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SUBSTANCES WHICH SUPPORT RESPIRATION AND METABOLIC RESPONSE TO ELECTRICAL IMPULSES IN HUMAN CEREBRAL TISSUES

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Suggestions have frequently been made that changes in cerebral activity of endogenous origin are reflected or induced by changes in the utilization of substrates by the brain. Substances which may act as substrates have been administered with therapeutic intent, for when changes in the utilization of substrate are caused experimentally, as in hypoxia, hypoglycaemia, and with some added drugs, large changes in cerebral functioning can ensue.

The main energy-yielding substrates for the human brain under normal circumstances have been found by studies of arterial-venous difference to be glucose and oxygen. Their interaction involves many intermediates which may themselves act as energy-yielding substrates, and under unusual conditions still further substances may act as major substrates for the cerebral tissues of experimental animals. In man, knowledge of which additional substrates will support cerebral activities is, however, limited, and results mainly from the administration of materials to subjects in hypoglycaemic coma. Some findings are discussed at the end of this paper. In experimental animals secondary changes inherent in such methods have been minimized by using hepatectomized or eviscerated subjects, or by perfusion techniques, but this could be done only exceptionally in man and does not yet appear to have been carried out.

By radically different techniques, biopsy material from cerebral surgery can afford excellent opportunities for examining the abilities of various substances to support cerebral activities. Such material is the basis of the present studies. These depend on previous findings (McIlwain, 1951a, b, c) that electrical impulses of types which excite motor or other responses when suitably applied to the brain *in situ* can be caused to induce metabolic changes in separated cerebral tissues *in vitro*. The requirements for such metabolic responses are remarkably close to those which modify electrical responses in the central nervous system *in vivo*. However, only certain substrates maintain tissues from laboratory animals in a condition permitting response to applied impulses (McIlwain, 1951c; Kratzing, 1953). A number of potential substrates have now been examined in this respect with human tissues.

EXPERIMENTAL

Tissues

Human tissues were available to us through the collaboration of Mr. Murray A. Falconer, Director of the Guy's-Maudsley Neurosurgical Unit. Advantage was taken of neurosurgical operations for leucotomy, lobectomy, and cerebral tumours to obtain specimens of macroscopically normal brain tissue. These specimens were removed from subjects under general anaesthesia, care being taken that their blood supply was not interrupted until the last possible moment (usually less than 30 seconds) before excision. The specimens were then placed in small, dry, glass or polythene containers. These were surrounded by crushed ice in a vacuum can and brought promptly to the laboratory. Here, arrangements for the metabolic experiment had already been made. The time between removal of the tissue, its preparation, and restoring to good metabolic conditions in experimental vessels, was between 25 and 90 minutes. During most of this time (noted in the individual experiments) the specimen was at 0°.

Cerebral specimens from rhesus monkeys were obtained under much the same conditions as those from neurosurgical operations. They were from animals in which small cerebral lesions had been induced for other purposes. The present specimens were remote from the site of such lesions. The cerebral cortex was again exposed while the animals were anaesthetized and the specimens removed and cooled promptly.

Except for the experiments of Table VI, guinea-pigs (adult) were killed by a blow on the back of the neck, the

skull opened and the brain removed, and prepared without cooling to 0°. Guinea-pigs were anaesthetized (only when noted) by breathing ether through a mask, adjusted manually to maintain somnolence and absence of response to touching the eye and to pressure on the paw.

Preparation of Tissues.—Human specimens which presented an area of cortex much larger than 3 sq. cm. were cut into two. Specimens or sections of specimens were freed from membranes and large blood vessels. Such sections or a whole guinea-pig hemisphere, while moist with the experimental saline, were cut at their cortical surfaces into slices of 0·3 to 0·4 mm. in thickness with razor blading and a recessed glass slide as guide. Slices were trimmed to a suitable size, adherent saline removed by draining on a glass surface, and the slices weighed. They were either (a) fitted to grid electrodes or (b) cut to about 20 fragments when ring electrode vessels were used. This fashion of handling the tissue yields weights (" wet weights ") which have been increased about 30% over those of the fresh tissues by adhering or absorbed saline. (Mean water content of tissue as received, 82·1% ; after preparation, 86·7%.) No allowance has been made for this in the tables or results section of this paper, but values corrected for such saline are quoted in the discussion.

Salines

Phosphate-glucose saline contained 134 mM-NaCl ; 5·4 mM-KCl ; 1·34 mM-KH_2PO_4, $MgSO_4$, and $CaCl_2$; 13 mM-glucose ; 10·4 mM-Na_2HPO_4 brought to pH 7·4 by HCl ; it was saturated with O_2. Bicarbonate saline contained 124 mM-NaCl ; 5 mM-KCl, 1·24 mM-KH_2PO_4, $MgSO_4$ and $CaCl_2$; 12 mM-glucose and 26·1 mM-$NaHCO_3$, and was equilibrated with 5% CO_2 in O_2.

When several substrates were being examined in the same experiment, tissues were cut and handled in salines without added substrate ; the substrates were placed in the main compartment of the individual vessels employed.

Apparatus

Orthodox manometric apparatus was used with the following additions. Grid electrodes were those of Ayres and McIlwain (1953) and fitted to vessels A (McIlwain, 1951a). Ring electrode vessels were vessels E (McIlwain, 1951b). Electrical impulses consisted of condenser discharges from the apparatus of Ayres and McIlwain (1953), giving 100 pulses/sec. at voltage and duration (time-constant) measured during the experiment and noted in the tables below.

Determinations

Oxygen pressure was determined manometrically each 5 minutes. At the end of the manometric measurements the set of vessels was maintained shaking in the thermostat, one after another removed, and a measured portion (usually 0·5 ml.) of the salines in which the tissue had been respiring was taken and treated with the copper-lime reagents for the determination of lactic acid according to Barker and Summerson (1941).

RESULTS

Unstimulated Respiration with Adequate Glucose : Nature of Specimens

Details are given in Table I of all specimens examined. They derive from 13 operations carried out over a period of 18 months. Some of the operations yielded specimens large enough to be divided into two, which are given separate numbers. They derive from two main cortical areas, frontal (8) and temporal (5).

TABLE I

ORIGIN OF TISSUES

Serial No.	Patient	Sex	Age	Diagnosis	Area Examined
1	K	F	43	Psychomotor epilepsy	Temporal
2	N	M	62	Depression	Frontal
3	N	M	62	Depression	Frontal, adjacent to 2
4	C	M	38	Cerebral tumour	Temporal, in sulcus
5	C	M	38	Cerebral tumour	Temporal, outer cortex
6	M	F	37	Depression	Frontal
7	M	F	37	Obsession	Frontal
8	M	F	37	Obsession	Frontal, adjacent to 7
9	S	M	35	Obsession	Frontal
10	K	F	37	Paranoid schizophrenia	Frontai
11	K	F	37	Paranoid schizophrenia	Frontal, adjacent to 10
12	N	M	56	Recurrent depression	Frontal
13	N	M	56	Recurrent depression	Frontal, adjacent to 12
14	A	F	45	Agitated depression	Frontal
15	A	F	45	Agitated depression	Frontal, adjacent to 14
16	M	F	14	Psychomotor epilepsy	Temporal
17	M	F	14	Psychomotor epilepsy	Temporal, adjacent to 16
18	T	F	53	Depression	Frontal
19	T	F	53	Depression	Frontal, adjacent to 18
20	M	M	45	Psychomotor epilepsy	Temporal
21	M	M	45	Psychomotor epilepsy	Temporal, adjacent to 20
22	W	M	23	Psychomotor epilepsy	Temporal

Initial Respiratory Rates

Part of the material from every operation from which specimens were available to us was examined under a uniform set of conditions ; other parts were examined in a variety of ways discussed below. The uniform conditions consisted of the measurement of the respiratory rate of two samples of the specimen in

the phosphate-buffered saline with 10 mM glucose as substrate. Six readings of oxygen pressure at five-minute intervals were made for this purpose. Thereafter one of the specimens was allowed to respire without further change for about 90 minutes longer, during part of which time electrical impulses were applied to the other. Lactic acid was then determined in each.

The initial respiratory rates are given in Table II, and to aid their interpretation corresponding values are quoted for guinea-pig cerebral cortex. These employed the same apparatus and batches of reagents and were carried out by the same observers at times in between the experiments with human tissues. They permit the following conclusions.

TABLE II

VARIATION IN RESPIRATORY RATES AMONG ADJACENT SAMPLES AND AMONG DIFFERENT EXPERIMENTS WITH HUMAN AND GUINEA-PIG CEREBRAL CORTEX*

	Man			Guinea-pig		
	No. of Observations	Mean	S.D.	No. of Observations	Mean	S.D.
A. Respiratory rate† (μmoles O_2 g./hr.)	36	40·8	8·9	24	62·9	5·6
B. Difference between the two members of pairs of observations on adjacent samples (μmoles O_2/g./hr.)	18	2·47	3·07	12	3·17	3·3
Mean differences B as % of mean rate A		6·0			5·0	

*With human tissues, the rates quoted are from all but one of the specimens available; one of a pair of determinations was spoiled. The 12 specimens yielded 18 pairs of values as six of the specimens were large enough to subdivide. With both human and guinea-pig tissues each pair of values which is compared derives from one experiment in which some four to eight samples of the tissue were handled at the same time and with most of the reagents in common. The differences from one pair to another are in the piece of tissue used for the samples, and in the batches of reagents.

† Specimens from frontal area: mean, 42·6 and S.D. 8·7; from temporal area, mean 39·2 and S.D. 9·4.

