Thiols and the Control of Carbohydrate Metabolism in Cerebral Tissues

By H. McILWAIN

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

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When cerebral tissues are incubated in glucose-containing media, addition of glutathione increases their aerobic glycolysis. This has led to suggestions that glutathione plays a part in the Pasteur effect (Geiger, 1935; Baker, 1937; Weil-Malherbe, 1938). The tissues' respiration and glycolysis in glucose-containing media are increased also by electrical pulses and by 20–100 mM-potassium salts, and these increases are smaller in the presence of cysteine (McIlwain, 1954, and unpublished work).

Cerebral respiration and glycolysis *in vivo* and in ordinary glucose media normally proceed at only $\frac{1}{2}$ – $\frac{1}{3}$ of their maximal rates, and the actions of the thiols mentioned could bear on the mechanism by which such control is effected. Functions in regulating intermediary metabolism have frequently been ascribed to glutathione (see Barron, 1951; Racker, 1954). Accordingly, the concentrations of oxidized and reduced glutathione in cerebral tissues have been determined under different conditions and metabolic effects of glutathione, cysteine and related compounds further explored. In particular, their effects on inorganic phosphate and creatine phosphate have been examined, because earlier studies gave evidence for control of carbohydrate metabolism in cerebral tissues through their inorganic and labile phosphates (McIlwain, 1952*a*, *b*; 1959*a*).

EXPERIMENTAL

Media and experimental details not otherwise specified were those of McIlwain, Buchel & Cheshire (1951) and McIlwain (1951).

Tissues, respiration and glycolysis. Guinea-pig or rat cerebral cortex prepared by dissection at room temperature was chopped (McIlwain & Buddle, 1953) or sliced while moistened with the medium of the experiment. The slices were trimmed to chosen sizes (60–100 mg.), drained by repeatedly touching a glass surface, and weighed and placed in 3 ml. of medium in conical manometric vessels of about 20 ml., within 20 min. of the death of the animal. Vessels were normally equilibrated with oxygen and shaken at 37° and oxygen uptake was measured by conventional procedures. In the experiments so indicated in Table 3, oxygen-free nitrogen was employed as the gas phase, but without reagents for absorbing oxygen. When KCl was added during an experiment it was present initially in a side arm as a M-solution, and 2:4-dinitrophenol as a mM-solution. Most experiments used six to eight samples of

tissue obtained from one animal, and of these samples one or two were used as controls in media without further additions, while additions were made to the others.

Lactic acid produced during metabolism was determined by applying the method of Barker & Summerson (1941) to samples of 0.5 or 1 ml. of medium removed promptly at the end of the manometric experiment. By employing the initial copper-lime precipitation, none of the substances added to the media was found to behave as lactic acid in this determination. In expressing lactic acid formation in μ moles/g. of tissue/hr., it was assumed that the rate of formation was uniform during the experiment; this assumption is an approximation (McIlwain, Anguiano & Cheshire, 1951). When potassium chloride or dinitrophenol had been added in the middle of the period of incubation, the effect of the added substance was calculated by comparing the lactic acid formed in such vessels with that formed in others, run in parallel, but to which the addition had not been made.

Tissue composition. When tissue phosphates were determined, slices which had been incubated in glycylglycine-buffered media were rapidly removed from the manometric vessels with a mounted bent wire, drained for 1–2 sec. on the side of the vessel and ground in 2 ml. of ice-cold 10% (w/v) trichloroacetic acid in a homogenizer tube. The slices were ground within 15 sec. of removing the manometric vessel from the thermostat. The procedure described by Heald (1954) was followed to obtain the barium salts of phosphates insoluble in water, and those soluble in water but insoluble in ethanol, and to determine inorganic phosphate in the former and creatine phosphate in the latter.

For determining glutathione, slices removed in the same manner were quickly rinsed (1–2 sec.) in glucose-phosphate media and ground in 1.5 ml. of ice-cold 3% (w/v) sulphosalicylic acid. Glyoxalase assay of the oxidized and reduced glutathione was completed as described by Martin & McIlwain (1959; see also Thomson & Martin, 1959).

RESULTS

Respiration and glycolysis

Several thiols have now been found to affect the respiration and glycolysis of cerebral tissues incubated in glucose-containing media. Aerobic glycolysis was increased by 10–30 mM-reduced glutathione and by comparable or lower levels of cysteine, homocysteine, mercaptoacetic acid and mercaptoethanol (Table 1). