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EFFECTS OF SOME INORGANIC SALTS ON THE METABOLIC RESPONSE OF SECTIONS OF MAMMALIAN CEREBRAL CORTEX TO ELECTRICAL STIMULATION

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The electrical activity of the central nervous system in intact animals is affected by changes in inorganic constituents of the blood or by application of inorganic salts in other ways (for a recent review see Heppenstall & Greville, 1950). The electrical changes are, however, complex, and the chemical composition of the fluid environment of the brain in such experiments is also complex and usually undetermined. Studies do not appear to have been made in simple media, on the response to stimulation of preparations of mammalian brain which do not themselves show spontaneous activity. The experiments with the central nervous system which are closest to giving such data are those of Libet & Gerard (1939) in which complex, spontaneous or induced electrical activity of the olfactory bulb of the frog brain was found to be affected by inorganic constituents of fluids in which it had been soaked. The deficiency with respect to the mammalian central nervous system has not been made good by perfusion techniques, which at present still require media containing proteins and red blood cells (Tschirgi, Gerard, Jenerick, Boyarsky & Hearon, 1949; Waelsch, 1951).

A technique has, however, been developed in these laboratories by means of which electrical impulses can be applied to slices of cerebral tissue while these are respiring in oxygenated saline solutions containing glucose as the only added organic constituent. The application of the impulses produces an increase in the rate of respiration and glycolysis of the tissue, and a concurrent fall in its creatine phosphate with rise in inorganic phosphate (McIlwain, 1951*a*, *b*; McIlwain, Anguiano & Cheshire, 1951; McIlwain & Gore, 1951). This same pattern of metabolic changes is induced in the brain by increased

activity *in vivo*. Its occurrence following electrical impulses *in vitro* may, therefore, reflect a state in the separated tissue which in some way corresponds to stimulation *in vivo*. The metabolic characteristics and the way they change under changed conditions are at present the only indications of this, but the correspondence here is considerable (McIlwain, 1951*a*, *c*, *d*).

This technique has now been applied to determine the effects of potassium, calcium, magnesium, ammonium, phosphate, sulphate, and chloride ions on the response to electrical impulses. In the absence of such impulses, certain of these salts affect respiration, glycolysis, or the phosphates of such tissue (McIlwain, 1952). Further such changes without, as well as with, electrical stimulation have also been observed during the present experiments. Except in perhaps one case, this did not prevent observation of the response to applied stimuli.

METHODS

Metabolic and chemical methods. Guinea-pig cerebral cortex slices were used in most experiments; details of their preparation and of the apparatus for manometric measurement of respiration during electrical stimulation are given by McIlwain (1951*a*). Vessels of type A[1] with tissue-holding electrodes, type D, were used. The tissue slices were trimmed to fit the holders and weighed 100–130 mg. At the end of an experiment the slices were rapidly transferred to 1·5 ml. of 10% trichloroacetic acid by the procedure described by McIlwain & Gore (1951). Inorganic phosphate and creatine phosphate were estimated in the trichloroacetic acid extract after Ca-ethanol fractionation (McIlwain, Buchel & Cheshire, 1951). Glycolysis was followed by determination of lactic acid (Barker & Summerson, 1941) in the saline. The experiments recorded in the tables were usually one of the two or three which were performed in media of a given composition, and which gave similar results. Values are not, however, averaged as the experiments were often of different duration, or with stimulated and unstimulated periods differently arranged, or were with tissue from rats and not from guinea-pigs.

Salines. The saline used in all control experiments contained 0·127 M-NaCl, 0·0051 M-KCl, 0·00273 M-$CaCl_2$, 0·00134 M-KH_2PO_4 and -$MgSO_4$, 0·05 M-glycylglycine brought to pH 7·4 with 0·05 M-NaOH and 0·013 M-glucose. This was the basic medium in which all tissue slices were cut and mounted in the tissue-holding electrodes. When the effects of the absence of any ion were being tested, the slices concerned were held in the deficient medium for 5 min before being transferred to the experimental vessels. Alterations were made separately in the concentrations of Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, PO_4^{3-} and SO_4^{2-} in the medium, and the exact concentrations used are given in the tables. In preparing a potassium-free medium, NaH_2PO_4 was added to maintain the phosphate concentration at 0·0127 M. Additional potassium was added to the medium as KCl. In some of the experiments made in the absence of Ca^{2+}, sodium oxalate at 0·014 M was added. The highest concentration of Ca^{2+} which could be tested without causing precipitation of calcium phosphate was 0·082 M.