(1) The mean respiratory rate of human cerebral cortex under the present conditions was 41 μmoles O_2/g./hr. or about two-thirds those of guinea-pig. (2) The pieces of human tissue used in any one experiment were as uniform in respiratory rate as those from guinea-pig. The average area of cortex examined was about 2 × 1·5 cm., and usually from this two to four successive slices of 0·35 mm. were cut. A piece much larger than this was divided into two and two experiments were run simultaneously with the different portions. Thus the pieces of tissue whose rates are compared in any one experiment had initially *in vivo* been in positions close to each other, usually adjoining. In about half the instances they came from different depths in the cortex. In this sense the pairs of determinations both with guinea-pig and human tissues were not duplicates. No attempt was made to subdivide the tissue so that each sample might be representative of the whole specimen as considerable subdivision was not desirable and greater value was attributed to the possibility of showing differences between different parts of the same human specimen. Such differences were not usually found. This can be seen by the magnitude of the differences between samples from the same specimen (Table II). With human material this averaged 6% of the mean respiratory rate, which was similar to the value of 5% with guinea-pig tissue. (3) The different pieces of human tissue obtained at different operations, however, varied among themselves in respiratory rate much more than did tissues obtained from different guinea-pigs. This conclusion is derived from Table II which shows the large scatter with human tissues; the extreme values were 54% and 140% of the mean. This is affected by factors which include the following:—(*a*) Variations from one experiment to another, assuming the tissue to be uniform: there appears to be no fully valid reason for this being greater with human than with guinea-pig tissue. (*b*) Differences in the handling of the specimens from the time of their removal to the beginning of measurements: the fashion of removing human tissues was different from that of removing guinea-pig brain. It might be said to be more satisfactory metabolically in that the subject was anaesthetized and blood circulation was maintained until within a minute before their removal, or less satisfactory in that material was kept, cool, for longer times between removal and the experiment. Some study has been made of these factors (McIlwain, Ayres, and Forda, 1952; and below) and they appear minor in comparison with the treatment of the tissue in cutting, weighing, and transference to the experimental salines. They, however, remain not fully studied experimentally. (*c*) Inherent differences in respiratory rate between the different specimens: variation here includes the two major possibilities of different respiratory activity in different normal cortical areas and of differences induced by pathological processes. Considering the first possibility, a clear answer can be given in terms of gross cortical anatomy. The specimens derived in about equal numbers from frontal and temporal areas, and no significant difference was shown between these two groups. Other differences in location of the tissue have not been noted systematically, but in two instances cortex from a given operation but cut from a

surface facing into a sulcus respired at a higher rate than samples from the same block but facing outwards towards the dura. The nature of the specimens precludes a clear conclusion as to whether this is typical of human cerebral tissues or whether it and the respiratory variations as a whole are pathologically conditioned.

Maintenance of Respiratory Rate

Respiration of specimens of human tissues after the first 30 minutes, quoted in Table IV, is seen to remain stable for at least a further 90 minutes. Values for 19 tissues showed a mean decrease of 2 μmoles/g./hr. in the fourth 30-minute period in comparison with the first. It is clear from this that the differences between different specimens, discussed above, were persistent ones and not characteristic only of the initial period of the experiments. In the 19 vessels to which impulses (see below) had been applied to adjacent pieces of tissue during the second and third periods, the mean final rate was 2·5 μmoles/g./hr. (S.D. 4·9) higher than the mean initial rate. The maintenance of metabolic rate with glucose as substrate is contrasted below with the behaviour towards some other substrates.

The respiratory rate was also examined in two of the specimens after portions had been kept in their container at 0° overnight. Specimen 10 after keeping for 18 hours had an initial rate 66% of the adjacent specimen examined promptly, and the rate was maintained during the subsequent 90 minutes. Specimen 18 after keeping for 19 hours gave a corresponding value of 79%, again maintained.

Unstimulated Glycolysis with Adequate Glucose

With the limited material available, experiments were not planned to yield rates of glycolysis continuously during the experiments. Instead, the lactic acid which accumulated in the vessels with glucose as substrate, was in almost all cases determined at the end of the experiment.

The 12 experiments then yielded a mean accumulation of lactic acid of 48 μmoles/g./experiment. The experiments were of 130 to 140 minutes' duration, except three experiments which differed from this by five minutes, and one which was of 190 minutes. This, expressed as a rate of accumulation (for observations on the validity of this see McIlwain, Anguiano, and Cheshire, 1951), is 21·3 μmoles/g./hr. (Table III). It is thus close to the value found for guinea-pig tissues. It corresponds to a rate of glucose utilization of 11 μmoles/g./hr., while the concomitant rate of respiration of human tissues accounts for about 7 μmoles glucose/g./hr. Individual values often differed considerably from the mean: their standard deviation was 19·1 μmoles/g./exp. or 9·5 μmoles/g./hr. Aerobic glycolysis is well known to be a labile property of cerebral tissues. Values obtained in these laboratories for the same property measured in guinea-pig cerebral cortex show (Table III) a greater uniformity with any one mode of preparation than did human tissues, but considerable differences when the manner

TABLE III

RESPIRATION, GLYCOLYSIS, AND RESPIRATORY AND GLYCOLYTIC RESPONSES TO APPLIED IMPULSES WITH GLUCOSE AS SUBSTRATE*

Tissue and Experimental Conditions	Mean Respiratory Rate			Mean Accumulation of Lactic Acid		
	(*a*) Unstimulated (μmoles/g./hr.)	(*b*) At 10 V. (% of *a*)	(*c*) At 18 V. (% of *a*)	(*d*) Unstimulated (μmoles/g./hr.)	(*e*) Increase with Applied Impulses (μmoles/g./hr.)	(*f*) Stimulated Rate (% of *d*)
Human, sliced, in grid electrodes	40·8 (40 ; ±8·9)	140 (5 ; ± 11·9)	144 (8 ; ± 11·3)	22 (8 ; ± 7·3)	18 (10 ; ± 10·3)	186 (10 ; ± 54)
Human, sliced, in concentric electrodes		156 (10 ; ± 31)	197 (8 ; ± 26)	21 (4 ; ± 12·4)	15·6 (5 ; ± 10·1)	182 (5 ; ± 46)
Guinea-pig, sliced, in concentric electrodes	60·8 (18 ; ± 6·0)	157 (8 ; ± 15)	193 (7 ; ± 25)	21 (7 ; ± 5)	39 (9 ; ± 12·3)	288 (9 ; ± 51)
Guinea-pig, chopped, in concentric electrodes	59·7 (18 ; 5·5)	133 (8 ; 14)	192 (7 ; 22)	35 (7 ; ± 5·6)	37 (9 ; ± 17·8)	203 (9 ; 43)

*The number of specimens and standard deviation are given in brackets below each mean value. Data with some individual specimens are given in Table IV. About 70 mg. moist weight of tissue was used in each experiment in 3·5 ml. saline. Values in column (*a*) are from the first 30 minutes' metabolism and (*b*) and (*c*) from subsequent periods during which some of the vessels were subjected to condenser pulses of time-constant 0·3–0·4 msec. at the peak potentials quoted. A further period without applied impulses usually followed. Lactate accumulating was determined at the end of the experiments; the quantity found in vessels without applied impulses, divided by the time of the experiment, have the values of column (*d*). The difference between pairs of vessels, with and without pulses, and divided by the time during which pulses were applied, gave values of column (*c*). Impulses were usually applied for 30 minutes at 10 V. then 30 minutes at 18 V., so that the increase in lactate is not maximal but is the result of maximal and submaximal stimulation.

of preparing the tissues was changed. The variation in glycolysis of human tissues is too great to be the subject of the type of consideration given to variation in normal respiratory rate.

Electrical Impulses on Metabolism with Adequate Glucose

Previous experiments (McIlwain and others, 1952) have shown that suitable electrical impulses can greatly increase both respiration and aerobic glycolysis in human cerebral tissues, as is the case with cerebral tissues from all other species examined. Such an increase was also invariably obtained with the present specimens. Summarized data are given in Table III and individual examples in Table IV. Results in Table III fall into two groups according to the electrode arrangements employed. Grid electrodes are seen to give a smaller but more consistent response than the concentric electrodes ; the performance and advantages of the two types are discussed elsewhere (Ayres and McIlwain, 1953). The mean response in concentric electrodes, as a percentage of the initial rate, was closely similar in human and in guinea-pig tissues. One importance of these results lies in their acting as positive controls to other vessels in the same experiment which contained other samples of the same tissue provided with other substrates or other quantities of glucose. For this reason, individual values for respiration and respiratory response are quoted in Table IV.

Metabolism with Various Substrates

These investigations have been carried out with a background of knowledge of how tissues from experimental animals react in the presence of the various substrates now examined in human tissues (McIlwain, 1951c ; Kratzing, 1953 ; see Himwich, 1951). Experiments in these laboratories with animal tissues were interpolated between those with human tissues and employed the same reagents and apparatus. When, therefore, a substrate behaved in much the same way with human tissues as it did with those from laboratory animals, it was not studied with human tissues in great detail. The limited amount of human tissue was used in preference for more detailed study of situations in which human tissue appeared to be exceptional.

Results with substrates which behaved with human as with animal tissues are given in Table IV. The ability of each substance to support normal metabolism and metabolism with applied impulses has been examined and compared with the performance of pieces of the same tissue specimen, with glucose as substrate.

In the absence of substrate, initial rates were lower than with glucose, and were poorly maintained whether or not impulses were applied (McIlwain, 1953 ; an example is given in Table IV).

Lactate.—Lactate at 30 mM, in two specimens maintained well a rate of respiration as high as did glucose. Moreover, it enabled the tissues to respond fully to applied impulses.

