readily pass through the walls of the glomeruli and account for the high excretion rate when silica is ingested or injected. Large polymers of silicic acid form adsorption complexes with polar and hydroxylic organic molecules which may be soluble in organic solvents such as ethanol and dioxan. Silicon also occurs in the tissues in a form which is not excreted and is not soluble in organic solvents. The nature of these complexes is under investigation.

SUMMARY

1. The silicon in tissues has been studied by injecting into rats silicate solutions containing ^{31}Si. Muscular tissue retains the least ^{31}Si, kidney tissue the most.

2. After drying the tissues, a part of the ^{31}Si can be extracted in ethanol, dioxan or (less effectively) ethyl acetate and ether. The ethanol-soluble silicon is probably present in micelles having organic groups adsorbed on silicic acid polymers.

3. The greater part of the ^{31}Si in the kidney occurs as inorganic silicate which is not extracted by organic solvents.

4. A part of the ^{31}Si is associated with the tissue, but is not soluble in ethanol.

The authors are indebted to the British Steel Founders' Association for a grant towards the cost of this research, and to Dr D. H. Tomlin for his assistance in assaying the solutions.

REFERENCES

Carman, P. C. (1940). *Trans. Faraday Soc.* **36**, 964.
Dean, E. W. & Stark, D. D. (1920). *Industr. Engng Chem.* **12**, 486.
Drechsel, E. & Winogradow, H. (1897). *Zbl. Physiol.* **11**, 361.
Holt, P. F. & Yates, D. M. (1950). *Biochem. J.* **45**, xxxii.
Holt, P. F., Yates, D. M. & Tomlin, D. H. (1951). *Biochem. J.* **48**, xliv.
Holzapfel, L. (1942). *Naturwissenschaften*, **30**, 185.
Holzapfel, L. (1943*a*). *Z. Ver. dtsch. Ing.* **87**, 606.
Holzapfel, L. (1943*b*). *Naturwissenschaften*, **31**, 386.
Holzapfel, L. (1947). *Naturwissenschaften*, **34**, 189.
Isaacs, L. (1924). *Bull. Soc. Chim. biol., Paris*, **6**, 157.
King, E. J. & Stantial, H. (1933). *Biochem. J.* **27**, 990.
Ohlmeyer, P. & Olpp, U. (1944). *Hoppe-Seyl. Z.* **281**, 203.
Veall, N. (1948). *Brit. J. Radiol.* **21**, 347.
Weyl, W. A. & Hauser, E. A. (1951). *Kolloidzschr.* **124**, 72.
Willstätter, R., Kraut, H. & Lobinger, K. (1929). *Ber. dtsch. chem. Ges.* **62**, 2027.

Induced Loss in Cerebral Tissues of Respiratory Response to Electrical Impulses, and its Partial Restoration by Additional Substrates

By H. McILWAIN and MARION B. R. GORE
Biochemical Laboratories, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, S.E. 5

(*Received* 15 *November* 1952)

Cerebral tissues are the most susceptible of those in the animal body to lowered blood levels of glucose. Cerebral activity rapidly fails with low glucose and if severe hypoglycaemia is maintained for periods between a few minutes and an hour, recovery may not be complete when glucose is again supplied. In the present study we have sought to reproduce in separated cerebral tissues a comparable loss of excitability in absence of glucose, a loss which was not restored on adding glucose alone. These conditions having been found, restoration of excitability by other means has been sought.

Experiments of this type have become possible through the development of metabolic means of measuring response by such tissues to their electrical excitation (McIlwain, 1951*a*). Applied impulses induce various metabolic changes in cerebral tissues (McIlwain, 1952). Of these changes, the increase in respiration and lactic acid formation were chosen for the present study for the following reasons. They are large and relatively easy to determine. They give a measure of the main energy-yielding reactions of the tissue. It is understandable that failure of these reactions should lead to irreversible loss of function in the tissue.

EXPERIMENTAL

Cerebral cortex. Cerebral cortex mainly of guinea pigs was prepared and mounted for electrical stimulation as described previously, using vessels A^1 and the tissue-holding electrodes type *D* (McIlwain, 1951*a*; McIlwain & Gore, 1951). The vessels were fitted to Warburg type manometers and changes in O_2 or CO_2 were followed at 37° by the usual

manometric techniques. A vessel normally contained a slice of about 100 mg. fresh weight with 5 ml. saline. Typical experiments comprised five or six vessels containing tissue from the same animal. Creatine and inorganic phosphates were determined in certain of the slices after their rapid removal from the electrodes (McIlwain & Gore, 1951) by extraction with trichloroacetic acid and separation by Ca-ethanol precipitation (McIlwain, Buchel & Cheshire, 1951).