When the Na^+-free solution was prepared, the correct osmotic pressure of the solution was maintained by increasing the glucose concentration to 0·25 M; the glycylglycine was brought to pH 7·4 with KOH and the corresponding amount of KCl omitted from the basic medium. In the Na^+-low medium, part of the tonicity contributed by the NaCl was replaced by glucose, 0·1 M, and part by glycylglycine, 0·15 M, the pH of which had been adjusted as usual with NaOH. The Na^+ contributed to the medium by the glycylglycine gave a final concentration 0·015 M. Additional Mg^{2+} was added as $MgCl_2$ and ammonium ion as NH_4Cl. Variations in the concentration of chloride ion in the medium during these changes were not compensated, for it was found (see below) that wide variations in $[Cl^-]$ could be introduced without altering the response of the tissue. Considerable variation in total tonicity of the medium was also found permissible, and

thermodynamic activities were therefore not taken into consideration in changing the salt concentrations of the media, but all salts were assumed to be equally ionized.

Impulses were alternating condenser pulses from the circuit described by McIlwain (1951*a*). Pulse frequency, 100/sec; time-constant, 0·5 msec; peak potential, 18 V, except when other values are quoted.

Procedure. Weighed slices were mounted in electrodes in a series of (e.g. six) vessels attached to manometers. These included vessels with the basal medium and others with media of changed composition. The air in the vessels was replaced with oxygen and the vessels placed in a thermostat at 37°; this point in the experiment was usually reached 25 min after death of the animal. Readings of oxygen pressure were taken each 5 min. About 30 min later (the exact time for individual experiments being given in the tables) impulses were applied to some of the vessels, again for 30 min periods during which 5 min readings were taken. Further periods with and without impulses followed in some cases. At the end of an experiment, tissue was first removed rapidly (McIlwain & Gore, 1951) from the vessels receiving impulses, and then from the others, for determination of the phosphates. Specimens (usually 0·5 ml.) of the fluid were then taken for determination of lactic acid.

RESULTS

Response in the basal medium. Impulses were applied to sections of cerebral cortex, while they were metabolizing in the basal medium, in each set of experiments. Results are not always included in the tables but are illustrated by Expt. 1, Table 1. Here, the rate of respiration is seen to increase by nearly 100 % on stimulation. Glycolysis increased similarly. This can be concluded from the difference between lactic acid values in Expt. 1*a* and *b*, which resulted from the application of stimuli for less than half of the duration of the experiment. Creatine phosphate fell and inorganic phosphate rose. The type of impulse applied in obtaining this result has been chosen, as a result of previous studies, as the minimum necessary to produce maximal effect on respiration. (Voltage/response curves are recorded by McIlwain (1951*a*) and McIlwain, Ayres & Forda (1952). Effects of impulses of different voltage-time characteristics are reported by McIlwain (1951*a*, *b*).) This arrangement with respect to stimulation in the present experiments made it likely that only certain types of action would be detected as resulting from the changed conditions which were being examined. Depression of activity or sensitivity in the tissue would be seen, as would any circumstances acting synergically with the impulse. Increased sensitivity of the tissue to the impulse would not be seen.

Varying tonicity of medium. The medium could be made considerably hypertonic without preventing response to electrical impulses. This has been examined in one instance by increasing the glycylglycine concentration from 0·05 to 0·15 M; in another by increasing glucose from 0·015 to 0·1 M (Expt. 2); and in another, by increasing NaCl from 0·127 to 0·142 M as well as glycylglycine from 0·05 to 0·15 M (Expt. 3). The tissue from media high in glucose showed a somewhat lower level of creatine phosphate than normal, and its response to impulses was less than normal. In high concentrations of glycylglycine, however, the creatine phosphate was high and the response to impulses

was normal. Slightly hypotonic media also permitted the tissue to respond normally. Lowering NaCl from 0·127 to 0·1 M without other adjustment was associated with normal levels of respiration, glycolysis, and the phosphates in the presence and absence of impulses.

Anions. It was convenient experimentally to be able to alter the concentration of chloride ion together with alterations in other constituents, as Cl^- was the main anion of the medium. The effects of change in $[Cl^-]$ itself were

TABLE 1. Metabolic response to stimulation and the effect of tonicity and of the anions of the medium. Guinea-pig cerebral cortex slices (100–130 mg) were used with 5 ml. of glycylglycine-glucose medium (see Methods) in each vessel. Concentrations are quoted only of the salts which were being altered in the particular experiments recorded. Experiments were timed from the moment when the vessels were placed in a thermostat at 37°

Expt. no.	Ion concn. M	Period min	Stimulation	Respiration μmole O_2/g/hr	Inorganic P μmole/g	Creatine P μmole/g	Lactic acid μmole/g, whole expt.
1*a*	No change	0–75	–	61	3·6	1·35	57
b	No change	0–40	–	60	4·0	0·65	80
		40–75	+	117			
2*a*	Na^+ 0·127	0–60	–	56	4·35	1·34	57
b	Na^+ 0·127 + 0·1 glucose	0–60	–	51	3·6	1·10	46
c	Na^+ 0·127 + 0·1 glucose	0–30	–	56	3·75	0·75	113
		30–60	+	84			
3*a*	Na^+ 0·127	0–55	–	60	3·8	1·65	65
b	Na^+ 0·142 + 0·15 glycylglycine	0–55	–	56	3·7	1·90	71
c	Na^+ 0·142 + 0·15 glycylglycine	0–25	–	60	3·3	1·39	121
		25–55	+	112			
4*a*	Na_2SO_4 0·124 (no NaCl)	0–90	–	58	3·6	1·25	78
b	Na_2SO_4 0·124 (no NaCl)	0–30	–	56	3·9	0·72	124
		30–60	+	120			
		60–90	–	61			
5*a*	SO_4^{2-} 0·00134	0–60	–	64	3·5	1·50	41
b	No SO_4^{2-}	0–60	–	62	4·0	1·51	33
c	No SO_4^{2-}	0–30	–	62	3·8	1·06	59
		30–60	+	111			
6*a*	PO_4^{3-} 0·00134	0–70	–	62	3·3	1·55	43
b	No phosphate	0–70	–	58	1·7	1·2	47
c	No phosphate	0–40	–	50	2·6	1·0	47
		40–70	+	78			

therefore examined. As indicated in the preceding paragraph, increase in $[Cl^-]$ from 0·138 to 0·154 M, together with an equivalent increase in $[Na^+]$ brought about no change in the characteristics under examination. A large reduction in $[Cl^-]$ also had little effect. This is shown in Expt. 4 when Na_2SO_4 replaced NaCl, so that the $[Cl^-]$ of the medium was lowered from 0·138 to 0·011 M.

Addition of Na_2SO_4 in Expt. 4 involved a large increase in the SO_4^{2-} of the medium, from 0·00134 to 0·078 M. The complete replacement of SO_4^{2-} by Cl^-, leaving the medium free from added $[SO_4^{2-}]$ was also examined. Expt. 5

shows that no significant change was induced in the characteristics measured, either in unstimulated or in stimulated tissue.

The concentration of phosphate, the other anion of the medium, was also varied. Its absence from the medium led, as would be anticipated, to lower concentrations of both inorganic and of creatine phosphates in the slice (Expt. 6) and also to a lowered response to electrical impulses. In the phosphate- and in the sulphate-free media, chloride concentrations had been increased from 0·138 to 0·140 or 0·141 M; but as indicated above, much larger changes in chloride can be made without alterations in the characteristics measured. Increase in the phosphate of the medium also caused little change in the effects of the impulses. When sodium phosphate replaced glycylglycine as the main buffer, the impulses led to the usual increase in respiration and glycolysis (McIlwain, 1951*a*). These experiments involved increase in the phosphate from 0·00134 to 0·012 M.

Sodium salts. Deficiency in sodium salts had a major effect on the metabolic changes induced in the tissue by electrical impulses. Absence of sodium salts from the medium made the tissue unresponsive. Its rate of respiration changed little or fell with time whether or not impulses were applied. The concentration of inorganic phosphate was unusually high in both presence or absence of stimulation, and the unstimulated rates of respiration and glycolysis were low. In Expt. 7, replacement of Na^+ was by glycylglycine, and $[Cl^-]$ was also lowered. However, glycylglycine had been shown in experiments described to be non-toxic and it had also been shown that Cl^- could be replaced by SO_4^{2-} without preventing stimulation. Thus, the metabolic changes in Expt. 7 were likely to be specific to the change in $[Na^+]$. This was confirmed by replacing NaCl by an equimolar solution of choline chloride, an expedient used by Hodgkin & Katz (1949). Here also lack of response to impulses was found. A respiratory rate of 59 μmole/g/hr instead of being doubled by impulses, fell, whether or not impulses were applied, to 48 μmole/g/hr.

When $[Na^+]$ was lowered rather than absent, intermediate effects were found. This is illustrated by Expt. 8. Considering the values in absence of stimulation, reduction of $[Na^+]$ from 0·127 to 0·015 M is seen to have lowered the creatine phosphate of the tissue only a little. The rise in inorganic phosphate was also less than in the Na^+-free medium. Impulses with 0·015 M-Na^+ then had little effect on respiration, but increased glycolysis and partly depleted the creatine phosphate.

The effect of impulses of varying intensity was examined in Na-deficient media. Fig. 1 compares the respiratory response so obtained with the response of tissues from the same animals examined at the same time in normal media. The deficient medium contained Na^+ at 0·015 M, for under these conditions the unstimulated tissue was more normal in respect to its

TABLE 2. Effects of sodium and potassium salts. Details as in Table 1. Expt. 1 of this table is the same as that of Table 1

Expt. no.	Ion concn. M	Period min	Stimulation	Respiration μmole O_2/g/hr	Inorganic P μmole/g	Creatine P μmole/g	Lactic acid μmole/g, whole expt.
7*a*	Na^+ 0·127	0–60	–	64	3·2	1·20	64
b	No Na^+	0–30	–	48	5·0	0·23	32
		30–60	–	41			
c	No Na^+	0–30	–	50	5·2	0·45	39
		30–60	+	38			
8*a*	Na^+ 0·127	0–70	–	53	4·1	1·74	46
b	Na^+ 0·015	0–70	–	44	4·5	1·42	37
c	Na^+ 0·015	0–35	–	51	4·1	0·72	61
		35–70	+	56			
1*c*	No K^+	0–75	–	56	2·9	0·85	53
d	No K^+	0–40	–	56	3·6	0·71	78
		40–75	+	98			
9*a*	K^+ 0·0067	0–70	–	54	4·0	1·74	49
b	K^+ 0·0267	0–70	–	90	4·05	1·29	65
c	K^+ 0·0267	0–35	–	90	3·9	1·20	69
		35–70	+	93			
10*a*	K^+ 0·0067	0–120	–	60	3·5	1·18	54
b	K^+ 0·1067	0–120	–	96–60	4·5	0·39	164
c	K^+ 0·1067	0–30	–	93	4·9	0·42	173
		30–60	+	90			
		60–90	–	86			
		90–120	+	76			

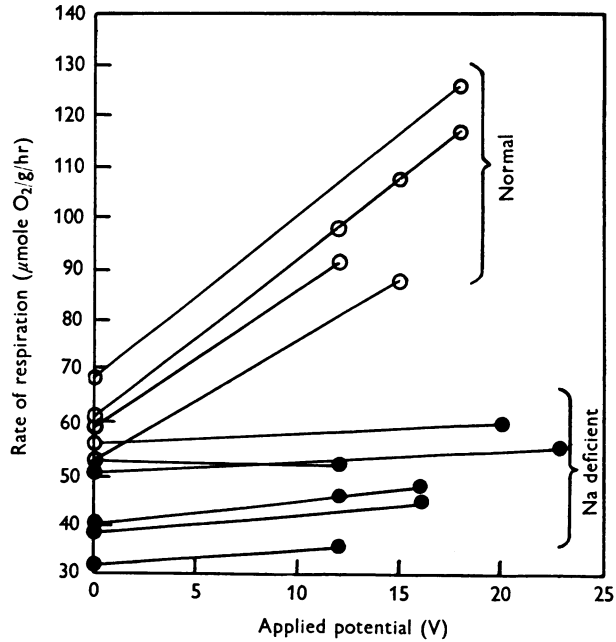

Fig. 1. Effect of the voltage of applied impulses on the respiration of cerebral cortex in a normal medium (0·127 M-Na^+), and in one with 0·015 M-Na^+. Potentials were applied as condenser pulses of time-constant 0·4 msec, 100/sec.

content of phosphates than when Na^+ was altogether omitted. Nevertheless, a great difference was seen in respiratory response at all voltages examined.

Moderate increase in the $[Na^+]$ normal to the medium had little effect. It was noted previously that change in NaCl from 0·127 to 0·142 M did not alter the characteristics studied. Also, replacement of chlorides by Na_2SO_4 at 0·124 M yielded a medium high in $[Na^+]$ in which the respiratory rate could however be doubled by impulses (Expt. 4).

Potassium salts. Absence of potassium salts from the medium had no marked effect on the rate of respiration or glycolysis of the tissue, but its levels of inorganic phosphate and of creatine phosphate were lower than in tissue in normal media. Electrical impulses in the absence of added K^+ (Expt. 1*d*, Table 2) induced the normal rise in respiration and glycolysis.

Increased $[K^+]$ is one of the circumstances noted above to alter the metabolic activities of the tissue, in the absence of any electrical impulses. Expts. 9 and 10 show this. Increase of $[K^+]$ from 0·0067 to 0·0267 M nearly doubled respiration and glycolysis, and led to fall in creatine phosphate. Electrical impulses then produced no further significant change. Respiration and glycolysis in absence of the impulses had already increased to rates as high as those normally induced by electrical stimulation. At 0·1067 M, K^+ induced rates of respiration which were as high as, but less stable than, those induced by 0·0267 M-K^+. Glycolysis was still higher and creatine phosphate lower, but again none of these was affected by the electrical impulses.

Calcium salts. The effect of absence of Ca^{2+} on the levels of energy-rich and inorganic phosphates in cerebral cortex slices has previously been investigated (McIlwain, 1952). In the present experiments, omitting Ca^{2+} from the medium (Expt. 11, Table 3) increased respiration and glycolysis and decreased creatine phosphate. Electrical stimulation caused a further rise in respiration with no change in creatine phosphate level. In Expt. 12, sodium oxalate was added to the calcium-free medium with the intention of making the absence of calcium ions from the tissue more complete. This caused, without impulses, a greater increase in respiration and glycolysis than was caused by simply omitting Ca^{2+}. Creatine phosphate level was reduced to one-third of that in the control slice. Inorganic phosphate level was also unusually low. Electrical impulses produced no further response.

Addition of calcium salts to three times their concentration in the control medium caused no change in the unstimulated respiration or glycolysis and the response to electrical stimulation was normal (Expt. 13).

Magnesium salts. Absence of Mg^{2+} from the medium caused a rise in respiration but not in glycolysis (Expt. 14), and decreased the creatine phosphate of the tissue. Impulses in absence of Mg^{2+} then produced a marked increase in respiration and glycolysis and a further loss in creatine phosphate.

An eightfold increase in the Mg^{2+} of the medium produced a slight fall in respiration and a marked drop in the inorganic phosphate of the slices. Electrical impulses then produced the normal rise in respiration and some fall in creatine phosphate, but no increase in lactic acid.

