee jerk was still present after 20 min., but respiration failed and death followed at 6¾ hours.

(2) Sodium diphenyl hydantoin (10 mg./kg.) by mouth was given to dog 60 on two successive days, followed by 5 mg./kg. on another two days. This dosage made the dog quiet and subdued; 0.3 mg./kg. MFA was then given by mouth, but the effect of the anticonvulsant was insufficient to prevent the onset of convulsions after 4 hours. The pattern of the fits was modified; they were shorter, of normal severity, and with less relaxation in between. The dog died after 4¼ hours.

Other measures to reduce muscular excitability, such as injection of magnesium sulphate and chloride and calcium gluconate, have been ineffective in preventing death in rabbits.

Oxygen and CO_2

Two batches of 10 rats injected with 5 mg./kg. MFA were used, the one as controls and the other kept in a small chamber in a continuous stream of O_2 and CO_2 for 5 hours. All the control rats had convulsions before those treated but the mortality was as follows:

Mortality	*Treated*	*Controls*
24 hours ...	7/10	2/10
36 „ ...	8/10	4/10
3 days	10/10	7/10

DISCUSSION

Methyl fluoroacetate is an exceedingly interesting poison because unlike most others it is about as toxic by mouth as by injection. The fact that doses of 0.1–1.0 mg./kg. by mouth will kill all the species of animals tested, except the monkey, mouse, and rat, shows that it is more toxic than strychnine or nicotine and brings it into the category of aconitine and the most poisonous substances known. Lethal concentrations in food, water, or milk were not detected either by smell or taste, even by animals such as the cat or dog with very keen senses.

Investigation of its pharmacological effects in the higher mammals shows that it is a powerful convulsive poison and careful clinical observation and cinematographic analysis of its action in monkeys strongly suggests that it acts predominantly on the cerebral motor cortex and on the rest of the central nervous system to a lesser degree. These suppositions are confirmed by a

comparison of the effects of nicotine, strychnine, and leptazol (metrazol) on the monkey. Briefly MFA has almost an identical convulsive pattern with that of leptazol. Both show a similar initiation of the convulsion by an apparent asymmetrical stimulation of the premotor cortex, demonstrated by conjugate deviation of the eyes and head rotation followed by a spread, at first unilateral and confined to the same side of the body, and then becoming generalized.

MFA however differs very much from leptazol in some respects; whereas the effect of leptazol is immediate, occurring within 5–9 sec. after intravenous injection, that of MFA is delayed for some 15–20 min. Further, leptazol usually produces one fit only and is rapidly destroyed in the body, whereas MFA almost invariably causes repeated convulsions either close together, lasting about 15 min. and followed by death, or more widely spaced and usually followed by recovery. The lethal dose by mouth and by injection is practically the same and the toxic symptoms appear after a similar time, whether it is given by mouth or by injection. Even intravenous injection has little effect on the time interval.

Investigation of the smaller animals led the Cambridge workers to compare its action with that of nicotine, and in some animals, even in some monkeys, the depressive action is sufficient to support this view; but whereas nicotine depresses the knee jerk in monkeys MFA does not and may even increase it. Again, MFA has a nicotine-like effect, raising blood pressure after big doses, but no effect after doses which produce convulsions and even death.

At first we thought its action was like that of strychnine; the type of convulsion in the rat was similar, and in the preconvulsive excitable stage in the cat, auditory stimuli demonstrated a nervous hyper-sensitivity. In the monkey, however, a real difference between strychnine and MFA was seen, convulsions being generalized and symmetrical with the former and asymmetrical to start with in the latter and almost Jacksonian in pattern. In the rat the convulsion is predominantly tonic and identical with that produced by electric shock.

In the monkey the repeated and severe tonic and clonic seizures, together with the conjugate deviation of the eyes, very strongly suggest a resemblance to the condition of status epilepticus in man. Evidence in man confirms this belief, and in fact repeated and severe fits indistinguishable from status epilepticus are observed.

Although MFA is a powerful convulsant in most animals, suggesting possibly that death is due to exhaustion and cerebral anoxia after repeated convulsions, there is considerable evidence against such a simple cause of death. Goats and horses do not convulse, and observation of these and other animals shows that death is due to a progressive failure of respiration, the heart continuing to beat for some minutes after breathing has ceased. In addition there is considerable haemoconcentration, but as there is no alteration in plasma proteins or evident oedema it is likely that this is chiefly due to contraction of the spleen and mobilization of red blood corpuscles from other depots. Many of the animals become very cold with apparent peripheral circulatory failure, this considerable loss of body heat being well marked in the horse. Further investigation on this line may possibly reveal some central effect on the heat regulating centre or mechanism. Changes in blood chemistry reported here are based on a very small number of animals only, but they reveal nothing which might be of value in the interpretation of the mode of action or in possible treatment.

If MFA were used as a poison for rabbits or rodents and other mammalian pests, there would always be the risk of accidental poisoning in man, especially as it is tasteless and odourless. With the incomplete evidence of its action available, but with the knowledge of the symptoms and probable cause of death, various therapeutic measures have been attempted. The difficulties are great: the first indication of poisoning in man is the onset of epileptiform convulsions after an initial period of nausea and mental apprehension; treatment then amounts to very urgent measures to control status epilepticus, and to prevent the developing depression of respiration; there is considerable danger that active attempts to control convulsions by drugs, which themselves may be depressive to the respiratory centre, will only accelerate death.

