LXXIII. THE RELATION BETWEEN CARBOHYDRATE METABOLISM AND THE FUNCTION OF THE GREY MATTER OF THE CENTRAL NERVOUS SYSTEM.

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It appears now to be an established fact that carbohydrate degradation plays, quantitatively, a dominant part in the metabolism of the grey matter of the brain. A number of authors have shown that lactic acid is produced from carbohydrate, and that glucose, lactic acid, and a number of other substances more or less closely related to glucose can maintain the oxygen consumption of brain tissue, *in vitro*, at a high level. (For review, see Holmes [1932, 1].) Finally, Himwich and Nahum [1932] have shown that, under most conditions, the respiratory quotient of the intact brain is unity.

An important problem, however, still awaits solution; namely, whether or not the actual functional activity of the grey matter depends directly upon carbohydrate breakdown and oxidation. It is evidently possible that, while carbohydrate metabolism is, in the long run, necessary if the neurones are to maintain their normal powers of function, activity may be immediately dependent upon some other, and hitherto unidentified, process.

The activity of peripheral nerves has been intensively studied from many points of view (see review by Gerard [1932]); we must, at least for the time being, assume that observations made on peripheral nerves apply equally to the phenomena of the central tracts; and there is nothing to suggest that either nerves or tracts are more than conductors of impulses set up by some outside machinery. It therefore seems safe to assume that the grey matter is responsible for initiating impulses, either spontaneously or in response to stimuli.

There is some evidence [Holmes, 1932, 2] which suggests that the chemical events so far identified as characteristic of grey matter may occur, not in the cells, but in the network of non-medullated processes that surrounds them and makes up the bulk of the grey matter. If this is true, then impulse initiation, if it be a function of the cell bodies, may be unconnected with carbohydrate metabolism; alternatively, of course, the cell body may serve a mere trophic function, the real seat of activity being the surrounding non-medullated network.

There is one group of phenomena which shows that normal brain function depends, ultimately, upon carbohydrate metabolism, namely the train of events known as the "hypoglycaemic response." Previous work [Holmes and Holmes, 1927; Holmes and Sherif, 1932] has shown that in hypoglycaemia the brain is suffering from actual carbohydrate starvation. But little help, however, is to be gained from a study of the subjective and objective phenomena associated with the condition. Though the final stage seems to be coma and death, all

manner of manifestations, including violent convulsions, may precede this terminal event [Wauchope, 1933]. The train of symptoms is, in any case, very far from suggesting a gradual suppression of function, such as might be expected from a gradual failure in the supply of some foodstuff directly necessary for activity. Such a suppression of function does, on the other hand, occur after the administration of anaesthetics, and here the blood-sugar is commonly raised; never, certainly, reduced. Carbohydrate oxidation by excised brain tissue can undoubtedly be reduced by anaesthetics—of the experiments showing this, the most recent are those of Quastel and Wheatley [1932, 1]. The concentration of anaesthetic employed is often high, but these authors claim (in opposition to Bülow and Holmes [1932]) to have demonstrated that such reduction occurs in the brain of the living anaesthetised animal.

It seemed likely that iodoacetic acid, which has proved of such assistance in attacking the problems of muscle physiology, might be of assistance in approaching those presented by the brain. By employing this substance, it seemed that it might be possible to discover whether the functional activity of the central nervous system could persist after the production of lactic acid from glucose had been suppressed. Krebs [1931] had previously shown that iodoacetate did, in fact, inhibit glycolysis by the cerebral grey matter, and that this inhibition resulted in a diminution of oxygen uptake. Similar results, with fluoride, had previously been obtained by Holmes [1930]; Kinnersley and Peters [1930] observed a diminution in the lactic acid content of the brains of pigeons killed with iodoacetic acid. Haldi [1932] reports that the *post mortem* lactic acid production in the brains of anaesthetised dogs, killed after (sometimes by) intravenous injections of iodoacetic acid, is sometimes, but not invariably, lowered or abolished.

In the present work, the lactic acid content of the central nervous system of frogs injected with iodoacetic acid has been found to have been reduced by some 80 %. It was observed, however, that both with frogs and with mammals death occurred fairly rapidly owing to the effect of iodoacetic acid on the heart. The circulation failed (in the mammalian experiments the blood pressure dropped suddenly to zero) and the ventricles, when examined, were found to be bloodless, pale and tightly contracted. (This recalls the work of Clark Eggleton and Eggleton [1932] on the frog's heart: these authors used isolated ventricles and found that rigor was quickly caused by oxygen lack in the presence of iodoacetic acid. The heart working *in situ* would, of course, make greater demands on the oxygen supply than would the isolated ventricle.) The existence of this rapid cardiac effect complicated the technique of the experiments here to be discussed, since it was evident that a perfusion method would have to be employed. Some preliminary work has already been done with mammals, which will be continued; for the moment, however, the problem has been approached by the easier route offered by perfusion experiments with frogs.

