Glucose Level, Metabolism, and Response to Electrical Impulses in Cerebral Tissues from Man and Laboratory Animals

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

(*Received* 11 *April* 1953)

When, in man, blood glucose is lowered by insulin from its normal level of some 90 mg./100 ml. (5 mM), cerebral activities become increasingly disturbed until at about 20 mg./100 ml. (1·1 mM) coma has usually ensued. At this point the electrical activity of the brain has greatly changed (the change commencing at some 2·5 mM, Hill, Loe, Theobald & Waddell, 1951) and its oxygen uptake has fallen. Kety *et al.* (1948) showed that the rate fell from 3·4 to 2·6 ml. oxygen/100 g./min., or by 24 %, at 1·1 mM; and to 1·9 ml. oxygen/100 g./min., or by 44 %, at 0·5 mM glucose. Considerable electrical and metabolic changes in cat brain followed the lowering of blood plasma glucose from 12 to 2·5 mM (Olsen & Klein, 1947).

It thus appears that hypoglycaemia by limiting the main substrate of the brain has limited the respiration, the energy derived by respiration, and the energy-consuming electrical activities of the brain. This might be shown to some extent with separated cerebral tissues; i.e. their respiration might fall with falling glucose below about 2·5 mM, becoming obviously lower at 0·5 mM. To judge by many accounts, it is generally believed that such relationships have been demonstrated, but this is not the case. In suggesting correlation *in vivo* and *in vitro*, Gellhorn (1938) quoted Holmes (1930), who showed a difference between the respiration of rabbit cerebral cortex in salt mixtures with no added glucose, and with glucose at 19 mM, but did not study intermediate levels, and Wortis (1935), who described increase in the respiration of a brain preparation between 2×10^{-5} and 0·06 mM, but reported no investigations with higher concentrations. Wortis (1935) suggested that his results agreed with some obtained by Holmes, possibly those quoted above, and with others of Dickens & Greville (1933); these authors, however, were again concerned only with the absence and presence of glucose at about 10 mM in salt mixtures. Dameshek & Myerson (1935), in similarly suggesting correlation, also quote Holmes (1930). More recently, Elliott & Henry (1946) found no fall in the respiration of cell-free suspensions of rat brain when glucose was reduced to levels as low as 0·2 mM, a fall being detectable only at a level estimated to be 0·1 mM. However, Maleci (1947) found the respiration of rabbit cerebral cortex to increase with increasing glucose up to 2 mM.

Certain points lacking in previous studies have now been examined. (*a*) The large fall in respiration *in vivo* during hypoglycaemia (Kety *et al.* 1948) was associated with large changes in electrical activity in the brain. In most of the brain, electrical activity is probably not spontaneous but induced. Methods have been developed in these laboratories (McIlwain 1951*a*, *b*; 1952) of electrically stimulating the metabolism of separated cerebral tissues, and these methods have now been applied with varying glucose levels. (*b*) Human tissues have been examined. (*c*) Tissue preparations which retain appreciable cell structure have been studied over a range of glucose concentrations between 0·3 and 15 mM and in a variety of media.

EXPERIMENTAL

Details given by McIlwain (1951*a*, *b*) were followed with respect to salines, preparation of animal tissues, manometric measurements, and determination of lactic acid. During preparation, the tissues were handled in media either glucose-free or containing the lowest concentration of glucose which was under investigation. Electrode vessels *E* (McIlwain, 1951*b*) were used unless others are specified. Condenser pulses were derived from the circuit of Fig. 1*B* of McIlwain (1951*a*) incorporated in an instrument capable of supplying four vessels simultaneously from the a.c. mains (Ayres & McIlwain, 1953). Typical experiments comprised four to eight vessels, each with 3·5 ml. of saline containing the chosen concentrations of glucose, and 70 mg. fresh wt. of tissue as slices 0·35 mm. in thickness cut with scissors to about 20 fragments. Respiration was measured at 5 min. intervals for periods of 30–40 min. Two, three or four such periods (see the Tables), with or without applied impulses, were included in each experiment. Respiratory rates are given in terms of the wet weight of the tissue, determined by draining and weighing slices which had been floating in saline after cutting. The water content of such tissue was 86·7 %, and of fresh cerebral cortex, 82·1 %.