Salines. All salines contained 134 mM-NaCl, 5·4 mM-KCl, 1·34 mM-KH_2PO_4, 1·34 mM-$MgSO_4$ and 2·7 mM-$CaCl_2$. Glycylglycine saline contained in addition 50 mM-glycylglycine, brought to pH 7·4 by NaOH, and was saturated with O_2. Phosphate saline contained 10·4 mM-Na_2HPO_4, brought to pH 7·4 by HCl, and was saturated with O_2. Bicarbonate saline contained 26·1 mM-$NaHCO_3$ and was saturated with 95% O_2 and 5% CO_2.

Glucose and other substrates when added during an experiment were placed initially in a side arm as neutral M solutions.

an approximate doubling in respiratory rate during the period of application of the impulses.

A *and* B, *Fig.* 1. Excised blocks from the cerebral hemispheres could be kept at 0° without added substrates for several hours without loss of excitability. That is, slices later prepared from such blocks and shaken in glucose-containing oxygenated salines at 37° respired at normal rates which increased with electrical impulses. If kept at 0° for 24 hr., some fall in initial respiratory rates and in excitability occurred (McIlwain, Ayres & Forda, 1952), but this method of depleting the tissue was not examined further as the loss in excitability was only partial and the conditions involved were remote from those of hypoglycaemia *in vivo*.

C *and* D, *Fig.* 1. Conditions closer to those of the body were therefore chosen. Under normal metabolic conditions at 37° but without glucose,

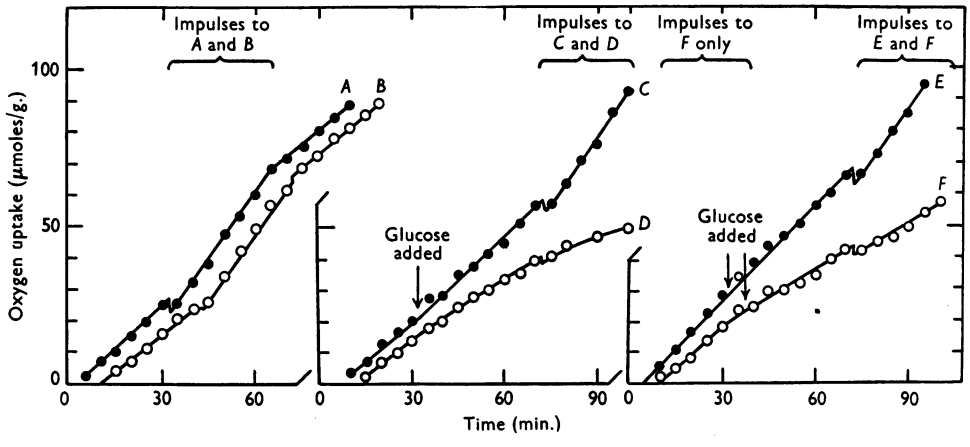


Fig. 1. Respiratory response of cerebral tissues to electrical stimulation after various treatments. Response is shown by increase in slope of the O_2-time curves. Their displacement on starting or stopping the impulses is a temperature artifact (McIlwain, 1951*a*). 5 or 10 min. have been added to the abscissae in the second of each pair of curves for clarity in the diagram. Glucose concentration, when added, 10 mM. *A*, normal tissue; and *B*, tissue kept at 0° for 2 hr., responding similarly to impulses in glucose saline. *C* and *D*, tissue in media without glucose does not show respiratory response to impulses (*D*); respiration gradually falls (*D*) unless glucose is added (*C*), when excitability also is restored. *E* and *F*, tissue initially without glucose, and impulses applied to *F*. Glucose then added to each, and impulses applied to each; *F* does not respond.

Stimulation. Except when otherwise described, stimulation was by alternating condenser pulses, 100/sec., at a peak voltage of 18 V., and of time constant 0·3–0·5 msec., from the apparatus of McIlwain (1951*a*) and later of Ayres & McIlwain (1953) which uses the same basic circuit but is supplied from a.c. mains.