TABLE IV

RESPIRATION OF HUMAN CEREBRAL CORTEX WITH VARIOUS SUBSTRATES IN PRESENCE AND ABSENCE OF APPLIED IMPULSES*

Substrate (mM)	Specimen (see Table I)	Respiration during Successive 30-min. Periods (μmoles O_2/g./hr.)			
		1st	2nd	3rd	4th
None	10	34	*32*	**24**	22
Lactate, 30	10	54	59	59	59
Lactate, 30	10	52	*69*	**79**	52
Lactate, 30	11	48	48	46	50
Lactate, 30	11	38	*44*	**54**	41
Glucose, 10	11	44	*54*	**66**	46
Pyruvate, 33	22	48	54	48	49
Pyruvate, 33	22	43	*71*	**83**	47
Pyruvate, 10	22	45	53	46	47
Pyruvate, 10	22	44	*55*	**60**	52
Glucose, 10	22	45	48	47	49
Glucose, 10	22	42	*56*	**61**	45
Succinate, 30	11	42	44	42	42
Succinate, 30	11	43	*41*	**46**	44
Fumarate, 20	17	45	36	27	27
Fumarate, 20	17	45	*39*	**39**	29
Glucose, 10	17	46	*66*	**63**	45
Fumarate, 20	18	55	45	39	36
Fumarate, 20	18	48	*54*	**40**	33
Glucose, 5·7	18	54	54	54	54
Glucose, 5·7	18	51	*81*	**81**	54
Citrate, 30	13	34	25	20	20
Citrate, 30	13	31	*35*	25	20
Glucose, 10	13	45	47	44	44
Citrate, 19	17	51	*36*	**36**	24
Citrate, 20	18	72	66	57	42
Citrate, 20	18	60	*54*	**42**	27

*Values in *italics* give rates during periods when impulses of peak potential 10 V. were applied to the specimens ; in **bold** type, when peak potential was 18 V ; time-constant, 0·4 m.sec.

Results with glucose as substrate are given with material from each operation and included for comparison with the response to the other substrates. For graphs of the course of experiments similar to these, see McIlwain and others (1952).

Pyruvate.—Pyruvate at 10 or 33 mM both maintained a normal respiratory rate, and permitted response to impulses as did glucose.

Succinate.—Succinate at 30 mM maintained respiration without permitting response to impulses.

Fumarate.—Fumarate at 20 mM did not maintain a normal rate of respiration. The rate fell with time much as occurred in the absence of any substrate. The fall was possibly delayed by applied impulses but these had little if any stimulating effect in tissue which responded well with glucose as substrate.

Citrate.—Citrate supported respiratory rates which were initially a little more or less than those with glucose, but these invariably fell during subsequent experimental periods. Impulses might a little delay or accelerate the fall, but when impulses were stopped the final rates were always lower than those of otherwise similar tissues which had not been exposed to impulses.

Glutamic Acid as Substrate

L-Glutamic acid maintained well the respiratory rate of human cerebral tissues and supported their respiratory response to applied impulses (Table V). It does not have these effects in tissues from experimental animals (McIlwain, 1951c). Glutamic acid has recently attracted much attention in relation to cerebral functioning. Its behaviour as substrate has accordingly been examined in the following ways.

(1) Four specimens of human cerebral tissues were examined and found to behave in the same way towards glutamates. The specimens included ones which were low, moderate, and high in their initial respiratory rates with either glucose or glutamate. Glutamate is not, therefore, acting only by making good some defect in tissues of unusual respiratory rate. (2) Respiration and respiratory response to impulses were maintained in human tissues supplied with a mixture of glucose and glutamate (Table IV). This, also, does not occur in tissues from the guinea-pig or rat. (3) Experiments such as those already reported with glutamic acid and tissues of guinea-pig (McIlwain, 1951c) were repeated concurrently with the present studies, using the same reagents and vessels. These included two samples of glutamic acid of different origin. Absence of response to glutamic acid was confirmed. (4) The treatment to which the human tissues had been subjected before the metabolic experiment was imitated with tissues from guinea-pigs and rats (Table VI). In one case the tissue, removed as usual from the experimental animal, was placed in one of the polythene containers employed with neurosurgical material and kept at 0° for one hour before slicing for metabolism. This caused no change. In other experiments a guinea-pig and a rat were maintained under ether anaesthesia for 30 to 40 minutes before tissue was taken; the tissue was then kept at 0° before preparation. The weight of tissue placed in the container used for this purpose was approximately the same as many of the neurosurgical specimens; this might have been important as volatile anaesthetic was smelt in each case on opening the container. Tissue from laboratory animals still gave no respiratory response with glutamic acid as substrate.

TABLE V

RESPIRATORY RESPONSE IN HUMAN TISSUES WITH GLUTAMATE AND GLUCOSE AS SUBSTRATES*

Substrate (mM)	Tissue Specimen	Respiration in Successive Periods (μmoles O_2/g./hr.)			
		1st (no impulses)	2nd	3rd	4th (no impulses)
Glucose, 10	2	29	29	29	29
Glucose, 10	2	26	*38*	55	33
Na-L glutamate, 15 ..	2	44	40	40	36
Na-L glutamate, 15 ..	2	39	*51*	54	33
Glucose, 15	9	35	*64*	66	34
Na-L glutamate, 15 ..	9	49	*49*	50	39
Na-L glutamate, 30 ..	9	49	*49*	58	45
Na-L glutamate, 30 ..	9	50	49	49	45
Glucose, 15	12	48	48	48	48
Glucose, 15	12	51	*108*	122	63
Glucose, 15 + Na-L glutamate, 30	12	50	50	47	47
Glucose, 15 + Na-L glutamate, 30	12	49	*93*	125	65
Na-L glutamate, 30 ..	12	55	60	58	58
Na-L glutamate, 30 ..	12	57	*81*	81	48
Glucose, 15	14	48	46	46	46
Glucose, 15	14	45	74	74	54
Na-L glutamate, 30 ..	14	51	60	60	58
Na-L glutamate, 30 ..	14	52	54	54	51
Na-L glutamate, 30 + glucose, 15	14	60	57	57	50
Na-L glutamate, 30 + glucose, 15	14	60	72	72	49

*Conditions and notation as in Table IV.

Cerebral Cortex from Rhesus Monkey

Two specimens were examined as described in Table VII. Each showed respiratory rates and respiratory and glycolytic responses of magnitudes

TABLE VI

RESPONSE TO IMPULSES WITH GLUTAMIC ACID AS SUBSTRATE IN TISSUES FROM LABORATORY ANIMALS TREATED TO SIMULATE CONDITIONS ASSOCIATED WITH NEUROSURGICAL SPECIMENS*

Material	Substrate (mM)	Respiration in Successive Periods (μmoles O_2/g./hr.)			
		1st	2nd	3rd	4th
Guinea-pig, cerebral hemisphere kept at 0° 1 hr. before cutting	Glucose, 15	69	73	66	68
	Glucose, 15	65	**121**	**122**	70
	Glutamic acid, 30	90	**74**	**58**	53
Guinea-pig under ether for 40 min. before death; hemisphere kept at 0° 105 min. before cutting	Glucose, 15	63	66	63	60
	Glucose, 15	59	**108**	**115**	63
	Glutamic acid, 30	87	**76**	**58**	42
	Glutamic acid, 30+glucose, 15	84	71	51	39
	Glutamic acid, 30+glucose, 15	83	**84**	**65**	47
Rat under ether for 30 min. before death; hemisphere kept at 0° for 90 min. before cutting	Glucose, 15	55	70	70	73
	Glucose, 15	64	**104**	**96**	72
	Glutamate, 30	71	**65**	**46**	52
	Glucose, 15 + glutamate, 30	75	71	65	50
	Glucose, 15 + glutamate, 30	70	75	47	39

*Conditions and notation as in Table IV.

TABLE VII

RESPIRATION OF CEREBRAL TISSUES OF RHESUS MONKEY ON APPLYING IMPULSES WITH VARIOUS SUBSTRATES*

Material	Substrate	Respiration in Successive Periods (μmoles O_2/g./hr.)				Lactic Acid (μmoles/g./whole experiment)
		1st	2nd	3rd	4th	
Anaesthetized with ether; right occipital cortex	Glucose, 0·015	61	63	63	60	55
	Glucose, 0·015	59	**81**	**83**	61	98
	Lactate, 0·03	63	60	60	58	—
	Lactate, 0·03	69	**75**	**78**	55	—
	Lactate, 0·03	63	**84**	**79**	57	—
	Succinate, 0·03	59	**50**	**40**	40	—
	Succinate, 0·03	60	**58**	**55**	45	—
Nembutal anaesthesia; frontal region	Glucose, 0·015	57	57	—	57	111
	Glucose, 0·015	63	**93**	—	62	140
	Glutamate, 0·03	57	56	—	52	—
	Glutamate, 0·03	57	**57**	—	54	—
	Glutamate, 0·03+glucose, 0·15	57	**66**	—	55	—

*Conditions and notation as in Table IV.

close to those found with tissues from other species. The rates of glycolyis of the second specimen were higher than usual, but glycolysis is a relatively labile property of cerebral tissues. Respiratory rates with lactate and succinate were similar to those described above with human tissues, and elsewhere with other species. Again, lactate but not succinate supported respiratory response. Results with glutamate and glucose were therefore of especial interest. In the instance examined, glutamate maintained respiratory rate for longer than it did with lower animals. No respiratory response to impulses was observed. A small response was, however, found with glucose and glutamate together as substrates. The tissue thus behaved towards glutamate in a way intermediate to those from man and from lower animals.

DISCUSSION

Metabolism without Applied Impulses

Study of electrically stimulated metabolism of cerebral tissues was the main object of the present work, but this was necessarily carried out in comparison with study of metabolism in the absence of applied impulses. Respiration of unstimulated human cerebral tissues with glucose as substrate has been measured by other workers with results similar to those of Table IV. Direct comparison cannot always be made as ratios for the fresh weight, wet weight, and dry weight of the tissues employed are not always available. Supposing those of page 257 to be generally applicable, the present value of 55 μmoles O_2/g. fresh wt./hr. for respiration with glucose as substrate may be compared with those of 53 μmoles O_2/g. fresh wt./hr. and 63 μmoles O_2/g. fresh wt./hr. of Elliott and Penfield (1948) and Elliott and Sutherland (1952). Media lacking calcium salts give higher rates (McIlwain, 1952): 87 μmoles O_2/g. fresh wt./hr. (Utena and Ezoe, 1952) and 89 (Elliott and Penfield, 1948).

The spread of values in the present study (S.D. 10·6) moreover is similar to that found by Elliott and Penfield (1948). In trying to assess whether such variability is inherent in normal cerebral cortex or peculiar to the tissues examined, it must be recalled that in each of these studies the tissues examined were removed for therapeutic purposes. Comparable respiratory data for localized cortical areas in a brain with convolutions or otherwise comparable to that of man do not appear to be available. *In vivo* a fall of 20 to 30% in human cerebral respiratory rate is associated with considerable functional changes. This is true of insulin hypoglycaemia (Kety, Woodford, Harmel, Freyhan, Appel, and

Schmidt, 1948a), pentothal narcosis (Wechsler, Dripps, and Kety, 1951), diabetic acidosis (Kety, Polis, Nadler, and Schmidt, 1948b), and uraemia (Heyman, Patterson, and Jones, 1951) and contrasts with the large range of values found for apparently similar tissues in the present studies. However, an almost comparably large scatter is found in the values observed in normal subjects *in vivo* (Kety and others, 1948a ; Heyman and others, 1951). The scatter is beyond experimental error and may or may not be due to causes similar to those operating in the separated tissue. Human cerebral tissues showing clear structural abnormalities have been examined within the same period as those of the present study, and have often shown respiratory rates markedly different from those of Table IV.

Respiratory rates with substances other than glucose as substrate were not available when the present experiments were carried out, but Elliott and Sutherland's (1952) publication now gives values with lactate and pyruvate which are similar to those of Table IV. Rates with succinate were higher initially but less stable than in the present studies. Rates which were markedly poorly maintained are quoted in Table IV with fumarate and citrate, substrates apparently not studied with human tissues by other investigators. Fumarate but not citrate gave poorly maintained rates with guinea-pig cerebral tissues (Kratzing, 1953).

Metabolism with Applied Impulses

Respiratory Rate with Glucose.—The mean rate with maximal electrical impulses (Table III) corresponds to about 110 μmoles/g. fresh wt./hr. This may be compared with the normal rate of cerebral metabolism *in vivo* which is some 3·3 ml. 0_2/100 g./hr. (Kety and others, 1948a and b) corresponding to 88 μmoles/g./hr. This is presumably an average rate for different parts of the brain which differ in respiratory rate when examined *in vitro* (Dixon and Meyer, 1936 ; Himwich, 1951 ; McIlwain, 1953). Suppose, to obtain an approximate estimate, that the brain consists of equal parts of grey matter and white matter, of which the grey has twice the respiratory rate of the white. Then that of the grey matter *in vivo* would be 118 μmoles/g./hr. Thus the respiratory rate of the separated tissue in the presence of applied impulses is one which corresponds more closely to that of the brain *in vivo* than does the rate as ordinarily determined. Other alterations also bring about higher rates *in vitro* ; for example, media low in Ca salts to which are added certain intermediates in carbohydrate metabolism (Krebs, 1950), or materials of natural occurrence such as serum. It is not known whether glucose remains the main substrate in such media, as is the case *in vivo* and in tissues electrically stimulated with glucose as the only added substrate.

That respiratory rate with applied impulses is closest to that *in vivo* is understandable as fluctuating electrical potentials are part of the normal environment of cerebral tissues *in vivo*. It is true that applied impulses *in vivo* can probably increase respiratory rate still further, but this takes place for a brief period only and is not to be compared with the sustained high rate normal *in vivo* or obtained with applied impulses *in vitro*. Metabolism at low glucose levels has also shown the electrically stimulated tissue to afford the closest parallel *in vitro* to the behaviour of the brain *in situ* (McIlwain, 1953).

Substrates Other than Glucose.—Metabolic rates with different substrates in the presence and absence of electrical impulses raise matters related to the mechanism of response to such impulses. Many of these have been considered elsewhere (McIlwain and Gore, 1953 ; Kratzing, 1953) in relation to tissues from laboratory animals, and the present discussion concerns points more specifically related to the findings with human tissues.