We are greatly indebted to Mr Murray A. Falconer, Director of the Guy's-Maudsley Neurosurgical Unit, for specimens of human cerebral tissues, which have been described by McIlwain, Ayres & Forda (1952) and McIlwain (1953).

Glucose was determined colorimetrically according to Nelson (1944), but with the following modifications which were found necessary on account of the composition of the media used and of the low concentrations of glucose in them. Portions of the mixture (0·25–1 ml.) were treated with the $CuSO_4$-Na_2SO_4 mixtures (to 2 ml.) in which the quantity of Cu salts was sufficiently increased to leave an excess after any combination with constituents of the medium. The tungstate reagent (0·1 ml.) was added, and samples as large as possible taken for determination. The glucose solutions taken for establishing the standard curve were prepared in the same medium as the solutions being determined, since constituents such as glycylglycine were found to influence the colour developed.

For experiments with serum, blood (30–60 ml.) was removed by syringe from the marginal ear vein of a rabbit, without anticoagulant, and allowed to clot during 3 hr. at 37°. The clot was broken and the whole centrifuged. The serum was placed in dialysis tubing and agitated in running water at 10–15° for between 5 and 24 hr. The glucose remaining was determined and found to be between 0·05 and 1·2 mM, depending on the time of dialysis. To such serum (8 ml.) was added in most experiments 2 ml. of a solution prepared from 10 ml. 0·77 M-NaCl, 0·4 ml. 0·77 M-KCl, 0·1 ml. 0·77 M-KH_2PO_4, and 0·1 ml. 0·77 M-$MgSO_4$; and also 0·15 ml. 0·11 M-$CaCl_2$, 0·75 ml. 0·5 M-glycylglycine, and the chosen quantity of glucose in 0·2 ml. water. In one instance after brief dialysis, the salts added were reduced to two-thirds of these quantities.

RESULTS

Metabolism of tissues in absence of applied electrical impulses

Respiration in oxygenated phosphate-salines. Respiratory rates were first determined under conditions ordinarily regarded as good ones for metabolic studies, with various concentrations of glucose in media containing a balanced salt mixture and buffered at pH 7·2 with phosphates. Respiratory rates in the absence of added glucose were clearly different from those in its presence (Table 1). The rate in absence of glucose was poorly maintained; at the end of 2 hr. it lay between 30 and 80 % of the initial rate, whereas with glucose little change was seen in that time.

However, very little glucose was needed to give the higher and maintained rates. It was difficult from the results of Table 1, to see a clear difference between the respiratory rates when the initial glucose concentration was equal to or greater than that of normal blood (about 5 mM), and when it was about 0·5 mM. This was true in spite of the decrease in glucose concentration brought about by its metabolism by the tissue. When the initial glucose

Table 1. *Cerebral tissues with varying initial glucose*

(Experiments were in phosphate-buffered salines. Grey matter from the outer convexity of the cerebral hemispheres to a depth of 1 mm. and sliced parallel to the outer surface was used in all cases. From guinea pig and rabbit, the specimens were representative of all such cortex; the human specimen was from the right temporal area of a girl of 14 years. Condenser pulses (100/sec., alternating, of time constant 0·4 msec.) were applied at 10 v when values for respiration are in italics, and at 18 v when in bold-face type. Values for lactic acid and glucose in bold-face type are from vessels exposed to pulses as indicated in the columns giving respiratory values.)

Species	Initial added glucose (mM)	Respiration (μmoles O_2/g./hr.) in successive 30 min. periods				Lactic acid (μmoles/g./expt.)	Glucose remaining at end of experiment (mM)
		1st	2nd	3rd	4th		
Guinea pig	0	55	53	46	36	3·8	—
	0	54	*53*	**35**	21	**2·1**	—
	0·67	61	56	54	54	21·2	0·22
	0·67	55	*68*	**90**	60	**18·3**	**0·17**
	2	61	*70*	**106**	59	**24·0**	**1·08**
	10	60	61	60	62	26·5	9·02
	10	57	*67*	**100**	62	**48·5**	**8·80**
Rabbit	0	43	29	26	12	<1	<0·1
	0	44	*35*	**33**	18	**<1**	**<0·1**
	0·38	47	47	47	47	5·02	<0·1
	0·38	42	*47*	**84**	39	**4·04**	**<0·1**
	0·54	50	50	48	50	14·3	0·20
	0·54	51	*55*	**92**	45	**9·2**	**0·15**
	3·8	60	60	60	60	19·0	3·17
	3·8	54	*61*	**102**	53	**26·4**	**3·06**
Man	0	36	36	**34**	30	0·3	<0·1
	0	40	*41*	**43**	32	**0·1**	**<0·1**
	0·57	47	47	47	47	7·6	0·12
	0·57	40	*55*	**70**	42	**4·8**	**0·05**
	1·43	38	38	38	38	18·4	1·04
	1·43	37	*41*	**85**	40	**21**	**0·98**
	3·80	43	43	43	43	15·9	3·28
	3·80	42	*51*	**90**	45	**23·9**	**3·15**

concentration was less than 0·5 mM its final value was about 0·1 mM.

Results from several experiments such as those of Table 1, were therefore combined and are shown in Fig. 1. This suggested that experiments with initial glucose concentrations above zero but below 1·5 mM might show lowered respiratory rates. To avoid situations in which glucose was excessively lowered by metabolism, only the initial rates, in experiments with more than 0·5 mM added glucose, were taken into consideration. These were subjected to the analysis summarized in Table 2. This showed, with a reasonable degree of certainty, that the respiratory rate in rabbit and human tissues was lowered by 9–12 % when the initial glucose was lowered from 5–15 mM to 0·5–1·5 mM. Also, the initial (first 30 min.) and final (fourth 30 min.) rates were compared in a series of eight experiments with initial glucose levels between 0·3 and 1·2 mM. The final rate was 98·5 % (standard error, 3·5) of the initial rate. Thus the lowered rate of initial metabolism at low glucose levels is maintained during the experiments and is not markedly affected by utilization of some of the added glucose.

Respiration in glycylglycine-buffered media and in air. Phosphate can affect the level of respiration in cell-free preparations of cerebral tissues (Banga, Ochoa & Peters, 1939; see McIlwain, 1952), though it is not ordinarily regarded as doing so with sliced cerebral tissues. This does not, however, appear to have been investigated at low levels of glucose, and was therefore studied by replacing phosphate with glycylglycine (see McIlwain, Buchel & Cheshire, 1951) at the same pH. No greater sensitivity to lowered glucose was found (Table 3), though rates throughout tended to be lower in the glycylglycine-buffered medium.

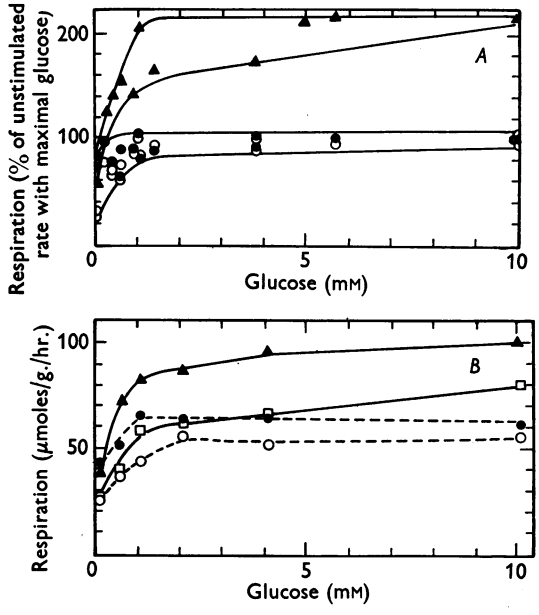

Fig. 1. Respiration of rabbit cerebral cortex with varying glucose concentrations in (*A*) phosphate saline; (*B*) a medium based on dialysed rabbit serum. Experiments were arranged as described in Table 2, and in *B* the ordinate gives actual respiratory rates as quoted in Table 1. In *A* are quoted the results of many experiments extending over some weeks and involving many animals. In different experiments the maximum unstimulated rate with the highest glucose level varied, and other rates were accordingly expressed in the figure as percentage of this rate. The lines in Fig. 1*A* are drawn to enclose the points: ●, initial unstimulated rate; ○, final unstimulated rate; and ▲, rate with tissues stimulated at a peak potential of 18 v. The lines of Fig. 1*B* are drawn through the experimental points; ●, ○ and ▲ as above; □, rate with tissue stimulated at a peak potential of 10 v.