RESULTS

Loss of respiratory response to electrical pulses

Fig. 1 illustrates conditions which were examined in obtaining from cerebral cortex a preparation which did not give the usual respiratory response to applied electrical impulses. The normal response is

respiration was initially at a rate slightly lower than normal, and fell with time. Applied impulses did not increase the respiratory rate. Addition of glucose after 30 min. at 37° arrested the fall. However, it also restored the ability of the tissue to respond to applied impulses.

E *and* F, *Fig.* 1. Cerebral tissues *in vivo* receive not only metabolic but also electrical influences from their environment. In examining their depletion *in vitro*, the experimental sections in absence of substrate were therefore exposed to electrical impulses of the type which would normally excite. When applied in the absence of substrate at the beginning of an experiment these led to little

Table 1. *Choice of conditions for depleting tissue*

(Guinea-pig cerebral cortex (about 100 mg.) was in tissue-holding electrodes immersed in 5 ml. glycylglycine-buffered salines without glucose, in manometric vessels. Glucose (0·05 ml. M) was present in the side arms and added at the beginning of the period indicated. Impulses were 100/sec., diphasic, peak voltage 18 V., and of time constant 0·4 msec. Respiration was obtained from manometric readings plotted as in Fig. 1.)

Exp.	Time and glucose (min. after placing at 37°)		Impulses (I, applied; 0, not applied)		Respiration (μmoles O_2/g./hr.)	
			a	*b*	*a*	*b*
1	0–15	None	I	0	46	44
	15–45	Added	0	0	48	44
	45–90	Added	I	I	62	70
2	0–30	None	I	0	46	46
	30–65	Added	0	0	46	46
	65–105	Added	I	I	42	70
3	0–35	None	0	0	38	37
	35–60	None	I	0	25	37
	60–90	Added	0	0	27	33
	90–120	Added	I	I	25	45
4	0–30	None	I	0	43	40
	30–60	Added	0	0	40	40
	60–120	Added	I	I	40	84

change or a slight fall in respiratory rate. If the impulses were then stopped and glucose added, little change occurred. However, an important difference was seen during the third period: impulses in the presence of glucose now failed to change the respiratory rate of the tissue.

Tissue so treated to impulses in absence of substrate constitutes the depleted preparation which is examined in the remainder of this study. Choice of the duration of treatment was made on the basis of experiments shown in Table 1. For depletion, 15 min. exposure to impulses was inadequate (Exp. 1); 30 min. was adequate in that later addition of glucose and application of impulses gave no respiratory response (Exp. 2). The intensity and type of impulses could also be varied, and some experiments in which this is done are described later. As however the period of 30 min., which was necessary for depletion with the type of impulses already employed, is of the order of time during which hypoglycaemia can become irreversible, the conditions of Table 1, Exp. 4, were considered satisfactory. The longer period in absence of substrate examined in Exp. 3 offered no advantage.

Glycolysis by depleted tissues

Lactic acid normally accumulates in oxygenated reaction mixtures containing cerebral tissues and glucose. This continued to take place with depleted tissues, though at a slightly reduced rate. Normal rates for cerebral cortex prepared in the same fashion as for the present experiments and at a concentration of about 20 mg./ml., were about 25 μmoles/g./hr. (McIlwain & Grinyer, 1950; McIlwain, Anguiano & Cheshire, 1951), but they fell during the course of an experiment. After incubation in the absence of substrate, values of 12–16 μmoles/g./hr. were found (Table 2) for the period of 60–90 min. after commencement of experiments. Thus temporary omission of glucose did not greatly affect the normal rates of glycolysis.

Moreover, glycolysis in the depleted tissue responded to applied impulses. A three- to five-fold increase is suggested by the values of Table 2. Exps. 1*d*, *e*, 2*d* and *e* show the formation of lactic acid to be little affected by the initial application of impulses which induced failure in respiratory response. This is shown also in Fig. 2. Here, increase in pressure, mainly due to formation of lactic acid, is seen to commence in depleted as well as normal tissue on addition of glucose, and to be accelerated by applied impulses.

The rate of lactic acid accumulation which was induced in reaction mixtures with the depleted tissues by the impulses varied with the buffer employed. It was higher with phosphate than with glycylglycine or bicarbonate (Table 2). In phosphate, the observed rate of 85 μmoles/g./hr. approached the maximum values obtained by electrical and other stimulation of cerebral cortex *in vitro*. This is of the order of 100 μmoles/g./hr. (McIlwain, Anguiano & Cheshire, 1951).

Partial restoration to depleted tissues of respiratory response to electrical impulses

One interest in the study of the depleted tissue lay in possible restoration of its full response to applied impulses. This was attempted by adding further substances to the glucose saline which by itself was adequate for response by normal tissue.

Table 2. *Lactic acid formation by treated tissue*

(Tissue and impulses were as in Table 1 except that phosphate replaced glycylglycine as buffer in Exp. 2, and bicarbonate in Exp. 3. Each vessel of each experiment was run for the three periods of 30 min. each, then promptly removed from the thermostat and samples taken for lactic acid. Each value given for lactic acid is the average of duplicate determinations; when two values are given they are from vessels run in parallel. The rate of lactic acid formation has been assumed to be uniform during each period and was calculated as follows. Taking Exp. 1 as example: *a*, 2/3 of observed value; *b*, 3·2 + 2 (6·4–4·7); *c*, 3·2 + (14–4·7); *d*, 12·5 + 2 (28–14); *e*, 12·5 + 2 (30·5–14).)

	Conditions			Lactic acid	
Exp.	1st period (I, impulses)	2nd period (G, glucose added)	3rd period (I, impulses)	Total formed during exp. (μmoles/g.)	Calculated rate of formation during last period (μmoles/g./hr.)
1*a*	—	—	—	4·7	3·2
b	—	—	I	6·4	6·6
c	—	G	—	14	12·5
d	—	G	I	28, 28	40·5
e	I	G	I	30, 31	45·5
2*a*	—	—	—	9·8	6·6
b	—	—	I	8·4	3·8
c	—	G	—	20	16·8
d	—	G	I	50, 57	83·8
e	I	G	I	53, 55	84·8
3*a*	—	—	—	11	—
b	—	—	I	8	—
d	—	G	I	44	—
e	I	G	I	32	—

Additions included (1) substances described as liberated from nervous structures on excitation, (2) substances concerned with oxidative and not glycolytic reactions with glucose, and (3) various other materials from natural sources.

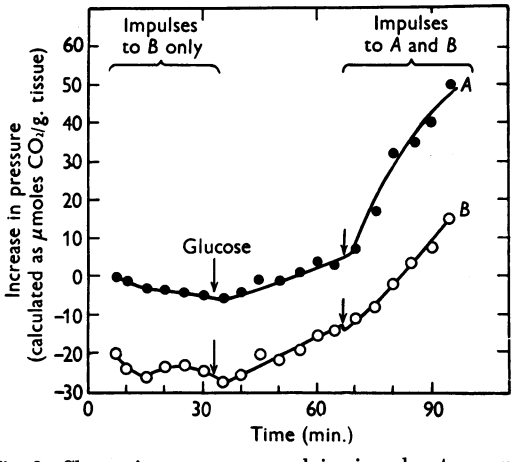


Fig. 2. Change in pressure on applying impulses to normal (*A*) and depleted (*B*) guinea-pig cortex in bicarbonate saline. Changes have been calculated as apparent μmole CO_2/g., using the K_{CO_2} of the vessels, and are largely due to the formation of lactic acid (cf. McIlwain, Anguiano & Cheshire, 1951). For clarity in the diagram, 20 μmoles have been subtracted from each ordinate of curve *B*.

An initial survey of such substances gave results which are summarized in Table 3. None of the substances of groups (1) or (3) of the preceding paragraph was effective; the first clearly active compound encountered was fumaric acid. The restoration of respiratory response brought about by fumaric acid was not complete, when examined under the conditions of depletion and testing which had been chosen on the basis of the experiments of Table 1. The conditions of depletion and of testing the tissue for respiratory response were therefore varied, and examples are given in Table 4. No effect of fumarate was found when (Exp. 1) conditions of depletion were mild, using a relatively low voltage and brief impulse. Here the tissue was relatively little depleted. The effect was seen when the impulses used were longer (Exp. 2) or of higher voltage (Exps. 3–5); the testing impulses were also varied in these experiments. No set of conditions led to a markedly greater effect of fumarate than was seen under the standard conditions adopted earlier. The results of Table 4 however showed that partial restoration of the depleted tissue by

Table 3. *Attempted recovery of metabolic response with various additional substances*

Substances (mM) *changing respiratory response by* <10%

Thiamine, 1·5; nicotinamide, 2·0 and 20; diphosphopyridine nucleotide, 0·1; adenylic acid, 10; adenosinetriphosphate, 10; choline chloride, 10; acetylcholine chloride, 0·5 and 5.

Guinea-pig and human blood serum, 10%; a 1:2 aqueous extract of guinea-pig brain, 10%.

Succinate, 10 and 40; glutamate, 10.

[*Substances* (mM) *increasing respiratory response by* >10%

Fumarate, 10 and 20; malate, 10 and 40.

Table 4. *Depletion and partial recovery with fumarate under various conditions*

(The main part of each experiment comprised measurement of respiratory rate of four pieces of cerebral tissue from the same animal during three successive experimental periods. Impulses were applied during the first period (to deplete) and during the last (to test); they were the same in each four vessels of any one period of an experiment but were altered from experiment to experiment and sometimes in the depletion period were different from those of the testing period. During the first period no substrate was present; it was tipped in a few minutes after impulses were switched off at the beginning of the second period and was either 0·01 M-glucose alone (in two of the vessels) or 0·01 M-glucose plus 0·01 M-fumarate (in the other two). Rates during the first period are not recorded, as the period was sometimes too brief to give dependable values; when obtained, values were comparable with those of Table 1.)

	1st period			2nd period		3rd period		Rate of respiration		
		Impulses				Impulses			3rd period	
Exp.	Duration (min.)	Peak potential (V.)	Time constant (msec.)	Duration (min.)	Fumarate added	Peak potential (V.)	Time constant (msec.)	2nd period (μmoles/ g./hr.)	(μmoles/ g./hr.)	Change from 2nd period (%)
1	15	14	0·1	45	0	20	0·3	52	79	+52
	15	14	0·1	45	+	20	0·3	62	99	+60
	30	14	0·1	30	0	20	0·3	49	73	+49
	30	14	0·1	30	+	20	0·3	44	58	+32
2	25	14	0·5	30	0	18	0·3	43·5	47	8
	25	14	0·5	30	+	18	0·3	54	69	28
	50	14	0·5	30	0	18	0·3	36	29	−19
	50	14	0·5	30	+	18	0·3	38	37	−2
3	25	20	0·1	55	0	20	0·1	48	48	0
	25	20	0·1	55	+	20	0·1	34	40	17
	50	20	0·1	30	0	20	0·1	45	48	7
	50	20	0·1	30	+	20	0·1	44	50	14
4	25	20	0·13	50	0	20	0·4	39	47	21
	25	20	0·13	50	+	20	0·4	55	87	57
	45	20	0·13	30	0	20	0·4	30	36	20
	45	20	0·13	30	+	20	0·4	33	38	16
5	30	18	0·4	30	0	18	0·4	33	35	6
	30	18	0·4	30	+	18	0·4	31	38	23
	30	18	0·4	30	0	18	0·4	42	44	5
	30	18	0·4	30	+	18	0·4	52	64	23

fumarate was not dependent on narrowly defined conditions of preparing and testing the tissue.

The results of Table 4 illustrate points which must be considered in expressing the ability of substances to restore excitability. Metabolic response to impulses has previously been expressed as a percentage of the rate immediately prior to their application, rather than by subtraction of one rate from the other. Under different experimental circumstances the percentage increase gives more consistent values for the effect of a given impulse. This applies to effects on tissue from different parts of the brain and from the brain of different animal species (McIlwain *et al.* 1952) and at different temperatures (unpublished). The last column of Table 4 accordingly gives such percentage changes in respiratory rate in reaction mixtures sometimes with and sometimes without fumarate. Fumarate has been considered to restore respiratory response when the percentage increase in presence of fumarate is greater than in its absence. Fumarate in some cases increased slightly the respiratory rate during the second experimental period, used as denominator in calculating the percentage. This fashion of expressing results would in such cases tend to minimize the restoring effect of fumarate. Similar considerations apply to the values of Table 5. Here, the significance of the difference between stimulation with and without added substance has been expressed as P values calculated by applying the t test. In doing this, the difference chosen has been that between the two percentage responses in a given experiment, and not that between the mean response, with and without addition, in the group of experiments with a given substance. This is because, in a given experiment, variable factors (e.g. the animal, time and temperature of preparing the tissue) operated equally on the experimental vessels with and without added substrate.

Other carboxylic acids, metabolically related to fumarate, were examined for restoration of the depleted tissue. L-Malate at 0·04 M had an effect comparable to fumarate (Table 5) but others had little or none. The respiratory response to impulses achieved by either of these compounds was about one-third of the normal response.