Von Ledebur [1932] has already carried out experiments on these lines. His results will be referred to again later; for the moment it will suffice to say that he concludes that perfusion of decerebrate frogs with Ringer solution containing iodoacetic acid abolishes the reflex excitability of the spinal cord. The technique employed was in most respects similar to von Ledebur's. (His paper was seen only after the work had been in progress for some months.) The frogs were decerebrated by cutting across the skull with scissors. They were perfused through the conus arteriosus with Ringer solution. The reservoirs of fluid were placed some 20 cm. above the cannulae, and the gas escaped to the air through a layer of mercury 0·5 cm. deep, thus ensuring a very rapid perfusion. In the

earlier experiments, the fluid contained 0·01 % $NaHCO_3$; after the oxygen had bubbled through it, the p_H was 8·0. For the majority of experiments, a Ringer solution was made according to the formula of Barkan, Broemser and Hahn [1921] and saturated with a mixture containing 97·5 % O_2 and 2·5 % CO_2. The p_H, measured by quinhydrone electrode, was 7·24. When iodoacetic acid was to be added to the perfusion fluid, a ligature was passed tightly round the hind-legs of the frog so as to include all the tissues except the sciatic nerve; this prevented the muscles of the hind-limbs from being poisoned by iodoacetic acid and left them free to contract as a result of a stimulus reaching them through the sciatics.

The method of testing whether or not the cord was active gave rise to some difficulty. Electrical stimuli are unsatisfactory in such conditions, for all the tissues are soaked in Ringer solution, and a response can always be obtained by increasing the strength of the current, since the latter will, if sufficiently strong, leak directly to the muscles. Mechanical stimuli, it was felt, were too variable in their effect. The method finally adopted was to inject 1 mg. of strychnine hydrochloride into the perfusion fluid, with a hypodermic needle, through the rubber tubing close to the cannula. If the animal then responded, by extensor spasms, to a mechanical stimulus, the cord was clearly still active, and the result was taken as a positive one. If only twitches, without sustained spasms, appeared, the result was classified as "doubtful." If no movements of the hind-limbs occurred, the response of the sciatics to electrical stimulation was tested, in case there had been a block in peripheral nerve conductivity. Only occasionally, in fact, did this seem to be the case, and it could commonly be accounted for by obvious mechanical injury to the nerves. If the sciatics were responsive to stimuli, it was assumed that the function of the cord had been abolished. The method of strychnine injection is admittedly a crude one, since a result was considered "positive" if the animal showed any extensor spasms, while, in fact, some frogs obviously convulsed for longer periods and more violently than others. It was not, however, practicable to differentiate quantitatively on these lines. In addition to observations on the activity of the cord, the amount of lactic acid in the central nervous system was estimated by pooling the central nervous systems of four frogs, excised at the end of the experiment, and working them up with trichloroacetic acid and subsequently estimating the lactic acid by the method of Friedemann, Cotonio and Shaffer [1927], the copper-lime precipitation, however, being omitted in many of the experiments for a reason to be mentioned later. The results obtained have a general bearing on the problem, which may be mentioned at once.

The lactic acid content of the frog's central nervous system, determined under these conditions, depends of course on the relative speed of three processes—lactic acid production by the tissue, the diffusion of lactic acid into the perfusion fluid and lactic acid oxidation by the tissue. The results obtained make it clear that the amphibian central nervous system, unlike the mammalian, produces lactic acid from sources other than the blood-sugar. This has, of course, previously been implied by the findings of Winterstein and Hirschberg [1925]. It probably also accounts for the very slow rate at which frogs react to insulin [Barlow, Vigor and Peck, 1931]. The perfusion fluid in these experiments contained no glucose, so that any lactic acid produced must have originated from stored precursor. At the end of 1 hour's perfusion, the lactic acid content was the same as that of the unperfused central nervous system. This shows that the lactic acid which had diffused or been oxidised must have been replaced by lactic acid newly formed in the tissue. In addition, while the central

nervous systems of frogs perfused with Ringer solution containing iodoacetic acid through which hydrogen is bubbled instead of oxygen have a very low lactic acid content, those perfused with hydrogenated Ringer solution without iodoacetic acid have a content a little higher than those treated with oxygenated Ringer solution. Evidently (since in both cases diffusion will occur, presumably at the same rate) lactic acid is replaced by fresh production in the absence of iodoacetic acid, but not in its presence. In the absence of an outside supply, the precursor must have been contained in the tissue.