Table 2. *Analysis of results at varying glucose levels*

(Results are derived from experiments such as those of Table 1, but in which the period of stimulation, at 18 v, followed immediately after an unstimulated one. Rates in later, unstimulated periods were not significantly different from those in the initial ones. Choice of the concentration ranges compared was made on the basis of Fig. 1 (see text). In assessing the significance of the differences, the result at low glucose level was subtracted from that at high glucose level in each individual experiment, and the variance of distribution of these differences from the mean difference, made the basis of determining *t*; *P* was read from tables. This was done, rather than comparing mean rates at high and low glucose, because several factors were common to each experiment but could not or could not easily be controlled throughout the series. These were the animals from which tissue or sera were prepared, and the time and details of handling the tissue.)

Species and medium	Rates compared	Mean rate with highest glucose (μmoles O_2/g./hr.)	Mean difference of rate with 0·5–1·5 mM glucose from rates with highest glucose (μmoles/g./hr.)	(% of highest rate)	No. of observations	Significance of difference (*P*)
Rabbit; phosphate saline	Initial	41	−3·75	−9·1	8	0·01
	Stimulated	88	−18	−20	4	0·01
Rabbit; serum preparations	Initial	53·3	−6·0	−11·3	8	0·01
	Stimulated	83	−26·6	−32	8	0·01–0·02
Human; phosphate saline	Initial	46	−5·5	−12	6	0·02–0·05
	Stimulated	94	−39	−41	4	0·01–0·02

Table 3. *Phosphate and oxygen levels on respiration of guinea pig cerebral tissues with varying glucose*

(Values are collected from experiments arranged similarly to Table 1, except that each involved three experimental periods of 40 min. and the rates in these periods are given successively in the columns headed (*a*), (*b*) and (*c*). Each rate is the average of two values in separate experiments. In vessels without impulses, the successive rates refer to the same pieces of tissue. As prolonged stimulation led to lower respiratory rates, the values in columns with impulses were derived from double this number of tissue specimens, half of which had been stimulated during period (*b*) and half during periods (*a*) and (*c*).)

Medium		Respiration (μmoles O_2/g./hr.)											
		In air						In oxygen					
		No impulses			With impulses			No impulses			With impulses		
Buffer	Initial glucose (mM)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)
13 mM-Phosphate	0·3	40	37	40	60	50	52	46	50	46	86	72	42
	1·0	42	51	42	62	80	70	52	50	50	102	95	93
	6·0	46	44	46	75	72	68	59	55	59	108	92	90
50 mM-Glycylglycine with 3 mM-phosphate	0·3	36	30	35	60	43	37	40	40	40	76	56	43
	1·0	44	37	44	73	65	55	44	40	44	86	88	68
	6·0	40	45	40	68	68	52	42	48	42	89	102	78

In intact animals, hypoxia acts synergically with hypoglycaemia in causing failure of cerebral activities (see Himwich, 1951). In the present studies, oxygen rather than air has been used, as high oxygen tension is normally regarded as necessary to ensure adequate oxygenation of slices of the present dimensions (Warburg, 1923; Field, 1948; for observations on stimulated tissue, see McIlwain, 1951*a*). To see whether a lowered oxygen tension sensitized the tissue to low glucose, comparison was made of respiratory rates in oxygen and in air (Table 3). No sensitizing was found. In air the rates were always somewhat lower than in oxygen.

Accumulation of lactic acid. Little lactic acid accumulated when no substrate was added, but it appeared in increasing amounts as the added glucose was increased from 0·3 to 10 mM, in tissue from guinea pig, rabbit, and man (Table 1).

