BRITISH MEDICAL JOURNAL

LONDON SATURDAY NOVEMBER 29 1952

THE PUZZLE FOR THERAPY IN FLUOROACETATE POISONING*

BY

Sir RUDOLPH A. PETERS, *M.C.*, M.D., F.R.C.P., F.R.S.

Whitley Professor of Biochemistry, University of Oxford

I have chosen to commemorate Bertram Louis Abrahams in this lecture by discussing one aspect of a problem upon which my colleagues and I have been working for some years. Though the field is therefore somewhat narrow, and though I cannot give to-day more than a progress report, I hope to show how biochemists can approach a therapeutic problem, and I felt that it would present the opportunity of alluding to a side of biochemistry (chemical physiology) which is of interest to clinical medicine and which has not yet received much attention in previous Abrahams lectures.

In this instance it seems easier to proceed from the particular to the general, and I shall therefore leave my general remarks until the end. It will make the biochemistry more intelligible to those unable to devote much time to reading it if I first make a few general remarks about aspects of modern biochemistry with which I shall have to deal.

Intermediary Metabolism

In the last ten years a great change has come over the subject of intermediary metabolism. It seems that at last, even if it be still on the horizon, a biochemical geography of the tissue cell can be envisaged in which the enzyme reactions concerned in degrading substances like sugars and fats can be tied to definite histological structures in the cytoplasm. I have been specially interested in these new developments because they have been much concerned with the oxidation of pyruvic acid, an intermediate stage in sugar metabolism, which I have studied with colleagues for many years, particularly in brain tissue. By 1939 we had realized that we were dealing with an organized complex of enzymes in this final metabolic stage (Banga *et al.*, 1939); and I felt that some structure was involved, though it appeared to H. M. Carleton and myself then that this was likely to be the nucleus (cf. Brachet (1950) for modern views on the nucleus).

The new developments indicate that it is a reasonable hypothesis to suggest that the enzymes concerned are in or closely associated with the mitochondria (Hogeboom *et al.*, 1948; Lehninger, 1951; Judah, 1951); in fragmented cells they centrifuge with the heavier parts of the cytoplasm and therefore are more strictly defined by calling them the "larger granules." For the purpose of this lecture I will call them mitochondria: part at least of the varying biochemical behaviour of tissue preparations can be explained by considering how far the mitochondria have remained intact in course of preparation of the tissue. For instance, normally in liver they are sausage-shaped (2–5 μ long, 0.5 μ wide) and stain with Janus green, and this is still their appearance if the tissue preparations are kept in strong sucrose solutions. A change of medium to phosphate-KCl leads to an alteration to a more globular shape, with loss of staining capacity; the structure behaves as if enclosed in a semipermeable membrane. It has been calculated (by Claude, 1949) that a mitochondrion in mammalian liver could accommodate at least one million protein molecules. There is therefore ample room for several sets of enzymes concerned with quite complicated metabolic changes.

It is a modern view that both carbohydrates and fats are reduced in the course of metabolism to fragments containing two carbon atoms. In the higher animal, for some reason that no one understands, this two-carbon acetyl-like fragment is not oxidized directly. Instead, it is condensed with a four-carbon acid (oxaloacetic acid) by an enzyme (known as the condensing enzyme) to form an acid with six carbon atoms—namely, citric acid (see Fig. 1). The existence of this condensing enzyme is certain, as it has now been isolated and crystallized by Stern *et al.* (1950). Once obtained, the citric acid is successively transformed via *iso*citric acid to a five-carbon and then a four-carbon acid again, with the elimination of the original two-carbon fragment as CO_2 and H_2O; the four-carbon acid so formed is ready then for the condensation of another two-carbon fragment. This cyclical process has been known as the tricarboxylic acid cycle. It had roots in earlier work, but the final form is due to Krebs (1950), of Sheffield, who saw that work by Martius and Knoop could be fitted to that of Toennisen and Brinkman and of Szent-Györgyi to form a whole.

There are strong reasons for thinking that the battery of enzymes needed for this purpose exists within the mitochondria; again, it has been calculated that there might be some 2,000 enzyme-organized units in the mitochondria: such figures are quoted only to orientate one's thought. This intrusion of citric acid as a stage in normal metabolism is most interesting; for 40 years, since the work of Thunberg, and of Batelli and Stern, it has been known that enzymes existed in the animal capable of dealing with it, and about 10 years ago it was found to be a constituent of bone and skin (Dickens). In this lecture I am not really concerned with history; considering the enzymic development as a whole, it is satisfactory to realize that the work of F. G. Hopkins (especially with W. M. Fletcher) had much to do with the earlier development, as also Garrod's conception of the enzymic cause of inborn errors; and other British work has continued their stimuli. Much credit must

*Bertram Louis Abrahams Lecture delivered at the Royal College of Physicians of London on July 3. This lecture was illustrated by lantern slides, only some of which are reproduced here.

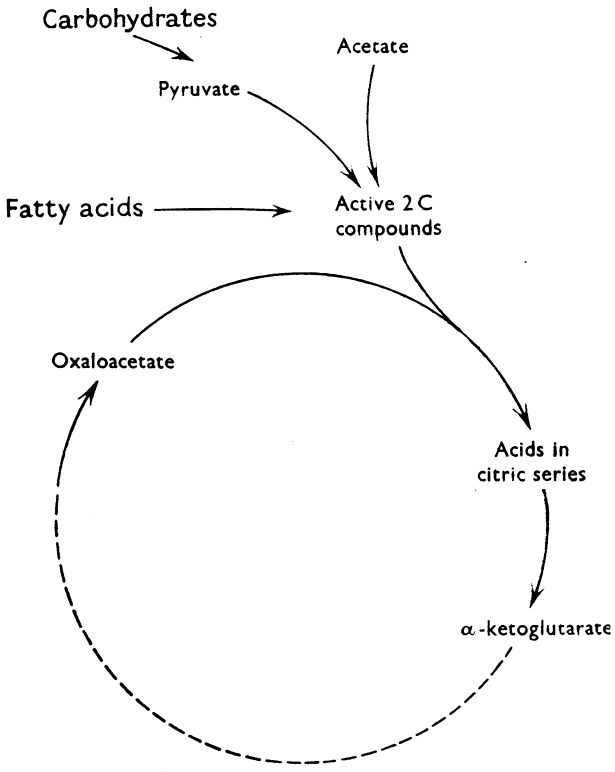

FIG. 1.—Showing the course of the tricarboxylic acid cycle.

also go to workers in Germany, Belgium, and the United States for other and for more modern developments. The idea that mitochondria were the seat of enzymic activity is an old one (Bourne, 1951). I hope to show that some of our own work has carried these concepts of the mitochondrial tri-cycle a little further by proving their existence *in vivo*. With this background, we can now embark upon the main story of this lecture.

Effect of Fluoroacetic Acid

Fluoroacetic acid ($F.CH_2COOH$) is chemically a simple substance (Swarts, 1896). It has been proposed as a rat poison (and has been known in the U.S.A. as compound 1080). About 20 minutes after giving a dose of 5 mg./kg. to a rat the animal begins to show signs of disturbance and may spread its feet out (Fig. 2). Without warning it may then suddenly convulse, and often becomes so rigid that it can be picked up by the tail. It may die in such a convulsion or in a subsequent convulsion. If this does not happen the animal becomes weak and usually collapses within the next 24 hours; this latter is probably due to heart failure.

Other animals show these signs differently; in the dog the effect is predominantly nervous, whereas in the rabbit it is upon the heart: for collected data see Chenoweth (1949). The dog is killed by 1/100 of the dose which kills the rat; this is a reason why it is not a good rat poison—for example, in a series of very carefully staged trials against rats the poison was picked up by and killed one calf, three dogs, four cats, six chickens, and 25 wild birds (Barnett and Spencer, 1949). Frogs and toads are extraordinarily resistant. Generalizing, there are two main classes of attack made by the poison in animals—an attack either upon the central nervous system or upon the heart.

Until comparatively recently, fluoroacetic acid was to some extent a chemical curiosity; but in 1944 Marais identified it with a well-known South African plant poison. According to Dr. du Toit, the director of the Onderstepoort Laboratory, this plant, *Dichapetalum cymosum* (Fig. 3), is so troublesome that large areas of the Transvaal in which it is localized have to be fenced off to prevent cattle eating the shoots, which are especially poisonous in spring and autumn.

According to Steyn (1928) signs may appear in sheep and goats as early as six hours after ingestion. The animal stops feeding and becomes uneasy. There are then nervous signs, hyperaesthesia, quivering of the muscles, exaggerated reflexes and swaying gait, and diarrhoea; it tends to lean up against walls, and finally becomes comatose and dies.

The finding of this curious substance in nature again underlines the old and well-worn saying that "there is nothing new." From the standpoint of agriculture alone it would be valuable to be able to cure these animals; but there is more than a hint, as I will mention later, that a similar poison may have been used on man. Hence it is also a human problem.

FIG. 2.—Rat in tonic convulsion due to fluoroacetate poisoning.

During the years 1941–3 fluoroacetic acid and related substances were investigated by physiologists, biochemists, and pharmacologists, as well as by organic chemists, because a knowledge of the behaviour was necessary for defence purposes. At this time it was learnt that man was, like the monkey, relatively insensitive to the poison: a particularly striking proof of this was given by Professor Adrian, who took with no harm a dose capable of killing a guinea-pig or a rabbit. The only therapy which could then be suggested was the early injection of a rapidly acting anaesthetic, followed by intramuscular injection of a cortical depressant (phenobarbitone, etc.) (Foss, 1948).

Some Curious Facts

About that time several other facts were learnt, some very curious. The C–F bond is very stable and is not taken to pieces in the body, unlike the C–I bond in iodoacetate. In a series of carbon compounds with increasing length of carbon chain there was a striking alternation of toxicity with this increase. It was somewhat like the Knoop rule for the fatty acids giving acetoacetic acid: compounds containing two, four, and six carbon atoms were toxic; those with odd numbers not so (Saunders, 1947). Furthermore,

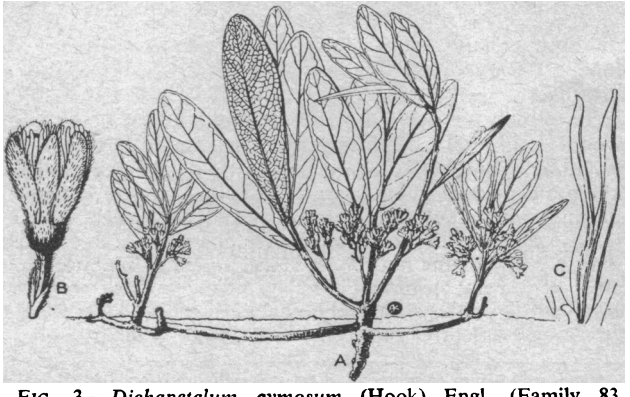

FIG. 3.—*Dichapetalum cymosum* (Hook) Engl. (Family 83. Chailletiaceae). A, plant, showing habit and underground stems; B, flower; C, bifid petal with scale at base. From Burtt Davy in *Journ. Ecol.*, Vol. 10.

fluoroacetate was quite harmless to isolated enzymes: this was especially worrying to biochemists like ourselves, who felt that in such a striking pathological condition the biochemical lesion must be enzymatic in origin, as appeared to be well proved for many arsenicals. One metabolic fact only was found by Bartlett and Barron (1947): under some conditions, in tissue slices, fluoroacetate could induce an accumulation of acetate, suggesting that it was blocking metabolism at the acetate stage.

This biochemical fact led to trial of acetate injections without therapeutic success; but similar ideas, with the addition of a suggestion derived from the fact that alcohol catalysed acetate metabolism in yeast, led Hutchens *et al.* (1949) to try the therapeutic effect of alcohol and acetate in combination and alcohol alone. This treatment is successful in some animals (mice and guinea-pigs) and not in others (dogs and monkeys). It was clear, however, that the only hope for rational progress in therapy lay in more information about the initial biochemical lesion.

Interesting Laboratory Findings

During the last five years we have been able, in my laboratory, to bring to light some new biochemical facts of interest. They may be summarized in the short statement that, though fluoroacetate is not toxic itself to any enzyme, it is converted *in vivo* by the synthetic action of tissue enzymes into a fluoro-compound which has a marked enzymic toxicity *in vivo* as well as *in vitro*. This is why, in my Croonian lecture last year, I called it a lethal synthesis (Peters, 1952). No longer, therefore, does this poisoning form an exception to the rule that a toxic condition of this nature is induced by an attack upon enzymes. This statement may now be expanded, and presented in two parts: the facts first, and then some biochemical explanations. It was found that, *in vitro*, kidney preparations poisoned with fluoroacetate accumulated relatively larger amounts of citric acid (Liébecq and Peters, 1949), and that, when animals such as the rat were given injections of the poison, even within an hour many tissues showed the accumulation of citric acid in large amounts (Buffa and Peters, 1949).

Based upon the ideas with which I introduced this lecture, a hypothesis could be constructed to account for these citric acid accumulations.* The fluoroacetate (or other FC–C fragments from longer-chain fatty acids) condensed like acetate to form a fluorotricarboxylic acid, which then blocked the further metabolism of the citric acid intermediate, so leading to the citrate accumulations (Fig. 4). A fluorotricarboxylic acid might be expected to be incompatible with an enzyme dealing with citric acid owing to its asymmetric C centres, whereas there seemed no reason why the condensing enzyme should distinguish between acetate and fluoroacetate.

At this stage it might be thought that therapy would consist in restoring the missing acid of the tri-cycle; we tried this, as did others, and an apparent temporary success was attained with the 5C acid α-ketoglutaric acid which on further investigations turned out to be wrong. These failures convinced me that we had to delve further into the biochemistry before we could decide even whether any logically designed therapy was possible.