These experiments do not offer any actual proof of the power of the central nervous system to oxidise lactic acid; this has, however, been so frequently demonstrated that it may be taken for granted. Table I shows the results

Table I. *Lactic acid content of central nervous system of frogs, mg./100 g. of fresh tissue.*

		After perfusion (1 hour)		
	Before perfusion	Oxygenated Ringer solution	Hydrogenated Ringer solution	Hydrogenated Ringer solution and I.A.A. 1/3000
	142	167	186	26
	145	144	137	23
	174	209	187	
	126	142	168	
	112	151	117	
	206	123		
	125	125*		
	150	98*		
	134			
Mean	146	145	159	24·5

* Perfused for 1¼ hours.

referred to above. The experiments on the effect of iodoacetic acid on the function of the central nervous system and on its lactic acid content gave the following results. The effect of iodoacetic acid on the function of the cord, as judged by the appearance or non-appearance of strychnine convulsions, was first tested by perfusion with Ringer solution containing neutralised iodoacetic acid at different concentrations. A fixed time of perfusion—75 minutes—was arbitrarily chosen. It at once became clear that there was no concentration of iodoacetic acid above which function was sharply abolished. By using a number of animals, however, and by observing the percentage which reacted, by convulsing, to strychnine after perfusion with any given concentration of iodoacetic acid, it was possible to obtain quite definite results. Eight frogs were used for each experiment, and each experiment was itself performed at least twice, so that each point on the curve represents, usually, results obtained from 16 animals. The results of the lactic acid determinations were striking. Iodoacetic acid in high dilution 0·001 %, definitely decreases the amount of lactic acid present in the central nervous system. A concentration of 0·0133 % reduces it to a low level, and further increases in concentration, even to a strength of 0·2 %, fail to depress it further, so that one suspects that the small residue of acid remaining (about 12·5 mg./100 g.) must either be produced from some different source, and one not affected by iodoacetic acid, or possibly, even, may not be lactic acid at all.

At an iodoacetic acid concentration of 0·013 %, at which the lactic acid has reached its permanent low level, 25 % of the frogs still convulse, and at

0·06 % convulsions are shown by some 12·5 %. Not, indeed, until a concentration of 0·1 % is reached, does activity seem to be finally suppressed, and, in fact, with two animals, brief convulsions have been observed with a concentration of 0·2 %. These results are shown in Fig. 1 and Table II.

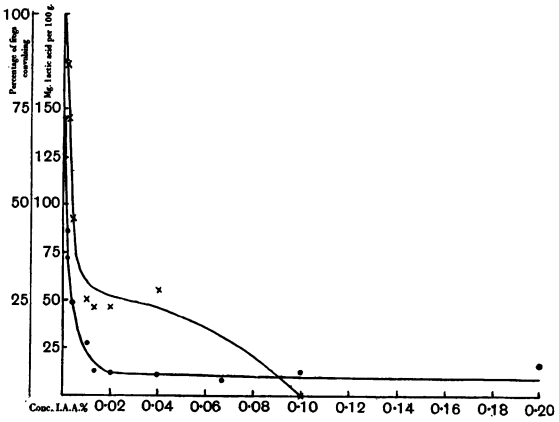

Fig. 1.

•——• Lactic acid mg./100 g. ×——× % frogs convulsing.

Table II. *Frogs perfused for 75 min. with Ringer solution containing bicarbonate 0·1 %, and saturated with 97·5 % O_2 + 2·5 % CO_2. p_H = 7·2.*

I.A.A. neutralised with sodium hydroxide.

Conc. of I.A.A.	Lactic acid mg. per 100 g.	% of frogs convulsing	No. of frogs used
0	145	100	32
0·001	86	87	16
0·002	73	73	16
0·004	48	47	16
0·01	27	25	16
0·0133	13	23	24
0·02	12	23	16
0·04	12	27	16
0·067	9	12·5	16
0·10	12	0	12
0·20	15	0*	20

* In one experiment, not forming part of this series, two frogs did show very brief convulsions after 1 hour's perfusion with 0·2 % iodoacetic acid.

Although there is a superficial agreement between the rate of suppression of lactic acid production and that of disappearance of function, as the concentration of iodoacetic acid is increased, closer scrutiny suggests that the agreement may be more apparent than real.