Effects of applied impulses

Respiration. Applied impulses had little effect on respiration in absence of added substrate (Table 1); they might initially delay, or later accentuate, the usual fall in respiration. With even small concentrations of added glucose, impulses stimulated respiration. There can be discerned from Table 1 and Fig. 1 an increase in this effect with increasing glucose, and its extent is made clear by the analysis of Table 2. The lowering of glucose from 5–15 mM to 0·5–1·5 mM in this case reduced respiration by 20–40 %. The experiments with human tissues showed the greatest degree of lowering with lowered glucose, but this does not necessarily reflect a greater sensitivity of human tissues to lowered glucose. The result is dependent on the actual concentrations, between 0·5 and 1·5 mM, which were examined, and these were not the same in the two groups of experiments.

Applied impulses were examined at two intensities, one of which gave maximal stimulation with adequate glucose, while the other gave a smaller response. Decrease in glucose concentration affected about equally the response to both intensities of impulse.

The effects of applied impulses were examined also in glycylglycine-buffered media and with air as gas phase. Neither of these procedures sensitized the tissue to lowered glucose (Table 3). Air markedly limited the effects of applied impulses.

Accumulation of lactic acid. Lactic acid, accumulating in reaction mixtures with tissues to which impulses had been applied, increased throughout the range of glucose concentrations examined (Table 1). Considering, however, the effect of impulses on accumulation at any one glucose level, this effect was not constant but was in some cases a fall and in others a rise. With low initial glucose, impulses decreased lactic acid accumulation while at higher glucose levels they increased it.

Experiments with some related substrates

To interpret some of the preceding results DL-lactic acid was examined as substrate for cerebral tissues (Table 4). In adequate concentrations, it increased the rate of respiration of the tissue and supported a still higher rate on application of impulses. The concentrations of acid required were, however, high in comparison with those of glucose. At 2 mM respiratory rates fell with time much as they did in absence of substrate (compare Table 1). The rates were better maintained by 6·7 mM lactate, but the effect of stimulation remained less than that at 20 mM when it nearly approached that obtainable with glucose as substrate. From these results it is seen that experiments with glucose as substrate are unlikely to be complicated by the produced lactate itself acting as substrate, unless it reaches markedly

Table 4. *Guinea pig cerebral tissues with varying substrates*

(Experimental conditions and recording of results as Table 1.)

Substrate	Initial concentration (mM)	Respiration (μmoles O_2/g./hr.) in successive 30 min. periods 1st	2nd	3rd	4th	Lactic acid (μmoles/g./exp.)
Lactic acid	2·0	54	45	32	25	—
	2·0	58	*56*	**44**	29	—
	6·7	75	76	69	74	—
	6·7	70	*89*	**89**	60	—
	20	76	74	73	61	—
	20	76	*96*	**101**	60	—
Fructose	0·66	50	47	34	22	4·6
	0·66	43	*42*	**35**	21	**0·3**
	2·0	43	41	40	32	4·9
	2·0	50	*58*	**57**	29	**3·3**
	10·0	76	75	82	78	16·5
	10·0	65	*87*	**113**	70	**19·0**
Fructose 1:6-diphosphate	0·66	35	30	19	11	<0·5
	0·66	37	*35*	**22**	10	**<0·5**
	2·0	38	28	17	9	3·1
	2·0	35	*35*	**28**	11	**1·0**
	10·0	50	48	42	27	12·0
	10·0	50	*59*	**36**	28	**5·8**

more than 2 mM in the reaction mixture, i.e. unless the rate of accumulation is more than $2 \times 3{\cdot}5/0{\cdot}07$ or 100 μmoles/g./expt. of 2 hr. with 70 mg. tissue in 3·5 ml. fluid. Presumably L-lactic acid is formed, so that the limit becomes 50 μmoles/g./expt. In fact, lactic acid accumulation in the present experiments was always below this value (Table 1).

With fructose as substrate (Table 4) all the phenomena described in relation to glucose were exhibited but the changes both in respiration and in lactic acid accumulation took place throughout at much higher substrate concentrations. With fructose diphosphate still higher concentrations were required.

Experiments with serum preparations

To assess the influence of some constituents of body fluids on the present findings, preparations from rabbit sera were incorporated in the media. The sera were first dialysed to remove most of their glucose, and inorganic salts were added to them as described in the experimental section. The effect of adding varying concentrations of glucose is shown in one instance in Fig. 1. The sera accentuated the fall in metabolism with lower glucose concentrations. Unstimulated respiration fell with time, not only in absence of glucose but also in its presence. The effect of impulses increased with increase in glucose even beyond 2 mM. An analysis of results with five different specimens of rabbit serum is included in Table 2. The decrease in respiration when glucose was lowered to 0·15–1·5 mM was not much more than occurred in saline media. The decrease of stimulated metabolism was appreciably greater than in saline, and the difference was probably real, for similar concentrations of glucose had been chosen in the two experiments.

DISCUSSION

Cerebral respiration in vivo *and* in vitro. Respiration of cerebral tissues as ordinarily examined *in vitro*, without electrical impulses, is thus much less sensitive to a fall in glucose concentration than is the brain *in vivo*. It is also much less sensitive than has been supposed by many of the workers quoted at the beginning of this paper. The dependence of respiration on the glucose level in the present experiments with sliced tissue from guinea pig, man, and rabbit is similar to that found with 'homogenized' rat cortex by Elliott & Henry (1946).

Electrical stimulation has, however, rendered the tissue much more susceptible to lowered glucose levels. With impulses, tissues with initially 0·5–1·5 mM-glucose respired at rates averaging 20–40 % less than those with optimum glucose. This is the degree of lowering in respiratory rate observed *in vivo* during hypoglycaemic coma. Failure of cerebral respiration in hypoglycaemia can thus be ascribed to metabolic peculiarities of excited cerebral tissues themselves towards glucose, independently of any effects from the rest of the body. As glucose is consumed more rapidly by the stimulated tissue, it is understandable that any deficiency should be more evident in stimulated than in unstimulated tissue. A more specific basis for this may be suggested as follows. Results with fructose indicate that the basis for the fall in respiration with lowered glucose

may lie in the first reactions undergone by glucose in the tissue, as later reactions are probably common to the two sugars. Omitting discussion of complicating factors (see Himwich, 1951) it can be seen that the range of concentration over which the respiration of cerebral tissues is sensitive to glucose and fructose is remarkably similar to the range over which their phosphorylation is sensitive to hexose concentration when catalysed by hexokinase in cerebral preparations. Harpur & Quastel (1949) quote this reaction as sensitive to glucose between 0·1 and 0·5 mM, while with fructose, the maximal rate is only being approached at 20 mM.

A parallelism can be seen between the relatively high concentrations of fructose and lactate required for respiratory response to impulses, and the lack of effect of these substances in the central nervous system when they are administered *in vivo*. High concentrations of fructose can support, with separated cerebral tissues, respiratory rates which are as great as those with glucose (Loebel, 1925; Dickens & Greville, 1933). The uptake of fructose by the brain is however markedly less than the uptake of glucose (Klein, Hurwitz & Olsen, 1946), and fructose does not support cerebral function in hepatectomized animals (Mann, 1927; Mann & Magath, 1922). Lactic acid also did not support activity in human subjects nor in dogs in insulin hypoglycaemia (Wortis, Bowman, Goldfarb, Fazekas & Himwich, 1941). The blood levels of lactate in these studies were usually less than 3 mM. At 6·7 mM-DL-lactate, the respiratory response of the separated tissue to electrical impulses was below optimum (Table 4). Lactic acid also did not restore the electrocorticograms to normal in eviscerated animals (Maddock, Hawkins & Holmes, 1939).