Inorganic and creatinephosphates in depleted tissues

The labile phosphates have already been determined in cerebral tissues prepared for metabolic work by slicing in the fashion used in the present experiments (McIlwain, Buchel & Cheshire, 1951; McIlwain, 1952; Kratzing, 1953). The creatinephosphate of the tissue from guinea pigs was then

Table 5. *Various added substances on recovery of respiratory response*

(The experimental arrangement was that of Table 4, with three successive 30 min. periods in each experiment. During the first and last impulses of 18 V. peak potential and 0·3–0·4 msec. time constant were applied. Glucose (to 0·01-M) with or without additional substrate was added at the end of the first period.)

Additional substrate (mM)	Change of respiratory rate in 3rd period (% of rate in 2nd period): Without additional substrate	With additional substrate	*P* (see text, p. 309)
Fumarate, 10	6, 5, 8, 11	23, 23, 28, 25	0·01–0·001
Fumarate, 20	20, 23, 9	37, 30, 17	0·05–0·02
L-Malate, 10	8, 0, 3, 9	18, 0, 8, 8	0·3–0·2
L-Malate, 40	14, 19, 7, −4	29, 33, 21, 31	0·05–0·02
Succinate, 10	12, 4	6, 2	—
Succinate, 40	6, 16, 8, 6	0, 18, 10, 7	—
Pyruvate, 10	3, 17, 5	−5, 8, 3	—
Lactate, 10	−6, 7	−11, 10	—
Lactate, 40	7, 11	8, 23	—
Citrate, 10	20	24	—
Citrate, 20	11, 23	12, 18	—
L-Glutamate, 10	6, −2, 10	0, −12, 8	—

Table 6. *Inorganic phosphate and creatinephosphate in depleted cerebral tissues*

(Tissues, vessels, and impulses were as described in Table 1; media differed only as described below. Glucose was added to 10 and fumarate to 20 mM.)

Treatment: First period of 30 min. Glucose	Impulses	Second period of 30 min.	Tissue constituents at end of experiment: Creatine-phosphate (μmoles/g.)	Inorganic phosphate (μmoles/g.)
0	0	No second period	0·31, 0·33	4·86, 5·52
0	+	No second period	0·32, 0·37	4·62, 5·60
+	0	No second period	1·52, 1·76	4·32, 3·85
+	+	No second period	0·42, 0·35	4·63, 5·05
0	0	With glucose	1·72, 1·44	3·53, 4·13
0	+	With glucose	1·44, 1·31	4·6, 4·88
0	0	With glucose and fumarate	1·26, 1·62	3·73, 4·09
0	+	With glucose and fumarate	1·29, 1·25	3·74, 3·9

shown to fall from its *in vivo* value of about 3 μmoles/g. to less than 1 μmole/g. on slicing and transferring to a physiological saline. If the slices were caused to respire in oxygen in salines without added substrate, creatinephosphate levels of about 0·3 μmole/g. were established. This is the condition of the tissue at the beginning of the present experiments (Table 6).

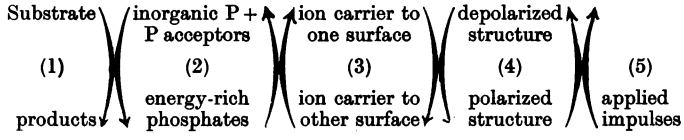
Addition of glucose-containing salines to tissue which had been without added substrates for some 10 min. after preparation, led to resynthesis of a large proportion of the creatinephosphate which it originally contained. Typical levels attained *in vitro* were of 1·5 μmoles/g. It was not however known whether this resynthesis took place in tissues which had respired for some 40 min. in absence of substrate, either with or without the application of impulses. This was the condition of the tissue in the present experiments at the end of their first period of metabolism. The results of Table 6 show that resynthesis did take place under these conditions. The levels of creatinephosphate attained did not greatly differ from those found when metabolism was throughout in glucose-containing solutions. Moreover, resynthesis to an only slightly smaller extent occurred following addition of glucose to tissues which had been depleted by impulses in its absence.

Lack of respiratory response in the depleted tissue was thus not due to lack of creatinephosphate. The addition of fumarate together with glucose, a mixture which partly restored the respiratory response, did not greatly affect the level of resynthesized creatinephosphate.

The inorganic phosphate of the slices showed under the conditions investigated (Table 6) relatively small changes, usually in a sense opposite to those of the changes in creatinephosphate.