TABLE 3. Effects of calcium, magnesium and ammonium salts. Experimental conditions as in Table 1

Expt. no.	Ion concn. M	Period min	Stimulation	Respiration μmole O_2/g/hr	Inorganic P μmole/g	Creatine P μmole/g	Lactic acid μmole/g, whole expt.
11*a*	Ca^{2+} 0·00274	0–60	–	58	3·6	1·90	40
b	No Ca^{2+}	0–60	–	80	3·5	1·19	52
c	No Ca^{2+}	0–30	–	82	3·9	1·22	56
		30–60	+	98			
12*a*	Ca^{2+} 0·00274	0–63	–	72	3·4	1·68	41
b	No Ca^{2+}; +0·0141 sodium oxalate	0–63	–	110	1·8	0·55	64
c	No Ca^{2+}; +0·0141 sodium oxalate	0–35	–	119	1·8	0·61	60
		35–63	+	105			
13*a*	Ca^{2+} 0·00274	0–80	–	72	3·2	1·84	42
b	Ca^{2+} 0·00822	0–80	–	68	4·2	1·68	48
c	Ca^{2+} 0·00822	0–35	–	72	4·3	0·60	74
		35–80	+	116			
14*a*	Mg^{2+} 0·00134	0–120	–	63	3·1	1·55	39
b	No Mg^{2+}	0–120	–	78	3·3	1·0	37
c	No Mg^{2+}	0–30	–	78	4·35	0·8	96
		30–60	+	145			
		60–90	–	82			
		90–120	+	118			
15*a*	Mg^{2+} 0·00134	0–60	–	68	4·2	1·72	20
b	Mg^{2+} 0·01134	0–60	–	50	2·5	1·66	22
c	Mg^{2+} 0·01134	0–35	–	57	2·6	1·24	21
		35–60	–	114			
16*a*	No NH_4^+	0–60	–	66	3·5	1·56	45
b	NH_4^+ 0·001	0–60	–	80	3·5	1·58	102
c	NH_4^+ 0·001	0–40	–	80	3·6	0·55	117
		40–60	+	120			
17*a*	No NH_4^+	0–60	–	68	3·8	1·65	49
b	NH_4^+ 0·01	0–60	–	88	4·0	1·0	120
c	NH_4^+ 0·01	0–30	–	88	4·1	0·93	121
		30–60	+	106			
18*a*	No NH_4^+	0–85	–	56	3·3	1·97	72
b	NH_4^+ 0·03	0–85	–	56–33	4·3	0·19	128
c	NH_4^+ 0·03	0–35	–	56	4·8	0·29	156
		35–85	+	42–33			

Ammonium salts. These are not normally added to the medium, but Weil-Malherbe (1938) found 0·001 M-NH_4Cl to increase respiration of cerebral cortex slices to a small extent and their aerobic glycolysis very markedly. This was found to be the case in the present experiments (Table 3); the phosphates were unchanged and the tissue was still able to respond to electrical stimulation by a further increase in respiration, and by a fall in creatine phosphate. Results with 0·01 M-NH_4Cl were very similar (Expt. 17). At 0·03 M, however,

the tissue became unresponsive to the impulses. This medium itself induced a very high rate of aerobic glycolysis, but respiration was not increased and fell gradually during the experiment. Creatine phosphate of slices in the presence or absence of impulses was very low and their inorganic phosphate high.

DISCUSSION

Changes observed during these experiments are summarized in Table 4. This does not describe many of the smaller differences noted in the preceding tables. It emphasizes, however, the following findings. (1) Of the metabolic characteristics measured respiration, glycolysis and inorganic phosphate have been observed both to increase and to decrease as a result of varying salt concentra-

TABLE 4. Summary of changes with changed media and electrical impulses. (N, normal; O, no change; I, moderate increase; II, large increase; D, moderate decrease; DD, large decrease)

	Respiration		Lactic acid formation		Inorganic phosphate		Creatine phosphate	
Medium	N	Change with impulses	N	Change with impulses	N	Change with impulses	N	Change with impulses
N; low K^+; high Ca^{2+}; N, high or low Cl^- or SO_4^{2-}	N	II	N	II	N	I	N	DD
Low Na^+	D	O	N	I	N	O	N	D
No Na^+	D	O	D	O	I	O	DD	O
High K^+	II	O	II	O	I	O	DD	O
No Ca^{2+}	I	I	I	O	N	I	D	O
No Mg^{2+}	I	II	N	II	N	I	D	D
High Mg^{2+}	D	II	N	O	D	O	N	D
10^{-2} M-NH_4^+	I	II	II	O	N	O	N	D
3×10^{-2} M-NH_4^+	N	O	II	O	I	I	DD	O

tions and the application of impulses. Creatine phosphate either remained unchanged or decreased. The pattern of changes induced by electrical stimulation is thus, even on this qualitative basis, one of fifty-four different possibilities; that is, it represents a well-defined group of changes. (2) The changes in individual metabolic characteristics which were induced by electrical impulses in normal media, in several cases did not appear in the different salt mixtures. However, in none of these was the effect of the impulses reversed; it either took its normal direction or did not occur. The group of changes associated with the impulses is thus a persistent one. (3) Media lacking or low in sodium salts were unique among the circumstances examined in giving normal or low metabolic rates in which very little or no increase was observed on applying electrical impulses. Media high in Mg^{2+} approached this in some of the characteristics measured. (4) The other circumstances in which electrical impulses were relatively ineffective were ones in which the metabolic changes which the impulses normally elicited had already largely taken place. This was the case with high $[K^+]$ or low

[Ca^{2+}], especially in the presence of oxalate; or, with some of the characteristics measured, in the presence of ammonium salts.

Effects of the individual salts may now be considered.

Sodium salts. Lack of sodium salts renders other excitable tissues unresponsive to impulses which otherwise stimulate. This effect was reported in muscle by Overton (1902), and in the squid axon by Hodgkin & Katz (1949). There is immediate analogy between these findings and our own. Comparison here and elsewhere must, however, be made with care because the data available in the different experiments is different in kind. In the present experiments response to impulses is judged by metabolic effect and the salts themselves produce some metabolic effects. Hodgkin & Katz observed lowered action potential in the axon with lowered [Na^+] and did not measure metabolic characteristics. It would be consistent with other data to suppose the lowered action potential to involve less expenditure of energy and thus less utilization of labile phosphates and a lower rate of respiration than normally follows stimulation. The lesser respiration and lesser fall in creatine phosphate were found in our experiments with cerebral cortex in media low in sodium salts, but no means was available for making a measurement corresponding to action potential (McIlwain & Ochs, unpublished).

Cerebral cortex in the media without sodium salts was even less responsive to impulses. Here, creatine phosphate was already low, a circumstance associated in several instances with lack of response to stimuli. It is also associated in many other circumstances with increased glycolysis, and an increase in glycolysis with lowering of [Na^+] can be reproduced in extracts of brain (Racker & Krimsky, 1945; Utter, 1950). Glycolysis of the slices in media without sodium salts was however low, and remained so on applying impulses.

The creatine phosphate content of peripheral nerve in sodium-free media does not appear to have been studied, but correlations have been reported between a component of the action potential of frog muscle, and its content of creatine phosphate (Ling & Gerard, 1949; Ling, 1950). This finding makes the analogy between our observations and those of Overton (1902) more complete. The excellent quantitative connexion between [Na^+] and action potential in Hodgkin & Katz's (1949) experiments makes it unlikely that creatine phosphate should be a limiting factor in that case. Neither is it a limiting factor in our experiments with 0·015 M-NaCl.

These further considerations thus tend to support rather than to minimize the immediate analogy between effects of sodium-lack in the present preparations of cerebral cortex, and those previously reported in muscle and peripheral nerve.

Potassium and calcium salts. Increased [K^+] has previously been observed to increase respiration and glycolysis in preparations of cerebral cortex

(Ashford & Dixon, 1935; Dickens & Greville, 1935). The present findings, including the changes in phosphate and the effects of the impulses, are closely analogous to previous findings with muscle. Hegnauer, Fenn & Cobb (1934) observed a range of concentrations of potassium chloride slightly higher than our own, to reduce both the excitability and the phosphocreatine of the muscle while its respiration rose. Lowered creatine phosphate is a factor common to many situations in which response of excitable tissues is depressed; suggestions have been made (McIlwain, 1952) as to how the lowering of creatine phosphate with high potassium and low calcium might be mediated. The changes in absence of added calcium were observed in the present experiments also. These therefore constitute a further situation in which lowered $[Ca^{2+}]$ has effects similar to increased $[K^{+}]$.

Magnesium salts. Administration of magnesium salts to yield serum levels of 4×10^{-3} to 10^{-2} M produced anaesthesia and depressed body temperature in rabbits and dogs (Taylor & Winter, 1929; Heagy & Burton, 1948). In investigating a possible basis for these effects, Peiss, Hall & Field (1949) found $[Mg^{2+}]$ at 10^{-2} M and higher to depress respiration of rat cerebral cortex slices. Anaerobic glycolysis, however, was greatly increased by magnesium salts between 10^{-3} and $1{\cdot}5 \times 10^{-2}$ M. Results of the present study reproduce the depression of respiration and show also that aerobic glycolysis, more relevant to conditions *in vivo* than the anaerobic reaction, is not at all increased by high magnesium. Moreover, with high magnesium, electrical impulses are only partly effective in bringing about the metabolic changes associated with stimulation *in vivo*. Respiration increased but aerobic glycolysis did not, and the changes in inorganic phosphate and in phosphocreatine were also less than normal. A much fuller picture of the metabolic depression by high $[Mg^{2+}]$ is thus given by the present results than was previously available.

Ammonium salts. The increases in respiration and glycolysis brought about by ammonium salts in the present experiments were similar to those reported by Weil-Malherbe (1938). In comparison with other circumstances in which glycolysis is increased, the increase with 10^{-3} M-NH_4Cl was associated with an unusually small change in phosphocreatine and in respiration. At 10^{-2} M-NH_4Cl, however, the more usual fall in phosphocreatine took place. When these changes had occurred, electrical impulses still had their normal effect of further decreasing phosphocreatine and increasing respiration.

Richter & Dawson (1948, 1949) have given evidence suggesting that epileptic and electrically induced convulsions might be mediated by liberation of ammonia in the brain. The concentration of ammonia in the blood and in the brain which was found to be associated with the beginning of convulsions caused by intraperitoneally administered ammonium chloride, was about 9 mg % or 5×10^{-3} M. At this concentration, therefore, we find most of the changes associated with electrically induced convulsions to have commenced,

but to be lesser in degree than could be induced electrically. A higher concentration of ammonium salts (3×10^{-2} M) in our experiments accentuated the changes brought about by the lower concentration, except with respect to respiration; but it made the tissue inexcitable. This concentration is above that which is toxic *in vivo*.

The present experiments as a whole therefore give further indications that the condition induced in separated tissues of the central nervous system by electrical impulses *in vitro*, is related to the conditions of increased activity which can be induced in the central nervous system *in vivo* and in muscle and peripheral nerve *in vitro*.

SUMMARY

1. Slices of guinea-pig cerebral cortex were examined in a medium containing 0·127 M-NaCl; 0·0051 M-KCl; 0·00273 M-$CaCl_2$; 0·00134 M-KH_2PO_4 and -$MgSO_4$; 0·05 M-glycylglycine and 0·013 M-glucose. Suitable electrical impulses increased their rates of respiration and glycolysis, and their content of inorganic phosphate while decreasing their content of creatine phosphate.

2. Considerable changes in tonicity of the medium and its content of sulphate and chloride ions did not change the normal metabolic characteristics listed above, nor the manner in which they changed with applied impulses.

3. Lowered concentrations of sodium ions had little effect on these characteristics in absence of impulses, but prevented most of the changes which normally followed the impulses.

4. Lowered concentrations of calcium salts or increased concentrations of potassium salts partly or wholly brought about the changes which could be induced by electrical impulses. The impulses then completed the changes which they normally induced.

5. Increased concentrations of magnesium salts lowered the respiration and inorganic phosphate of the tissue, and decreased the changes which were normally caused by electrical impulses in glycolysis, inorganic phosphate and creatine phosphate.

6. Added ammonium salts partly induced the changes normally brought about by impulses, and partly prevented the impulses from having their normal effects.

7. No circumstances examined caused the impulses to reverse their usual action; this either took its normal course, or did not occur.

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