GLUTAMIC ACID AND GLUCOSE AS SUBSTRATES FOR MAMMALIAN BRAIN.

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Effects of substances on the respiration of unstimulated portions of mammalian brain have been used in the past as aids to understanding or suggesting their actions on the brain *in vivo*. The present paper gives data with respect to glucose and glutamic acid, which show some of the limitations of such interpretations. It also describes the application of new experimental means of examining the metabolic effects of added substances in relation to the functional activity of the brain.

A link between respiration and functioning of tissues of the central nervous system exists in the phosphorylation which normally accompanies respiration, and which maintains the energy-rich compounds which are utilized in supporting activities of the brain. Methods have been devised in these laboratories by means of which both the maintenance of these compounds and the response of the tissue to electrical stimulation can for the first time be studied *in vitro* (McIlwain, 1951*a*; McIlwain, Buchel and Cheshire, 1951). We have therefore applied them in the present investigation to glutamic acid, as the effects of this substance on the central nervous system have been complicated by its effects on other systems of the body. During recent years its administration has been reported to affect a number of aspects of behaviour or of mental phenomena in experimental animals and in man (for assessment of these reports, see Weil-Malherbe, 1950; Milliken and Standen, 1951). As one of the bases for such effects, the ability of glutamic acid to support the respiration of separated portions of mammalian brain has been quoted (see, for example, Gadson, 1951). We now find, however, that glutamic acid does not maintain the energy-rich compounds of the tissue, nor does it enable the tissue to respond to electrical stimulation *in vitro* as does glucose.

Experimental.

Tissues (mainly cerebral cortex) from guinea-pigs and rats were used, prepared as described elsewhere (McIlwain, 1951*a*, *b*; McIlwain, Buchel and Cheshire, 1951), where descriptions of experimental salines are also given. Respiration was measured manometrically, and glycolysis by chemical determination of lactic acid. Inorganic phosphate and phosphocreatine were determined in the tissues after extraction with trichloroacetic acid; this and the conditions of electrical stimulation of the separated tissues are decribed in the papers quoted. L(+) glutamic acid, taken to pH 7·3 by NaOH, was used.

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Results.

Respiration.—The present experiments were carried out with cerebral cortex, in saline solutions buffered at pH 7·3 either with phosphate or with glycylglycine. In all cases respiration with glutamate as substrate was initially higher than with glucose as substrate. The initial part of Fig. 1 and values of

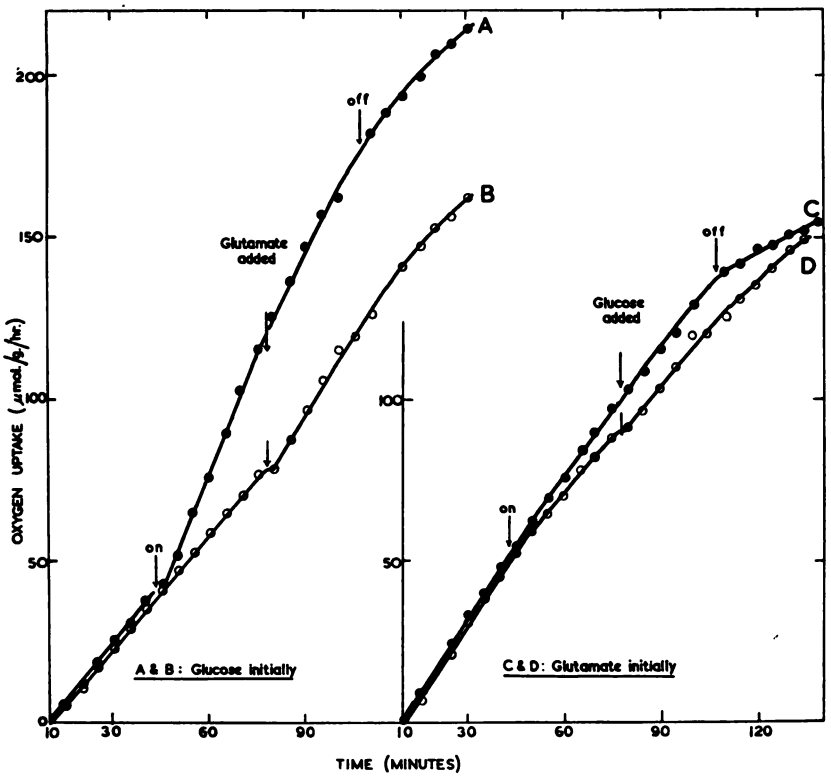


Fig. 1.—Respiration of fragments of rat cerebral cortex in phosphate-saline with glucose and glutamate as substrate, with and without electrical stimulation (condenser pulses of peak voltage 25, time-constant 0·4 msec., 100/sec). A and B: with glucose initially and glutamate added at 78 min. C and D: with glutamate initially and glucose added at 78 min. A and C were stimulated electrically between 44 and 108 min.

Tables I and II illustrate this; in some 16 experiments the rate during the first hour with glutamate was about 125 per cent. of that with glucose. Similar results have been found previously with tissues from various species and in various media (Krebs, 1935; Weil-Malherbe, 1936).

In the course of two or three hours respiration with the two substrates became approximately equal. Respiration with a mixture of both substrates was usually slightly higher still, but again fell with time. A marked effect of adding glutamate to tissue respiring in glucose is shown in Fig. 1, B.

Phosphates.—Certain phosphorus derivatives in the brain of intact animals show large and rapid changes with changes in the state of activity of the central nervous system. Prominent among these derivatives are inorganic phosphate

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TABLE I.—*Phosphates of Cerebral Cortex Slices Respiring with Glucose and Glutamate as Substrates.*

Species.	Substrate.	Repiration (μmol. O_2/gm./hr.).	Inorganic phosphate (μmol./gm.).	Creatine phosphate (μmol./gm.).
Rat	Glucose	91	3·45	1.14
,,	,,	93	3·4	1·10
Rat	Glutamate	105	4·6	0·31
,,	,,	110	5·05	0·26
Guinea-pig	Glucose	66	2·95	1·16
,,	,,	61	3·2	1·12
Guinea-pig	Glutamate	75	4·4	0·29
,,	,,	79	5·0	0·33

Slices were in glycylglycine-buffered salines (1 ml./20 mgm. slice), and were extracted after respiration at 37° on O_2 for 40 min. Substrates, 0·015 M, were present throughout.

and phosphocreatine (for references see McIlwain, 1950; Richter, 1951). Phosphocreatine falls and inorganic phosphate rises with increased central activity. During the preparation of slices for metabolic experiments the concentrations of these substances in the tissue change greatly; creatine phosphate falls to some 0·3 μmol./gm. and inorganic phosphate rises to some 6 μmol./gm. Metabolism with glucose as substrate partly restores these substances to their levels in the normal tissues (McIlwain, Buchel and Cheshire, 1951). Examples are shown in Table I in which creatine phosphate has been raised to 1·1–1·2 μmol./gm. and inorganic phosphate lowered to 2·9–3·4 μmol./gm. by respiration with glucose as substrate. Glucose has thus restored the tissue to a condition in which it can respond to stimulation (see below).

Respiration with glutamate as substrate did not have this effect. Creatine phosphate remained at the low level of some 0·3 μmol./gm. found in the absence of substrate or of oxygen. Also, inorganic phosphate was not lowered to the same extent as in the presence of glucose. Glutamate, moreover, lowered the creatine phosphate and increased inorganic phosphate when added to tissues already respiring in glucose. The concentration of glutamates in these experiments were ample for high rates of respiration and were typical of those used by previous workers. Lesser concentrations of glutamate and also glutamine had lesser effects on the phosphates (unpublished).