It is often stated [*e.g.* Quastel and Wheatley, 1932, 2] that the effect of iodoacetic acid depends upon the time during which the tissue is exposed to its action. With this in view, it was decided to see whether the effects upon function and lactic acid production could be more clearly dissociated in point of time than they could be in point of concentration of iodoacetic acid. Experiments, therefore, were performed in which the concentration of iodoacetic acid was fixed, but the frogs were perfused for 20, 40, 75 and 100 minute periods respectively. The results are shown in Figs. 2 and 3. The lactic acid is reduced, by

the end of 20 minutes, to a low level, where it remains. The reduction is somewhat greater in the presence of 0·04 % iodoacetic acid than in the presence of 0·01 % iodoacetic acid. But, in both cases, all the frogs are capable of convulsing at the end of 20 minutes; the disappearance of function is a gradual affair, and indeed, function is by no means abolished even after 75 minutes' perfusion. The rate of disappearance of function does not differ greatly with the two concentrations employed.

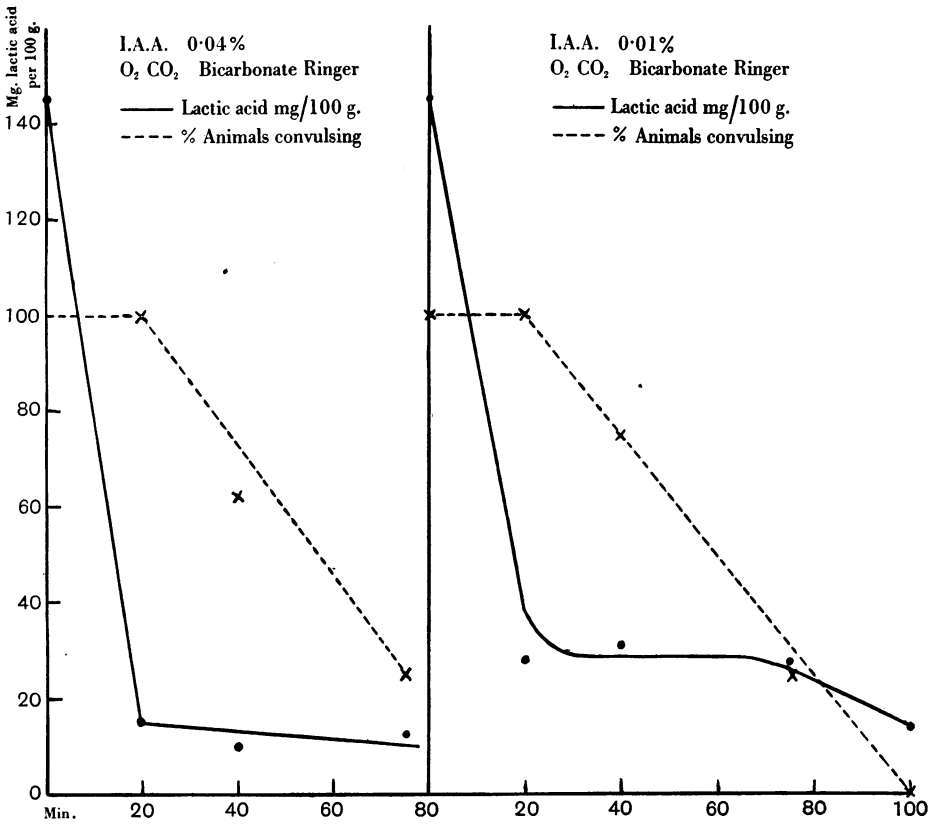

Fig. 2.

This dissociation in time seems to suggest very strongly that the two effects vary independently—in other words, however the function of the central nervous system may ultimately depend upon lactic acid formation or lactic acid oxidation, immediately it does not do so.

It must, of course, be remembered that the lactic acid estimated is that present in the central nervous system at the moment at which the perfusion is stopped, and its amount is the resultant of the three processes previously discussed—production, and removal by oxidation and diffusion respectively. The possibility, therefore, clearly cannot be ignored that, in spite of the presence of the iodoacetic acid, lactic acid is still being produced and oxidised at a rate sufficient to allow of activity being maintained. If the low figures consistently found after perfusion with iodoacetic acid do in truth represent lactic acid,