The preceding discussion has been concerned only with average responses to varying glucose levels whereas response at a given level varies with different individual tissue specimens (Fig. 1). The glucose level at which hypoglycaemic symptoms become evident *in vivo* also varies considerably with different subjects (see, for example, Sendrail, 1947) in a manner probably controlled endocrinologically. The phosphorylation of glucose is a likely point for such control to be exerted (Weil-Malherbe & Bone, 1951), and it is noteworthy that cerebral tissues from animals in different endocrinological conditions may retain metabolic differences during examination *in vitro* (see, for example, Eisenberg, Gordan & Elliott, 1949). In distinction to Weil-Malherbe's findings, however, present sera preparations appeared to act in one direction only: they rendered the tissues more susceptible to lowered glucose, while hexokinase was sometimes inhibited and sometimes accelerated by sera. The preparation of sera carried out in the present study, and the greater complexity of the system employed, may contribute to this difference. Different serum specimens have behaved differently with tissues from the same animal, not all giving the type of dependence of Fig. 1*B* in which respiration can be increased by increasing glucose up to 5 mM. This result differs from that with rabbit tissues in saline media (Fig. 1*A*). Hypoglycaemic signs in some but not all human subjects may commence even at blood levels of over 3 mM (Hill *et al.* 1951), and to such phenomena the gradual increase in metabolic activity with the serum of Fig. 1*B* presents a parallel; another possible basis is given below.

Glycolytic and respiratory responses. Lowering the glucose level has an interesting differential effect on the glycolytic and the respiratory responses to applied impulses. These are both present with glucose at 2 mM or higher, and both absent when no glucose is added; but at levels of 0·3–1 mM, a respiratory response occurs without any increase in accumulation of lactic acid. Accumulation may in fact be less when impulses have been applied, though lactate concentration is too low for it to serve as substrate when it is added as such. Endogenous lactic acid may possibly be metabolized when that added is not, but the results are more simply explained by diversion of glucose catabolism to the oxidative pathway at the stage of pyruvic acid. Oxidation of pyruvate by cerebral preparations is maximal at less than 10^{-3} M substrate, while $1–2 \times 10^{-2}$ M of lactate is required (Gavrilescu, Meiklejohn, Passmore & Peters, 1932; Elliott, Scott & Libet, 1942). Such dependence has been determined more accurately with systems from muscle, and pyruvic oxidase activity found to be half-maximal with 2×10^{-5} M-pyruvate, while lactate is required at 10^{-2} M or higher for half-maximal oxidation; also, low concentrations of pyruvic acid inhibit its oxidation (Green & Brosteaux, 1936; Socquet & Laidler, 1950; Jagannathan & Schweet, 1952). The glycolytic response to impulses in cerebral tissues is also more sensitive than the respiratory response when glucose catabolism is limited by iodoacetate (Heald, 1953).

The glycolytic response to applied impulses can increase with increasing glucose even beyond 2 mM, when the respiratory response is at its maximum. Glycolysis in the retina is believed to maintain certain components of the electroretinogram not maintained by respiration (Noell, 1951). The sensitivity of cerebral glycolysis to the glucose level at 2–4 mM may therefore explain the development of hypoglycaemic symptoms while cerebral respiration remains unchanged. This is considered in more detail elsewhere (McIlwain, unpublished).

SUMMARY

1. Respiration of human and of other cerebral tissues examined as slices in nutrient salines, fell by 9–12 % when glucose was lowered from 5–15 mM to

0·5–1·5 mM. This is the range of blood glucose levels in which hypoglycaemic changes, including a large fall in respiration, become evident *in vivo*.

2. Sensitivity of the tissues to low glucose was not increased by replacing oxygen by air, nor by lowering the concentration of inorganic phosphate in the surrounding media.

3. Electrical stimulation of the metabolism of separated tissues rendered them much more susceptible to a fall in glucose level. Respiration of stimulated human cerebral cortex was lower by 30 % at 0·5–1·5 mM than at 5–15 mM.

4. Preparations of rabbit sera rendered the electrically stimulated respiration of rabbit cerebral tissues still more susceptible to lowered glucose levels.

5. The aerobic accumulation of lactic acid in glucose-containing saline media also became more susceptible to lowered glucose levels when the tissues were subjected to electrical impulses.

6. Respiration with lactate as substrate increased as lactate concentration was increased between 2 and 6 mM in absence of stimulation, and at substrate concentrations up to 20 mM with applied impulses. With fructose as substrate both the respiration and the accumulation of lactic acid increased with increasing fructose level up to at least 10 mM, in presence and absence of applied impulses.

I am indebted to Mr A. J. McNeil and to Mr P. J. W. Ayres for their technical assistance during part of this work. Part of the apparatus was made available by a grant by the Board of Governors of the Bethlem Royal and Maudsley Hospital from the Hospital's Research Fund.