Response to electrical stimulation.—Isolated portions of tissue from the central nervous system, respiring in glucose, were found to respond to suitable electrical impulses by large increases in respiration and glycolysis (McIlwain, Anguiano and Cheshire, 1951). The respiration which took place in the absence of added substrate was not increased by electrical stimulation. The increase with glucose is illustrated by some of the results of Fig. 1 and Table II. Fig. 1 shows the course of respiration of fragments of rat cerebral cortex; A and B are similar until at 44 min. electrical pulses are applied to A, when its rate of respiration doubles. In Fig. 1 the slopes of the lines give rates of respiration, and such rates are recorded in Table II. This table shows the increase in respiration following a variety of different types of impulses.

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TABLE II.—*Effects of Electrical Changes on Respiration with Glutamate and Glucose.*

Electrical arrangements.				Respiration in successive periods (μmol. O_2/gm./hr.).		
Vessel and electrodes (see McIlwain, 1951*a* and *b*).	Impulse and voltage (a.c. : alternating current ; C : condenser pulses).	Tissue.	Substrates (M).	Unstimulated.	Stimulated.	Unstimulated.
A, A	a.c. ; 2·5 V.	Slice, fixed	Glucose	63	98	62
A, A	a.c. ; 2 V., 3 V.	,, ,,	Glutamate	77	70	60
A′, C	a.c. ; 2·5 V.	,, ,,	,,	75	72	63
A′, F	a.c. ; 2·5 V.	,, ,,	,,	68	70	63
E	C ; 24 V. (0·5 μsec.).	Slice, fragments	Glucose	64	110	63
E	C ; 24 V. (0·5 μsec.).	,, ,,	Glutamate	68	68	63
E	C ; 24 V. (0·5 μsec.).	,, ,,	Glucose and glutamate	68	66	59
E	C ; 24 V. (0·1 μsec.).	Slice, fragments	Glucose	63	93	63
E	C ; 24 V. (0·1 μsec.).	,, ,,	Glutamate	69	67	60
E	C ; 24 V. (0·1 μsec.).	,, ,,	Glucose and glutamate	72	70	61

Slices of guinea-pig cerebral cortex in phosphate-buffered glucose salines (approx. 1 ml./20 mgm. slice) were shaken in O_2 at 37°. The periods were of 40 min. each, commencing about 30 min. after death of the animal. The electrical charges applied were alternating current (a.c.) at 50 cyc./sec. or condenser pulses (C), of which there is quoted the peak voltage, and time taken to fall to one-third of the peak voltage. Substrate present throughout were all at 0·015 M. Slice fragments had a mean weight of about 2 mgm.

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Impulses which were effective with glucose as substrate had, however, little or no effect when glutamate was used as substrate. Thus in Fig. 1 lines C and D diverge only a little during the application of impulses to C, between 44 and 78 min. There was also little or no response with the variety of impulses of Table II. These included changes of between 2 and 24 V., lasting 0·1 to 20 msec., and frequencies of 50 or 100 sec. For choice of these values see McIlwain (1951*a*). Any effect of stimulation in the presence of glutamate appeared to be to delay the fall in respiration which normally occurred during experiments with glutamate as substrate. When the current was switched off respiration in most cases fell.

Impulses applied to tissue metabolizing in the presence of both glucose and glutamate were also ineffective, or nearly so (Table II). Similar results were given when the order of adding the substrates or applying the current were varied. Thus, addition of glutamate to tissue which was already responding to stimulation by any increase in respiration caused, if anything, a small fall in respiration (Fig. 1, A). This was in marked contrast to the effect of adding glutamate to unstimulated tissue respiring in glucose, when respiration increased (Fig. 1, B). Again, when glucose was added to a slice which was respiring in glutamate and to which impulses were being applied, any increase in respiration was very small (Fig. 1, C). The impulses were, however, maintaining the respiration to some extent, as it fell when the impulses were stopped.

Discussion.