DISCUSSION

In assessing the way in which tissues unresponsive to electrical impulses might differ from those which respond normally, it is necessary to have some picture of the normal linkage between applied impulse and metabolic response. Ideas presented previously (McIlwain, 1951*a*, *b*; McIlwain & Gore, 1951) may be summarized diagrammatically as follows:



Each of the numbered systems is of considerable complexity and the scheme is considered in more detail elsewhere (McIlwain, 1953).

In the depleted tissue, substrate is provided and labile phosphates are not seriously abnormal. The transport system (3) is presumably capable of maintaining a polarized membrane, as the glycolytic response to applied impulses is normal. Some stage of system (1) has apparently failed, and the stage is specific to respiration as distinct from glycolysis. Alternatives to this conclusion require glycolysis to be linked to functional activity independently from respiration and not through a common system such as (2) above. Failure in respiratory stages is, however, confirmed by the partial recovery following addition of fumarate or malate to glucose-containing media. The most likely role for these additions is as catalysts in the tricarboxylic acid cycle. Thus, fumarate added as the only substrate to cerebral cortical slices supports respiratory rates only a little higher than those found with no substrate. It contributes little to labile phosphates of such tissue and does not itself result in response to applied impulses (Kratzing, 1953). Alone in cerebral breis or suspensions fumarate yields pyruvate (Long, 1945) but added in catalytic quantities with pyruvate as substrate it markedly accelerates respiration (Banga, Ochoa & Peters, 1939*a*–*c*). Removal of pyruvate then increases while citrate and α-ketoglutarate accumulate (Coxon, Liebecq & Peters, 1949).

The deficiency in fumarate, induced in breis by dilution or dialysis, now appears to have been induced in the depleted tissue, and to do this both application of impulses and absence of added substrate were necessary. Both circumstances can be understood to increase the metabolism of whatever substrates are available and to increase the likelihood of mechanical loss from a tissue by its depolarization, but the manner of loss has not been investigated. Fumarate occurs in appreciable quantities in normal cerebral tissues as also does fumarase, which makes it understandable that fumarate and malate should act similarly. That citrate did not act as did fumarate may be related to a similar specificity in catalysing respiration in finely ground cerebral tissue (Banga *et al.* 1939*c*).

Requirement of additional substances for the increased respiration associated with impulses raises the question of the possible difference of such respiration from that of unstimulated tissue. Such difference seems unlikely. Loss of ability to respire more rapidly with impulses was gradual (Tables 1 and 4), and in choosing conditions for depletion the gradual loss was interrupted at an arbitrary point. That respiration can then be raised by an added catalyst could be due to increase in quantity of a catalyst already there, quite as well as by addition of one pertaining to a different metabolic route and totally absent.

SUMMARY

1. The ability of cerebral cortex slices to increase respiration with glucose as substrate, on applying electrical impulses, survived several adverse conditions. These included keeping the tissue for some hours at 0°, the prolonged passage of impulses with substrate, or metabolism in absence of substrate.

2. Adequate application of impulses in absence of substrate changed the tissue so that its respiration no longer increased when glucose was provided and impulses were applied.

3. Tissue so treated largely retained its ability to form more lactic acid from glucose in response to impulses.

4. Respiratory response could be partly restored to the changed tissue by addition of fumaric or malic acids to normally adequate metabolic mixtures. Many other additions were ineffective.

5. Inorganic phosphate and creatinephosphate have been determined in the initial and changed tissue, in the presence of various substrates, and have aided interpretation of the change induced by impulses in absence of substrate.

We are greatly indebted to the Board of Governors of the Bethlem Royal Hospital and the Maudsley Hospital for a grant from the Research Fund of the hospitals for purchase of apparatus used in this work, and to Mr P. J. Ayres for assistance during part of it.