Many substances increase the respiration of cerebral tissues in the absence of applied impulses but have little effect on the brain *in vivo* (see Himwich, 1951). In contrast, the present findings show that the substances which will support the respiratory response to applied impulses are more limited. Although limited they include some substances for which evidence of action *in vivo* is debatable. Thus Himwich, Bowman, Daly, Fazekas, Wortis, and Goldfarb (1941) and Wortis, Bowman, Goldfarb, Fazekas, and Himwich (1941) found little or no change in cerebral metabolism or recovery from insulin hypoglycaemia in subjects to whom about 10 g. of lactates or pyruvates were administered. Other experiments (Goldfarb and Wortis, 1941) have suggested a small increase in arterial-venous difference in oxygen to follow administration of pyruvate. The hypoglycaemic situation may not be a satisfactory one in which to observe the effects of carbohydrate intermediates, though findings (Stone, 1938 ; Klein and Olsen, 1947) with experimental animals without insulin administration suggest poor penetration to the brain to be important in the failure of the carboxylic acids as substrates. Pyruvate has been reported to antagonize barbiturate depression (Westfall, 1946), an observation consistent with its acting as substrate though other intermediation is possible.

These considerations are emphasized by the findings with glutamate. Under suitable conditions in experimental animals, administered glutamate or glutamine may reach the brain (Waelsch, 1949; Tigerman and MacVicar, 1951; see also Schwerin, Bessman, and Waelsch, 1950; Friedberg and Greenberg, 1947). In human subjects, a variety of effects involving the central nervous system have been found to follow administration of the acid. These include amelioration of petit mal and the associated electrical characteristics of the brain (Price, Waelsch, and Putnam, 1943; Pond and Pond, 1951). No effect as anticonvulsant could be found in experimental animals (Toman and Goodman, 1948), a difference of interest in relation to the present findings of greater metabolic activity of the acid in human cerebral tissues. The effect of the acid on intelligence in mentally defective subjects has been reported (Albert, Hoch, and Waelsch, 1946, 1951; Zimmermann, Burgemeister and Putnam, 1947 and 1948) and not always paralleled in (presumably mentally normal) rats (Stellar and McElroy, 1948). Again, such a species difference is understandable in terms of the present findings but others must be taken into consideration. Weil-Malherbe (1950) and Milliken and Standen (1951) give relevant experimental work and assess other studies not quoted here.

A third group of studies on administered glutamic acid has shown indubitable effects on the central nervous system, of which, however, a major part is indirect. The acid will rouse subjects from insulin hypoglycaemic coma (Mayer-Gross and Walker, 1949) but only in association with adrenalin release and a resulting increase in the blood glucose level (Weil-Malherbe, 1950). Nevertheless, the blood glucose level at the time when such subjects were aroused with glutamic acid, was less than the level associated with arousal following administration of glucose itself (Mayer-Gross and Walker, 1949). This leaves scope for an action of glutamic acid as substrate capable of supporting cerebral activities such as has been found in the present study.

Succinate has been reported to restore cerebral functioning following administration of barbiturates (Soskin and Taubenhaus, 1943; Castex, Camponovo, and Labourt, 1948; Ghilardi Mezzich, 1949) and in alcoholism (Katz, 1952). For these effects the present studies offer no parallel. The former report was not substantiated by Lardy, Hansen, and Phillips (1944).

SUMMARY

Human cerebral tissues removed during operations for prefrontal and temporal lobectomy afforded about 40 specimens for determining the initial respiratory rate in a phosphate-buffered saline with glucose as substrate. The mean rate so obtained was of 55 μmoles 0_2/g. fresh tissue/hr. The variation in respiratory rate was relatively large (S.D. 10·6) but rates were not systematically different in tissue from frontal and temporal areas. The rates changed little if at all during the first two hours' metabolism.

Applied electrical impulses increased respiration to up to 110 μmoles 0_2/g.hr., which is close to the probable respiratory rate of human cerebral cortex *in situ*.

Aerobic accumulation of lactic acid with glucose as substrate proceeded concomitantly with normal respiration and at about 25 μmole/g. fresh wt./hr. This also could be doubled by applied electrical impulses.

The respiratory rate of human cerebral tissues in a phosphate saline without substrate was lower initially than when glucose was present and fell further after 30 minutes' metabolism. Electrical impulses effective with glucose were then without action. Fumarate as the only substrate was without effect on the lower rate, on the fall in rate, and on the rate with applied impulses.

Succinate as the only substrate maintained respiration without permitting response to impulses. Citrate maintained less stable rates which were affected little by impulses. Lactate and pyruvate maintained well the respiratory rate of the tissues and permitted clear responses to applied impulses. Reaction to these substrates is similar in human tissues and in those from experimental animals.

With glutamic acid as substrate, human tissues behaved differently from those from other animals examined. Respiratory rate was maintained and responded to applied impulses.

Comparative observations were made on normal and treated guinea-pig tissues and on two specimens from rhesus monkey.

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