The first task was to prove that a synthesis had really taken place. This took us much longer than we thought it would, and I will not weary you with the details of the isolation. Suffice it to say that it involved shaking ground kidneys of rabbits, guinea-pigs, or dogs in a suitable buffered medium with a substrate (fumarate) and fluoroacetate, and then separating this from the mixture in a yield of not more than 1–2 mg. of the fluoro-compound which is inhibitory to citric acid metabolism from kidneys of two rabbits. In this way, and using some modern techniques, we got some 7 mg. crystals of a fluorotricarboxylic acid about 11 months ago:

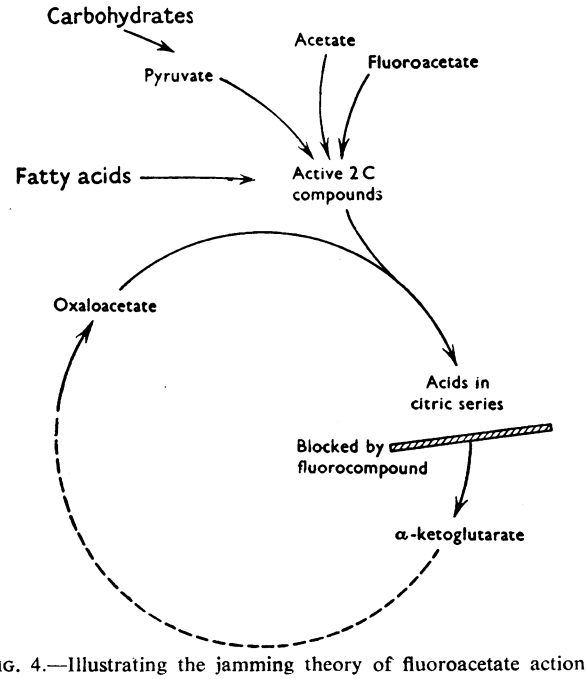

FIG. 4.—Illustrating the jamming theory of fluoroacetate action.

thereby we proved the synthesis, but we did not prove the nature of the acid (Peters *et al.*, 1952).

That it is actually one of the possible fluorocitric acids is made more likely by some recent work. D. G. A. Rivett, of South Africa, now working in the Ministry of Supply Experimental Station at Porton, has recently provided me with a specimen of sodium fluorocitrate prepared from the ester of synthetic fluorocitric acid with which our own preparation could be compared. Preliminary tests by R. Wakelin and myself upon the synthetic fluorocitrate indicate that it has about half the inhibitory activity of our isolated fluorotricarboxylic acid. This would be expected if the synthetic salt is a mixture of four diastereoisomers, two of which are active. This further evidence is strong support for the idea that our compound is actually fluorocitric acid.

The Inhibited Enzyme

The next question which arises is, What is the enzyme which the fluorocitrate inhibits? To answer this we had again to probe more deeply into the detailed behaviour of these metabolic enzymes. There are only two possible ones—aconitase and *iso*citric dehydrogenase (Fig. 5); of these, aconitase is the one attacked; no effect has ever been found on the *iso*citric dehydrogenase. Preliminary work in my laboratory (with Lotspeich and Wilson) showed that the fluoro-inhibitor could inhibit the enzyme aconitase competitively (Peters, 1952).

During the last year Dr. J. F. Morrison has spent a long time in the purification of aconitase and has got further in this than others before him. As the behaviour of the "inhibitor" to the enzyme is fundamental to the therapeutic problem, some points in his work can be mentioned.

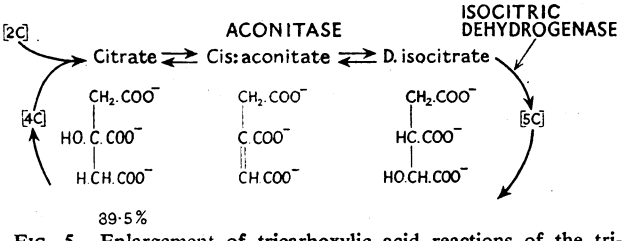

FIG. 5.—Enlargement of tricarboxylic acid reactions of the tricarboxylic acid cycle.

*This hypothesis was first advanced independently about the same time (June, 1948) by Liébecq and Peters (1949) and by Martius.

The enzyme has the peculiarity discovered in America (by Dickman and Cloutier) that it requires ferrous iron and cysteine for its activity. The new point has arisen (unpublished) that the purified enzyme is less inhibited by a given amount of fluoro-inhibitor than the cruder aconitase with which the work was done last year. The meaning of this is not yet clear; the fundamental attack upon the aconitase must involve other factors still to be understood. Therefore, though the biochemical lesion has now been found to be in the aconitase, there are details not yet definite regarding the basic mode of attack.

We now come to a curious feature of the story. We realized some two years ago that much more of the fluorocitrate (at least 10 times the amount) was needed to interfere with the isolated soluble aconitase enzyme than with the enzyme as we found it in the kidney preparations, which had been used as a guide in the isolations. This difference is clearly fundamental to our understanding of the phenomena. Going back to the remarks in my introduction, there is one great difference between the soluble aconitase enzyme preparation and that in our test kidney systems : in the latter the enzyme is still organized in the mitochondrial tri-cycle. The magnified effect of the fluoro-inhibitor might be due to the fact that it is exerted in the box enclosed by the mitochondrial envelope. In the ground kidney which we use in phosphate-KCl solution there is evidence that the mitochondria are partly damaged; in undamaged mitochondria citrate penetrates the permeability barriers with greater difficulty. I believe that it is this trapped fluorocitric acid synthesized *in situ* which is somehow responsible for the tissue-poisoning and pathological abnormalities.

Remarks About the Toxicity of the Citric Acid Itself

Turning now to the toxic signs, I have suggested elsewhere (Peters, 1952) that there are two possible reasons for the toxicity *in vivo*: (1) the interference with the supply of energy through the retardation of normal intermediary metabolism at the aconitase stage, and (2) an actual toxicity of the accumulating tricarboxylic acid, owing to its combination with calcium (or other divalent metals). Upon analogy with the motor-car, this would mean in the one case cutting off the petrol supply, and in the other perhaps a dirty sparking-plug.

In regard to (1) there is an analogous case: deficiency of vitamin B_1 blocks the pyruvate stage and can so induce convulsions in pigeons. There can be, however, considerable argument whether (2) is a contributory factor. I do not think it could be expected on physiological grounds that there would be a direct relation between the concentration of citric acid and toxic signs; such a view makes no allowance for physiological adjustment. We have to remember that the action would be on Ca^{++} *inside* cells, about which little is yet known. In favour of (2) we have the facts that toxicity is practically always preceded by an increase of citrate in the tissue, that citrate is well known to combine with divalent ions like Ca, that Sprague-Dawley rats have never survived a dose of fluoroacetate sufficient to cause the citrate concentration of spleen, heart, and kidney to exceed 1.7 mg./g. fresh tissue (Lindenbaum *et al.*, 1951), and that fluoroacetate-poisoned animals are more susceptible to injections of KCl, suggesting an alteration in ionic balance (Chenoweth *et al.*, 1951). Against (2) it may be said that intravenous calcium gluconate and magnesium injections do not cure the convulsions (Eeg-Larsen and Naess, 1951); the question is whether this Ca or Mg enters the tissue cells. Then there is certainly no direct correlation between concentration of citrate in the brain of rats and the actual onset of convulsions.

In the dog, according to Kandel and Chenoweth (1952), convulsions can occur when there is little general increase in brain citrate; the latter argument would be improved if the existence of specific increases in the lower parts of the brain could be excluded. An extreme case is presented by *Xenopus laevis*, which is abnormal in tolerating large doses of poison; this animal's heart can function normally for days with a high concentration of citrate. In this animal, as a secondary effect, Banister and Foulkes (unpublished—see Peters, 1952) have found a gradual entry of Ca ion into the poisoned heart.

Clearly, more work is wanted to settle these matters; but I shall be much surprised if the accumulations of citrate do not prove to enhance the toxic effects. Quite recently we have been able to get typical convulsions in pigeons in 12 minutes and 20 minutes respectively by injections of some 100 μg. of fluorocitrate into the subarachnoid space; in both these cases a definite rise in citrate had occurred. Since saline injections are harmless, this experiment is a particularly striking demonstration of an *in vivo/in vitro* biochemical correlation, and goes far to prove that the actual toxic agent is fluorocitrate.

Returning to the therapeutic problem, we can see that, fundamentally, what is required is to reverse the inhibition of aconitase produced by the fluorocitric acid. Theoretically, the tissues may be said to do the best they can, because the mere accumulation of tricarboxylic acids should in itself stop the inhibitory action. It would be a good thing to increase this, but we do not know yet how much tricarboxylic acid can be present at any moment in mitochondria without disrupting them. Attempts to cure poisoned rats by giving them cis-aconitate have failed, and this we can understand because even fluorocitric acid does not get into undamaged mitochondria at all easily. It is relevant to recall that experiments by Lindenbaum *et al.* (1951) have shown that with sublethal doses in rats the citric accumulations gradually disperse in 24–36 hours. Hence, the F citric must slowly get away. Our best chance is therefore at present to keep the poisoned animal alive somehow until the F citric can get away.