then there is ground for thinking that production and removal may still go on at a constant rate. It then, however, becomes difficult to account for the gradual and more slowly occurring failure in function. If there is enough lactic acid production and oxidation to maintain function at the end of 20 minutes, why should it not persist unimpaired for 75 minutes, since during that time there is no evident change in the level of lactic acid? A more probable explanation seems to be that function is directly connected neither with the production nor with the oxidation of lactic acid, but depends on some process which is more slowly suppressed by iodoacetic acid. The situation, unfortunately, is not clarified when one examines the effect of adding lactate to the perfusion fluid. As has just been pointed out, it is still conceivable that function may depend on the oxidative removal of lactic acid. If, however, sodium lactate is added to the perfusion fluid together with iodoacetic acid, the former in increasing concentrations, the effect of the iodoacetic acid in abolishing function is not in any way modified until concentrations as high as 0·5 % of lactate are reached. There is then a partial reversal of the iodoacetic acid effect, which, however, is evident even with high (0·2 %) concentrations of iodoacetic acid. If this result is due merely to the presence of lactate in the central nervous system, there is no obvious reason why it should not occur with much lower concentrations of lactate in the perfusion fluid, which, it is easy to show, raise the lactic acid content of the central nervous system to a marked degree. These points are illustrated in Table III.

Table III. *Perfused with O_2/CO_2 bicarbonate Ringer.* p_H *7·24.*

I.A.A. neutralised with sodium hydroxide.

Conc. I.A.A. %	Conc. lactate %	Lactic acid in C.N.S. mg./100 g.	Frogs convulsing / total used	Time min.
0·2	0	14	$\frac{1}{4}$	60
0·2	0·25	110	$\frac{4}{7}$	60
0·2	0·5	175	$\frac{2}{4}$	60
0·2	0	17	$\frac{0}{24}$	75
0·2	0·5	171	$\frac{4}{14}$	75

Feng [1932] has recently claimed that the abolition of conduction in peripheral nerve, which occurs as a result of treatment with iodoacetic acid, can be delayed by the immersion of the nerve in solutions containing lactate. He found it necessary to use 0·4 % iodoacetic acid, that is double the maximum concentration here employed. Possibly, therefore, the two phenomena may be unrelated. Moreover, in the present work, the excitability of the sciatics was tested at the end of each experiment, and the presence of a nerve block thus excluded. Gerard has, however, stressed the relative impermeability of the nerve sheath to many substances; and this impermeability may account for the high concentrations of iodoacetic acid which Feng found to be necessary. No such connective tissue sheath surrounds the white medullated fibres of the cord, which may therefore be more accessible to that substance.

In the present experiments, both cord and the upper portions of the sciatic nerves were perfused, and care was taken to dissect away the urostile and to stimulate the nerves close to their exit from the sacrum, since the procedure of tying off the legs prevents the perfusion fluid from reaching the lower portions of the nerves. Gerard (personal communication) has confirmed Feng's findings, and has shown, in addition, that added lactate will maintain the oxygen uptake

of nerve poisoned with iodoacetic acid, though it does not affect that of normal nerves. The fact that the failure, both of conductivity and of oxygen uptake, is gradual is in accordance with the low metabolic rate displayed by nerves. Von Ledebur [1932] also found that added lactate maintained the oxygen uptake of the frog's spinal cord after treatment with iodoacetic acid.

In view of the recent work of Winterstein [1930] the possibility cannot be entirely ignored that nerves may be sensitive to electrical stimulation when they are insensitive to stimuli originating in the grey matter. Apart from this, however, and considering the rest of the evidence together (bearing in mind, too, the fact that the spinal cord has a much richer vascular supply than peripheral nerve, so that probably the tracts in the cord are more sensitive to perfusion with iodoacetic acid than are the sciatic nerves), it seems possible that the failure in function observed really represents a failure in conduction, partly or wholly central (*i.e.* in the tracts of the cord). But if this is so, the probability seems to be strengthened that the grey matter itself is less sensitive even than appears from these experiments to the cessation of lactic acid production, and to its absence from the rôle of a substrate for oxidation, for otherwise, with its relatively high metabolic rate[1], one would expect an early and complete cessation of its function under the influence of iodoacetic acid.

The effect of substances other than lactic acid upon the function of the central nervous system has been tested. Pyruvate, succinate and (as was to be expected) glucose exert no protective effect against I.A.A. Methylglyoxal, however, has some potency in this respect and at lower concentration than lactate. In higher concentration (*e.g.* 0·5 %) it is, in fact, itself toxic, since it throws the whole animal into rigor. The methylglyoxal used was prepared from *iso*nitrosoacetone, made from ethyl acetoacetate according to the directions of Charrier [1907]. The methylglyoxal was prepared by distilling the *iso*nitrosoacetone with nitrosylsulphuric acid [Hoffman and Neuberg, 1930], a 1–2 % solution being obtained. The strength was determined by treatment with a known amount of hypoiodite and back-titration with thiosulphate. In addition, a few determinations were made by means of the *p*-nitrophenylosazone.