REFERENCES

Ayres, P. J. W. & McIlwain, H. (1953). Unpublished.
Banga, I., Ochoa, S. & Peters, R. A. (1939*a*). *Nature, Lond.*, **144**, 74.
Banga, I., Ochoa, S. & Peters, R. A. (1939*b*). *Biochem. J.* **33**, 1109.
Banga, I., Ochoa, S. & Peters, R. A. (1939*c*). *Biochem. J.* **33**, 1980.
Coxon, R. V., Liebecq, C. & Peters, R. A. (1949). *Biochem. J.* **45**, 320.
Kratzing, C. C. (1953). *Biochem. J.* **54**, 312.
Long, C. (1945). *Biochem. J.* **39**, 143.
McIlwain, H. (1951*a*). *Biochem. J.* **49**, 382.
McIlwain, H. (1951*b*). *Brit. J. Pharmacol.* **6**, 531.
McIlwain, H. (1952). *Symp. biochem. Soc.* **8**, 27.
McIlwain, H. (1953). To be published.
McIlwain, H., Anguiano, G. & Cheshire, J. D. (1951). *Biochem. J.* **50**, 12.
McIlwain, H., Ayres, P. J. W. & Forda, O. (1952). *J. ment. Sci.* **98**, 265.
McIlwain, H., Buchel, L. & Cheshire, J. D. (1951). *Biochem. J.* **48**, 12.
McIlwain, H. & Gore, M. B. R. (1951). *Biochem. J.* **50**, 24.
McIlwain, H. & Grinyer, I. (1950). *Biochem. J.* **46**, 620.

The Ability of some Carboxylic Acids to maintain Phosphate Levels and support Electrical Stimulation in Cerebral Tissues

By C. C. KRATZING*

Biochemical Laboratories, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), Maudsley Hospital, London, S.E. 5

(*Received* 15 *November* 1952)

In normal circumstances the energy requirements of mammalian brain are provided by glucose which is metabolized either completely to carbon dioxide and water or partially to lactate and pyruvate (for references, see Himwich, 1951). Glucose also enables creatinephosphate to be maintained in separated cerebral tissues (McIlwain, Buchel & Cheshire, 1951) and supports the additional respiration induced in such tissues by applied electrical impulses (McIlwain, 1951*b*). The complete oxidation of glucose in cerebral tissues can proceed through the tricarboxylic acid cycle (Coxon & Peters, 1950; Coxon, 1952; for earlier work see Quastel, 1939) and the present investigations were carried out to determine whether carboxylic acids intermediate in glucose catabolism would maintain creatinephosphate or allow response to applied impulses. Glutamic acid has already been examined from this point of view (McIlwain, 1951*a*; 1952*a*).

METHODS

The saline contained 0·128 M-NaCl, 0·0051 M-KCl, 0·00128 M-KH_2PO_4, 0·00128 M-$MgSO_4$, 0·05 M-glycylglycine (brought to pH 7·4 with NaOH) and 0·00275 M-$CaCl_2$. In some experiments, specifically mentioned in the text, this concentration of $CaCl_2$ was reduced by half and in some omitted completely; a corresponding volume of NaCl was added. In a few cases, also specially noted in the text, the glycylglycine was replaced by an equal volume of Na_2HPO_4 (brought to pH 7·4 by HCl) to give a final concentration of 0·01 M. The substrates used were either glucose or a carboxylic acid (pyruvic, succinic, α-ketoglutaric, citric or fumaric). The latter were added to the medium as solutions of the Na salt prepared by neutralizing the free acids. Glucose was added to 0·0056 M; and the carboxylic acid to 0·0056 or 0·02 M. Media were oxygenated at 37°.

Guinea-pig cerebral cortex slices were cut parallel to the outer surface and weighed as described by McIlwain (1951*b*). Slices which were to be exposed to impulses were in the type *D* tissue-holding electrodes which were used in conjunction with the type A^1 electrode vessels described by McIlwain (1951*b*). Control slices were usually freely suspended in the medium.

In most cases alternating electrical impulses were applied from a condenser as described by McIlwain (1951*b*), with a pulse frequency 100/sec., time constant about 0·1 msec., peak potential 15–20 V. Some of the slices respiring in pyruvate were activated by an applied voltage of 2·5 V. supplied by a transformer run from the 50 cyc. a.c. mains. The results of experiments using this form of excitation have been included with those obtained with condenser pulses as the responses from the two impulses were similar quantitatively.

At the end of the experiment, the slices were rapidly removed from the electrodes or vessels in the manner described by McIlwain & Gore (1951). Phosphates were determined either by a chemical method involving Ca-ethanol separation (McIlwain *et al.* 1951), or by the enzymic method described by Kratzing & Narayanaswami (1953). In a few experiments, creatine was determined as creatinine (McIlwain *et al.* 1951).

Synthetic phosphopyruvate was prepared by the method of Baer & Fischer (1949). The variation in the amounts of

* Present address: Biochemistry Department, Australian National University, Canberra, Australia.