The fundamental therapeutic problem we have here is the inverse of that with the virus. With the virus we want to penetrate the cell wall and the virus envelope in order to attack its structure without causing permanent damage to the cell organization. To reverse fluoroacetate poisoning we want to increase the concentration of a normal cell constituent without upsetting tissue relationships. How to undo this locked biochemistry is still the unsolved puzzle.

Mitigation of the Effects of the Poison by Protection

Since we cannot yet reverse the biochemical lesion we are driven back to any possible preventive measures. The first thing which occurs to mind is whether the condensation to fluorocitric acid can be stopped. In this connexion Kilby and Carpenter, of Adrian's team (unpublished results), found in 1943 that pyruvate postponed the toxic action of fluoroacetate upon the isolated rabbit heart; more extensive independent experiments in the U.S.A. gave similar answers (Chenoweth, 1949). Though it was unknown then that fluorocitrate was formed, a reference to Fig. 4 will show that pyruvate via its C–C fragment might well compete with fluoroacetate for entry into the tri-cycle. More recently Chenoweth *et al.* (1951) have had a striking therapeutic success with glycerol monoacetate, which they introduced upon the general idea that substances yielding acetate during metabolism seemed to antagonize fluoroacetate in isolated tissue preparations. Good results were obtained in rabbits, in dogs, and even in monkeys; abnormalities of cardiac rhythm, etc., were reversed. It seems that the good results of this compound are due to its capacity for penetrating cells and producing "nascent" acetate.

As the theory of using a C–C compound for protection depends upon the idea that it prevents the toxic substance from exerting its action, it follows that, according to our jamming theory, it should stop the formation of the fluoro-inhibitor. As we were in a position to test this directly, I have recently done a series of experiments with Mr. R. Wakelin, the gist of which is here given. Turning the tables, the fluoro-inhibitor can be used as a specific agent for jamming tissue changes at the citrate stage. In this way the synthesis of citrate from various substances can

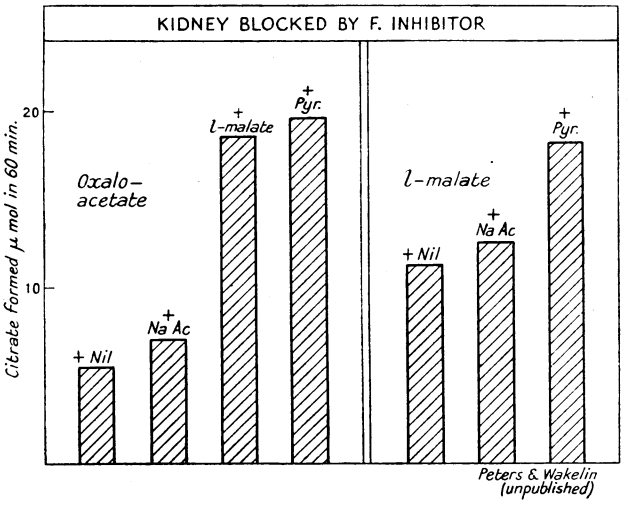

FIG. 6.—Showing the amount of citrate formed in 60 minutes by identical amounts of kidney homogenate in the presence of oxaloacetate and L-malate as substrates and with additions of sodium acetate, L-malate, and pyruvate.

be studied in the organized enzymes of the mitochondrial kidney preparations. Fig. 6 thus shows the formation of citrate from oxaloacetate, L-malate, and various other additions. The best combinations were oxaloacetate + pyruvate and malate + pyruvate. On the whole these experiments, with some exceptions, are consistent with recent work upon the role of coenzyme A and the tricarboxylic acid cycle.

Naturally I thought that synthesis of the fluoro-inhibitor would occur similarly; but, surprisingly, experiment has given a different result. By the biochemical device of blocking the kidney preparations with malonate, synthesized fluoro-inhibitor can be estimated in presence of free fluoroacetate. In this way we have found that the 4C acid which produces most fluoro-inhibitor is not oxaloacetate but L-malate. In fact, the presence of oxaloacetate decreases the yield. Fig. 7 summarizes the experimental facts. The observations are curious and may mean ultimately that we shall have to take into account alternate modes of entry (such as that shown in Fig. 8) into the tri-cycle. Especially is this likely because we have not been able to show any formation of fluoro-inhibitor from oxaloacetate + fluoroacetate by the pigeon liver preparation (Stern and Ochoa, 1949), which produces citrate well from oxaloacetate and acetate.