Estimations of lactic acid were made on the central nervous system of frogs perfused with methylglyoxal and iodoacetic acid. If the copper-lime technique is employed, a large amount of lactic acid is produced from unchanged methylglyoxal. The copper-lime procedure was therefore omitted, without any alteration in the values obtained when methylglyoxal was not used. A similar device has been resorted to by Haarman [1932].

These estimations show that, in the presence of methylglyoxal, there is a small increase in the amount of lactic acid in the central nervous system. The increase is much less than that obtained by perfusion even with concentrations of lactate that have no effect in preventing the inhibition of convulsions by iodoacetic acid. Its effect is clearly, therefore, not due merely to an increased production of lactic acid, which, perhaps, is the first solution that suggests itself. Indeed, it introduces a further complication. Dudley [1931] has presented strong evidence that the action of iodoacetic acid in muscle is to prevent the production of lactic acid by inhibiting the enzyme glyoxalase, which converts

[1] It is worth noting, for instance, that Hoffmann, Holzlöhner and Leegaard [1932] find that the frog's spinal cord produces heat at the rate of 580–2324 microcalories per g. for each second of stimulation. For medullated nerve, at 20°, the rate appears, from Hill's [1932] data, to be some 160 microcalories per g. for 1 second's stimulation. Since a large portion of the spinal cord must be white matter, the heat production of grey matter must be very large indeed compared with that of white.

methylglyoxal into lactic acid. Even with high concentrations of iodoacetic acid, some slight increase occurs in the lactic acid of the central nervous system, but it seems more reasonable, in view of Dudley's results, to suppose that methylglyoxal is effective by virtue of the fact that it follows some other metabolic pathway.

Table IV.

Conc. I.A.A. %	Conc. methylglyoxal %	Lactic acid in C.N.S. mg./100 g.	Frogs convulsing / total used	Time of perfusion (min.)
Frogs perfused with O_2/CO_2 bicarbonate Ringer p_H 7·2:				
0·2	0·05	26	$\frac{0}{3}$	60
0·1	0·05	22	$\frac{0}{4}$*	75
0·1	0·06	21	$\frac{2}{4}$	75
0·1	0·06	22	$\frac{0}{4}$	75
Frogs perfused with oxygenated bicarbonate Ringer (no CO_2) p_H 8·0:				
0·0333	0·034	66	$\frac{3}{4}$	60
0·0333	0·06	57	$\frac{4}{4}$	60
0·0333	0	24	$\frac{1}{4}$	60
0·05	0·05	34	$\frac{2}{4}$	60
0·05	0	12	$\frac{0}{4}$	60
0·05	0·05	29	$\frac{2}{4}$	60
0·05	0	8	$\frac{1}{4}$	60

* All showed marked twitchings. No actual convulsions.

Lohmann [1932] has found that glutathione is a coferment for the glyoxalase reaction, and Quastel [1933] and Dickens [1933] that iodoacetic acid inhibits this action of glutathione. An attempt, however, to restore the function of the central nervous system previously destroyed by 1 hour's perfusion with 0·2% iodoacetic acid, by subsequent perfusion with glutathione (0·05 %) and with glutathione 0·05 % *plus* methylglyoxal 0·05 % was unsuccessful: but the point clearly needs further investigation.

The dissociation in time between the depression of lactic acid production by the central nervous system and failure in its function makes it possible that, as in muscle, some other mechanism, such for instance, as the breakdown of phosphocreatine, may intervene between the two processes. The nerve cells may be able to function for a time without lactic acid, just as the muscle can contract without it, because, as in muscle, function may depend directly upon the breakdown of phosphocreatine and only indirectly on the formation or oxidation of lactic acid. An obvious step, therefore, was to investigate the behaviour of labile phosphate in the presence of iodoacetic acid. Gerard and Wallen [1929] have found that peripheral nerve contains labile phosphate and Gerard and Tupikow [1930] that it contains labile creatine; these are in equivalent amounts, and there is no reasonable doubt that they represent phosphocreatine. Stimulation of the nerve causes phosphocreatine to break down, and Gerard [1932] has recently discussed the evidence suggesting that the process is connected with impulse propagation. The next step seemed, therefore, to be to investigate the creatine phosphate content of the frog's central nervous system, and to try to discover what the effect upon it of iodoacetic acid might be. To this end, labile phosphate was estimated by the method of Eggleton and Eggleton [1929], the only important modification being that the PO_4 was estimated by the method of Fiske and Subarrow [1925] instead of by that of Briggs. The figures for the content of the central nervous system, removed immediately after decerebration, are given in Table V. As the central nervous systems were dissected out, they were quickly weighed and placed in a beaker containing