There is some evidence that if sublethal or subconvulsive doses are taken by mouth at daily or less frequent intervals it has a cumulative effect, and this is confirmed by Kilby and Kilby (1943) for inhalation by guinea-pigs, but they suggest that in rats the reverse is true, and that rats develop tolerance to repeated exposure to small doses by inhalation.

The most effective drug for raising the threshold of convulsions in epilepsy and electrically induced fits in man (Hemphill and Walter, 1941) is sodium diphenyl hydantoin (dilantin). According to Golla (1943) this drug does not alter the explosive motor discharge from the cortex in epilepsy, but prevents its radiation through the central nervous system, unlike sodium luminal, which is thought to reduce

the actual motor discharge in the cortex and not to influence the spread. Unfortunately dilantin has a *p*H of 11.4 and is only administered by mouth, the full effect taking about 6 days to develop ; therefore it is quite ruled out as a method of treatment for poisoning with MFA.

Sodium phenobarbitone or sodium luminal can be injected intramuscularly or intravenously ; one dog survived three lethal doses of MFA when the barbiturate was administered repeatedly, starting 30 min. after the animal had swallowed the poison and before the onset of toxic symptoms ; convulsions were prevented and yet fatal depression of respiration did not ensue.

The application of intravenous anaesthetics has to be pushed to full doses in order to produce anticonvulsant effects, because animals, even under chloralose or nembutal anaesthesia, still develop convulsions when MFA is given.

So far, in dogs, which were the most convenient and the most susceptible animals, no method of treatment has been effective once the convulsive phase or epileptiform state has been reached, and the technical difficulties of therapy, involving intravenous injection into a dog in convulsions, limit other possible lines of treatment. However, reviewing one's present knowledge of MFA the following lines of treatment for man are suggested:

1. Early intravenous injection of a rapidly acting anaesthetic such as pentothal sodium or evipan sodium followed by

2. intramuscular injection of a more prolonged acting cortical depressant, such as sodium phenobarbitone or sodium luminal, or rectal avertin ;

3. very careful supervision of respiration supplemented by adequate oxygen therapy with BLB mask and use of Bragg-Paul and/or Eve methods of artificial respiration ;

4. possible use of intravenous hypertonic glucose as in status epilepticus ;

5. careful use of tubocurarine chloride to control convulsions.

Summary

1. The toxicity of MFA by mouth and subcutaneous injection has been determined for a variety of animals. There is considerable variation of dosage from 0.1 mg./kg. in the dog to 10–12 mg./kg. in the monkey and the order of decreasing susceptibility is: dog, guinea-pig, cat, rabbit, goat, and probably horse, rat, mouse, and monkey.

2. The toxicity by inhalation for the rat and mouse has been investigated more fully than for other animals: the LD50 for rats is 450 mg./cu.m. for 5 min. and for the mouse above 1,000 mg./cu.m. for 5 min.

3. The pharmacological effects of this substance by mouth and by injection in all the animals investigated are described. In most animals it is a convulsant poison and causes progressive depression of respiration. It is toxic by inhalation, injection, and by mouth, but not when applied to the skin.

4. The effect of MFA is compared with those of nicotine, strychnine, leptazol (metrazol), picrotoxin, and electrically induced convulsions in the rat, cat, and rhesus monkey. The convulsive pattern is considered to be similar to that of leptazol.

5. Post-mortem findings are briefly described, but little is found except signs of asphyxia.

6. In a small number of rabbits, dogs, and goats estimations have been made of blood sugar, haemoglobin, plasma proteins, non-protein nitrogen, and serum potassium, calcium, chloride, and inorganic phosphate. Apart from the terminal rise in non-protein nitrogen and potassium, blood changes include a rise of 20–60 per cent in Hb, up to 90 per cent in blood sugar, 70–130 per cent in inorganic phosphate and a less significant rise in serum potassium.

7. MFA, like leptazol, acts on the whole central nervous system, but the higher centres are more sensitive than the lower.

8. Graphic records show that M.F.A. stimulates the rate and volume of respiration and then causes failure of respiration, probably central in origin. Blood pressure is little affected by small doses, but very large doses have a nicotine-like action.

9. MFA appears to accentuate the knee jerk until irradiation of the stimuli is so facilitated that convulsions occur.

10. Nervous conduction in the reflex arc of a spinal cat is increased and the threshold stimulus lessened.

11. In rats the electric convulsive threshold is reduced about 10 times by MFA.

12. As MFA is both a powerful convulsant and a respiratory depressant, the difficulties of treatment are stressed, but suggestions for treatment in man are made.

13. Since MFA is about equally toxic by mouth and by injection, and is not readily detected or destroyed, it presents a serious hazard as a food and water contaminant if used as a poison for rodents and other vermin.

The chemical estimations of plasma proteins, non-protein nitrogen, calcium, and potassium were made by Dr. F. C. Courtice, and estimations of chloride and

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