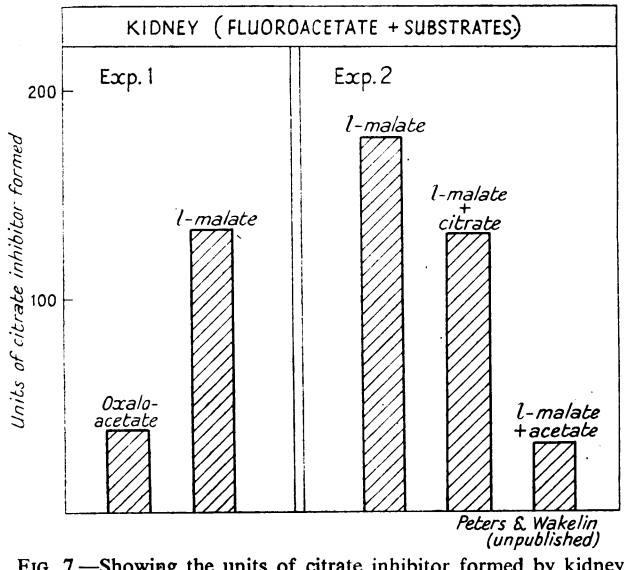

FIG. 7.—Showing the units of citrate inhibitor formed by kidney homogenates in the presence of fluoroacetate and various substrates in two experiments.

Whether our new facts can be fitted into the "old bottles," or whether they will necessitate some modifications of existing theory on the tri-cycle, must be left to the future. Meanwhile, the system makes it possible for us to study what substances prevent the entry of fluoroacetate into the cycle: both acetate and pyruvate in small amounts stop this synthesis, and, as would be expected, citrate can also inhibit slightly. It is therefore now an idea firmly based on biochemical experiments that –C–C substances, if administered early enough and with a sufficiently maintained concentration, should stop the formation of the fluorotricarboxylic acid and, as this is responsible for the effects, the toxic signs. One prerequisite *in vivo* must be penetration to the active centre (in the mitochondria), and this is evidently why the monoacetin discovery of Chenoweth and colleagues is of so much practical value.

Before coming to my concluding remarks, there is one related piece of work which is of interest.

Dichapetalum Toxicarium

Up to this point I have omitted any allusion to the poison from any other plant than the African Gifblaar. In the Sierra Leone district there is another plant of the same genus, known as ratsbane, which has been used by those euphemistically called the local doctors and which was generally believed to be responsible for "broke back," the cause of many deaths. I have alluded to this at more length in my Croonian lecture. The signs of poisoning seemed to be so like those of fluoroacetic acid that investigation was needed. Through the kindness of Sir John Simonsen, F.R.S., I have received some of the seeds, and can state upon the basis of some preliminary experiments that these contain a poison which kills rats, with production of large citrate accumulations in the tissues. Hence it may well be that this is also a fluoro-compound, and that "acetyl-like" compounds could also be used in these cases for protective therapy.

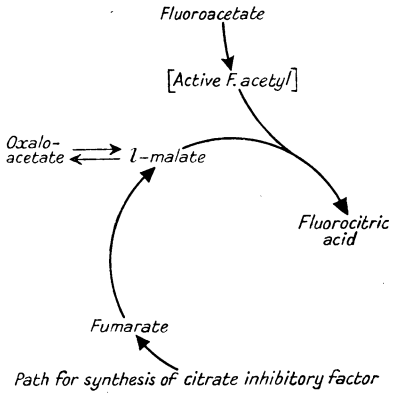

FIG. 8.—Summary of experimental facts regarding the synthesis of the fluoro-inhibitor from fluoroacetate.

Applications in Biochemistry

Dissection by the use of poisons has been employed in the past by physiologists; Langley, for instance, used nicotine to map out the autonomic nervous system. Toxic agents are now being employed by biochemists with equal success to map out the course of metabolic events *in vivo*. In fluorocitric acid we have a new inhibitor which is apparently selective for the aconitase stage of the tri-cycle. In this lecture I have been able to report for the first time the use of this inhibitor to study citrate formation in the mitochondrial organization. Already it appears powerful enough to show up the new point that L-malate may form an alternative path of entry to the classical oxaloacetate.

Some General Points

I have left the general points until the end. Summing up, I have shown how an attempt has been made in this instance to put therapy upon a logical basis by defining as clearly as possible the biochemical lesion. We have learnt that in fluoroacetate poisoning we have a unique case at present, in which a compound penetrates two permeability barriers, that of the cell and of its mitochondrial constituent, and is

then synthesized by the organized enzymes present into a fluorocitric acid which cannot during life easily leave the mitochondria. Put dogmatically, it is a case of lethal synthesis, in which the effect of the toxic agent is magnified by the structural biochemistry of the cell. It is a protective synthesis in reverse, and must be distinguished from toxic conversions, of which several examples could be quoted. From the proved reversal of arsenical poisoning by British anti-lewisite, there is no theoretical reason why an antidote should not be able to cope with the double permeability barrier, because this must happen when dimercaptopropanol removes arsenic from the cells. Unfortunately, the latter still remains the only example of a therapeutic agent developed on logical lines from biochemical principles; because, in fluoroacetate poisoning, though we now know more definitely what must be done for reversal, this knowledge has only intensified the puzzle and difficulty of reversing the biochemical lesion.