Table V. *Labile phosphate, expressed as mg. P/100 g. in C.N.S. of frogs removed immediately after decerebration.*

No. of frogs	Species	Labile P, mg./100 g.
8	English *temporaria*	3·92
12	,,	3·24
6	Hungarian *esculenta*	5·01
4	,,	3·80
4	,,	3·23
	Mean	3·84

iced, oxygenated Ringer solution, to minimise the chance of creatine phosphate breaking down during the period of 7–10 minutes necessarily consumed by dissecting eight or more central nervous systems. To minimise this delay, large Hungarian frogs, four of which yield enough tissue for estimation, were used in some experiments. The amount of labile PO_4 was extremely low, and the colours available for comparison in many instances were so pale that the results cannot be regarded as more than approximate.

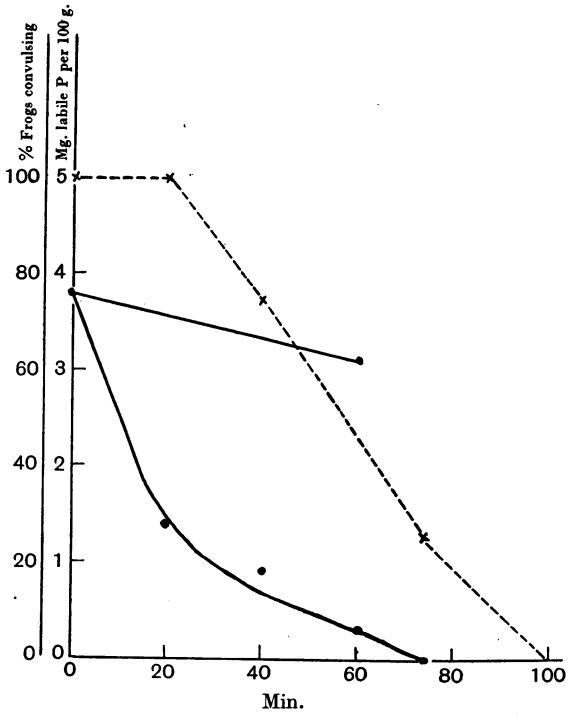

Fig. 3.

I.A.A. 0·01%. O_2/CO_2 bicarbonate Ringer. —— Labile P mg /100'g. ---- % animals convulsing. Upper continuous line represents effect of perfusion without I.A.A.

Perfusion of the central nervous system for 1 hour, without the addition of iodoacetic acid and without the administration of strychnine, caused a decrease in the content of labile P, as is shown in Table VI. All these animals, of course, were very active at the end of the perfusion.

The effect of iodoacetic acid on the labile P is to cause a diminution in its amount. The figures obtained are shown in Table VII and Fig. 3. The "activity" curve is the same in Fig. 2 (0·01 % iodoacetic acid), and there is considerable

Table VI. *Frogs perfused for 1 hour with O_2/CO_2 bicarbonate Ringer solution.*

No iodoacetic acid. No strychnine.

No. of frogs	Species	Labile P, mg./100 g.
10	English *temporaria*	2·92
10	,,	4·26
10	,,	2·50
10	,,	3·05
	Mean	3·18

similarity between the fall in labile P, shown in Fig. 3, and that of lactic acid as shown in Fig. 2. The amount of labile P has become undetectable before function has disappeared, and there seems no more evidence in favour of activity depending directly on the presence of creatine phosphate than there is that it is associated directly with lactic acid production or oxidation.

Gerard has lately found (personal communication) that the creatine phosphate content of nerves soaked in Ringer solution containing lactate falls more slowly than it does if the nerves are kept in lactate-free Ringer. It will be seen from Table VII that, after 40 minutes' perfusion with 0·01 % I.A.A. and 0·5 % lactate, the creatine phosphate content of the central nervous system is higher than after perfusion by I.A.A. alone.

Table VII.

Time of perfusion min.	I.A.A. 0·01 %. No strychnine injected	Perfused with I.A.A. 0·01 %. Strychnine injected	I.A.A. 0·01 % + Na lactate 0·5 %. Strychnine injected
20	1·30 *E*	1·59 *E*	—
40	0·949 *T*	0·596 *T*	1·10 *T*
	0·728 *T*	—	1·24 *E*
	0·959 *T*	—	—
60	0·10 *E*	0 *E*	1·38 *E*
		0·40 *T*	0·89 *T*
75	0	—	—

T indicates English *temporaria*. *E* indicates Hungarian *esculenta*.