REFERENCES

Ayres, P. J. W. & McIlwain, H. (1953). *Biochem. J.* **55**, 607.
Banga, I., Ochoa, S. & Peters, R. A. (1939). *Biochem. J.* **33**, 1980.
Dameshek, W. & Myerson, A. (1935). *Arch. Neurol. Psychiat., Chicago*, **33**, 1.
Dickens, F. & Greville, G. D. (1933). *Biochem. J.* **27**, 832.
Eisenberg, E., Gordan, G. S. & Elliott, H. W. (1949). *Science*, **109**, 337.
Elliott, K. A. C. & Henry, M. (1946). *J. biol. Chem.* **163**, 351.
Elliott, K. A. C., Scott, D. B. McNair & Libet, B. (1942). *J. biol. Chem.* **146**, 251.
Field, J. (1948). *Meth. med. Res.* **1**, 289.
Gavrilescu, N., Meiklejohn, A. P., Passmore, R. & Peters, R. A. (1932). *Proc. Roy. Soc.* B, **110**, 431.
Gellhorn, E. (1938). *Arch. Neurol. Psychiat., Chicago*, **40**, 125.
Green, D. E. & Brosteaux, J. (1936). *Biochem. J.* **30**, 489.
Harpur, R. P. & Quastel, J. H. (1949). *Nature, Lond.*, **164**, 693.
Heald, P. J. (1953). *Biochem. J.* **55**, 625.
Hill, D., Loe, P. St J., Theobald, J. & Waddell, M. (1951). *J. ment. Sci.* **97**, 111.
Himwich, H. E. (1951). *Brain Metabolism and Cerebral Disorders*. Baltimore: Williams and Wilkins.
Holmes, E. G. (1930). *Biochem. J.* **24**, 914.
Jagannathan, V. & Schweet, R. S. (1952). *J. biol. Chem.* **196**, 551.
Kety, S. S., Woodford, R. B., Harmel, M. H., Freyhan, F. A., Appel, K. E. & Schmidt, C. F. (1948). *Amer. J. Psychiat.* **104**, 765.
Klein, J. R., Hurwitz, R. & Olsen, N. S. (1946). *J. biol. Chem.* **164**, 509.
Loebel, R. O. (1925). *Biochem. Z.* **161**, 219.
McIlwain, H. (1951*a*). *Biochem. J.* **49**, 382.
McIlwain, H. (1951*b*). *Biochem. J.* **50**, 132.
McIlwain, H. (1952). *Symp. Biochem. Soc.* no. 8, 27.
McIlwain, H. (1953). (Unpublished observations.)
McIlwain, H., Ayres, P. J. W. & Forda, O. (1952). *J. ment. Sci.* **97**, 674.
McIlwain, H., Buchel, L. & Cheshire, J. D. (1951). *Biochem. J.* **48**, 12.
Maddock, S., Hawkins, J. E. & Holmes, E. (1939). *Amer. J. Physiol.* **125**, 551.
Maleci, O. (1947). *Boll. Soc. ital. Biol. sper.* **23**, 58.
Mann, F. C. (1927). *Medicine, Baltimore*, **6**, 419.
Mann, F. C. & Magath, T. B. (1922). *Arch. int. Med.* **30**, 171.
Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
Noell, W. K. (1951). *J. cell. comp. Physiol.* **37**, 283.
Olsen, N. S. & Klein, J. R. (1947). *Arch. Biochem.* **13**, 343.
Sendrail, M. (1947). *L'hyperinsulinie*. Paris: Masson.
Socquet, I. M. & Laidler, K. J. (1950). *Arch. Biochem.* **25**, 171.
Warburg, O. (1923). *Biochem. Z.* **142**, 317.
Weil-Malherbe, H. & Bone, A. D. (1951). *J. ment. Sci.* **97**, 635.
Wortis, J., Bowman, K. M., Goldfarb, W., Fazekas, J. F. & Himwich, H. E. (1941). *J. Neurophysiol.* **4**, 243.
Wortis, S. B. (1935). *Arch. Neurol. Psychiat., Chicago*, **33**, 1022.