On the other hand, if we can catch it in time, we now have good biochemical reasons for the therapeutic use of substances which can prevent the entry of fluoroacetate into the tri-cycle. In so far also as citric acid accumulation is induced in the poisoning, it is a medico-legal point that we have some means of detecting the criminal use of such a substance of this type, though the estimation should be done soon after death; thus biochemistry has aided forensic medicine.

In a way, in so far as the virus grows inside the cell, it much resembles the large cell granule; hence in the case of this obscure poison attacking the mitochondria we have a somewhat close analogy to what we should like to do with the intracellular virus. In one case, indeed, low concentrations of fluoroacetate have been used to inhibit the production of influenza virus in the lungs of mice. At any rate, we know that there are agents, like chloramphenicol, which are toxic to the virus and must be able to penetrate to it. There is a valuable thought here for cancer research, which has been exploited in part by the attempt to synthesize radioactive substances which will specifically attack the cancer. It may be noted that 5-fluoronicotinic acid has been found by Hughes (1952) to stop bacterial growth by inhibiting cozymase synthesis; this may well turn out to be a case of a lethal synthesis.

No longer need therapeutic medicine be the slave of empiricism. Though the failure of the logical approach has so often in the past led to total discouragement, there must come the time when the biochemical complexities in living matter are sufficiently understood and related to the physiology to enable a start to be made. From work in the last 25 years it can be claimed that there are signs of a change based upon improved knowledge of the intracellular enzymes and their collective functioning.

I know that much of medicine must remain an art, and that it would be indeed somewhat dull if it all became a science. These biochemical analyses take a long time, I fear, and are only too apt to give the impression that the biochemist fiddles while the disease does its work. Yet I believe that each case thoroughly studied teaches us much and will accelerate our advance to the goal of logical therapy.

I wish to express my thanks to the President and Council for the great privilege of delivering this lecture. I am also grateful to the Chief Scientist, Ministry of Supply, for permission to quote work by Dr. Rivett, and to the Editor of the *Journal of Ecology* for permission to publish; and to the Editor of the *Proceedings of the Royal Society* for permission to reproduce Fig. 2.

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SUBCONVULSIVE ELECTRICAL STIMULATION IN TREATMENT OF CHRONIC NEUROSIS

BY

A. SPENCER PATERSON, M.D., F.R.C.P.Ed. M.R.C.P., Dipl.Psych.

Physician in charge of Department of Psychiatry and Lecturer in Medical School, West London Hospital

AND

ARTHUR CONACHY, M.R.C.S., L.R.C.P., D.P.M.

Dan Mason Research Fellow, West London Hospital

A certain proportion of patients seen at a psychiatric out-patient department are ill enough to require more intensive treatment than superficial psychotherapy and advice. This group includes patients with severe anxiety and psychosomatic symptoms such as tachycardia, epigastric pain, asthma, stammer, etc. Others are unable to free themselves from some antisocial habit, such as exhibitionism, while others have become ill through some frightening experience. It is often impracticable to send such cases to a mental hospital, and so some more radical form of treatment such as intense abreactive therapy may be employed. This development is largely due to the work of British psychiatrists, particularly Horsley (1943), and its use has widely spread in different countries. Sargant (1949) has described in detail the emotional outbursts which can characterize the changeover from the neurotic reaction pattern to a more normal adjustment. Many of the drugs used for this purpose, however, have certain disadvantages. Barbiturates, for instance, may merely make the patient sleepy, while amphetamine is apt to be too lasting in its excitatory effects. Treatment with carbon dioxide is often refused by the patient, as it is so unpleasant.

One of us (A. S. P.) was therefore interested to observe in the Veterans Hospital, Lyons, New Jersey, in 1950 the use of an electric current of low intensity applied to the heads of patients after light anaesthesia. In about 80% of cases the patient showed a marked emotional response, which was generally in the nature of weeping and sobbing, and only occasionally of rage (Alexander, 1950; Hirschfeld, 1950). The Reiter B machine was used by these workers, and one of these instruments was brought back to this country. This is therefore a preliminary paper reporting our findings with this treatment, which could also, however, be administered by any electronarcosis apparatus.