In view of Gerard and Wallen's figures for the creatine phosphate content of nerve, it seems an open question whether the creatine phosphate content of the central nervous system may not, in fact, be confined to the white matter. It is unlikely that the medullated tracts differ from nerve in respect of their content of the substance, yet the average figure for the frog's central nervous system is 3·84 mg./100 g. while, according to Gerard and Tupikow [1930], that for frog's nerve is about 9·5 mg./100 g. It is difficult, if not impossible, accurately to decide the relative proportions of grey and white matter in the frog's central nervous system, and the figure given here for its labile phosphorus content is certainly an approximate one; still, it is evident that if the amounts of grey and white matter were equal the figures would at least be consistent with the

possibility that the creatine phosphate content of grey matter is zero, while that of white matter is similar to that of nerve. Mair and Lorrain Smith [1912] found that the dried white matter of adult human brain contained 60 % of chloroform-soluble substance, while the dried grey matter contained 30 %. If one is prepared to make the (doubtful) assumption that these figures hold good for the central nervous system of the frog, it would be possible to calculate the relative proportions of grey and white matter present from the percentage of chloroform-soluble substance present in the whole tissue, after it had been dried. Two determinations, for each of which eight central nervous systems were used, gave the following figures. Dry weight as percentage of wet weight, 19·8, 18·4. Chloroform-soluble substance as percentage of dry weight, 46·8, 45·6; mean 46·2. This gives a ratio of grey matter/white matter of 0·852:1. If all the labile phosphorus is in the white matter, then its concentration is 9·01 mg. per 100 g., a figure close to that obtained by Gerard for nerve. I have no wish to emphasise this figure, however, for it is founded on a very questionable assumption and on determinations which, owing to the small quantity of material available, cannot be accurate.

Just before this paper was written, the author had the privilege of reading, in typescript, a paper by Chang and Gerard, the contents of which have already twice been referred to as a "personal communication." The authors describe experiments showing that the addition of lactate to peripheral nerve will much delay, though it will not permanently prevent, the diminution in oxygen consumption and power of conduction and the increase in phosphocreatine breakdown, which occur as the result of treatment with iodoacetic acid[1]. These results are, in many respects, very suggestive of those which are described in this paper. The partial reversal of the effect of iodoacetic acid on the capacity of the central nervous system to respond to strychnine by the addition of lactate to the perfusion fluid, might well be ascribed to an incomplete protection of the medullated tracts against the effect of the poison. It was found, it is true, that the sciatics responded to electrical stimulation at the end of the experiment, but the experiments were all of much shorter duration than were Gerard's (60–75 minutes for the most part, as against 2½ to 12 hours). As has been earlier suggested, it is quite conceivable that the larger vascular supply of the cord renders its tracts far more accessible to perfused iodoacetic acid than are peripheral nerves, even when they also are perfused. If, however, the failure which is observed is a failure of conduction in the tracts, it may well be that the grey matter itself may, in reality, have continued to be capable of function for a longer time even than these experiments suggest.

There seems to be no doubt that lactic acid production, in the frog's central nervous system, is carried on at the expense of precursor stored in the tissue itself, and not, as in the mammalian central nervous system, at the expense of the blood-sugar. This may or may not indicate a fundamental difference in the chemical machinery underlying function in the two types, but at least it makes it certain that the state of affairs cannot be assumed to be the same in the two instances without further investigation.

[1] The experiments of Chang and Gerard, as well as those of Feng, raise the question as to whether nerve cannot, after all, oxidise lactic acid. In this connection, it is worth noting that Holmes [1930] found that the white matter of the central nervous system could do so to a slight extent. At the time, this result, in view of those previously obtained with nerve, seemed inexplicable; but in the light of these recent findings it becomes of more interest.

SUMMARY.

1. The capacity of the frog's central nervous system to respond to strychnine injections by convulsions is abolished by iodoacetic acid, but perfusion for $1\frac{1}{4}$ hours with 0·1 % iodoacetic acid is necessary for complete abolition. Perfusion for the same period with a concentration of 0·01 % reduces the lactic acid content to a very low level.

2. The capacity of the central nervous system to respond to strychnine is intact after 20 minutes' perfusion with 0·04 % iodoacetic acid, at which time the lactic acid content has been reduced almost to the minimum level reached.

3. The abolition of central nervous system function by iodoacetic acid is partially prevented by the presence of 0·5 % sodium lactate and 0·05 % methylglyoxal.

4. The "labile phosphorus," presumably representing the phosphocreatine, of the central nervous system, is reduced by iodoacetic acid more rapidly than the function of the tissue is affected.

5. It is suggested that the effect of iodoacetic acid may be chiefly on conduction in the white matter, and that the activity of the grey matter depends immediately neither on phosphocreatine breakdown, nor on lactic acid formation or oxidation.

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