Although glutamic acid has supported the respiration of cerebral tissues, it has not otherwise reacted as might be expected of a substance supporting the activity of the brain. It has not increased, but has decreased, the tissue-content of the energy-rich creatine phosphate. It has not enabled the tissue to respond metabolically to electrical stimulation, but has prevented response in preparations otherwise capable of doing so. Of these findings, the increased respiration has been known previously. It has been suggested that this increase was related to improved behaviour or mental functioning following administration of glutamic acid to rats, to defective children, or to subjects in hypoglycaemic coma. Our other findings might, on similar grounds, lead to opposite expectations. The reasoning, however, is tenuous. Weil-Malherbe (1950) has concluded that certain of these actions may be secondary to the release of adrenalin which glutamic acid brings about in intact animals.

The present findings may, however, offer a basis for the anticonvulsive actions of glutamic acid. Though these actions are surpassed by those of synthetic anti-convulsive drugs, glutamic acid reduces the number of *petit mal* seizures in a proportion of individuals (Price, Waelsch and Putnam, 1943; see Pond and Pond, 1951). The reduction in electrical excitability of the tissue which we observe gives an immediate basis for anticonvulsive action. Feeding extra glutamic acid is reported not to affect electroshock seizures in various experimental animals (Toman, Swinyard and Goodman, 1946), but to raise the threshold to electroshock in pyridoxine-deficient rats (Davenport and Davenport, 1948). Intravenously administered glutamic acid possibly reaches

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the cerebral tissues of mice (Waelsch, 1949; Schwerin, Bessman and Waelsch, 1950), but this has not been demonstrated in the rat following intravenous (Friedberg and Greenberg, 1947), oral or parenteral (Tigerman and MacVicar 1951) administration. Variable access to the tissue may explain the variable findings in man. Here orally administered glutamic acid is known to reach the blood stream (Bessman, Magnes, Schwerin and Waelsch, 1948; Pond and Pond, 1951), but has not been determined in the brain itself.

Independently of such interpretations, the present findings show an excellent correlation between the tissue level of creatine phosphate (which is known to support the activity of the brain *in vivo*) and the ability of the tissue to respond to electrical stimulation. It is especially noteworthy that glutamic acid lowers the creatine phosphate of tissues already respiring with glucose, and greatly reduces their excitability.

The present findings do not mean that glutamic acid or its amide glutamine are unimportant in nervous tissues, but only that under the conditions employed in the experiments the substances cannot be supposed to be major energy-yielding fuels. This is, however, a significant limitation. The conditions include those under which glutamic acid increases the respiration of cerebral tissues. Moreover, there are indications that glutamic acid and glutamine do not serve as major energy-yielding substrates for the brain *in vivo*. The oxygen uptake of the brain determined *in vivo* by arterial-venous difference is nearly equivalent to the glucose oxidized (Himwich and Himwich, 1946), though glutamic acid and glutamine are also available in the blood stream and in the brain. The glutamic acid content of the brain of insulin-hypoglycaemic rats is lower than normal (Dawson, 1949), but general blood-amino-acid levels are also lower. If the lower level in the brain implies usage by the brain, insulin coma shows that the usage is not adequate for maintaining normal cerebral activities. Mayer-Gross and Walker's (1949) experiments are also consistent with this view in indicating that any action of administered glutamic acid in insulin hypoglycaemia, apart from that secondary to change in blood glucose, is a relatively small one, and is shared by substances which do not act as energy-yielding substrates in the brain. In these experiments, contributions to the effects of glutamic acid which were not due to changes in blood glucose may have been made by other changed blood constituents such as pyruvic and α-ketoglutaric acids (cf. Villano and Rota, 1947).

SUMMARY.

1. Glutamic acid, unlike glucose, does not maintain the creatine phosphate of separated portions of mammalian cerebral cortex.

2. Cerebral tissues respiring with glutamic acid as substrate give little or no response to electrical stimulation. Response is given with glucose as substrate, but not with a mixture of glutamic acid and glucose.

3. Respiratory findings with cerebral tissues and glutamic acid therefore do not necessarily imply that it can act as an energy-yielding substrate for the brain. The present results may be related to the small degree of anti-convulsant activity shown by glutamic acid.

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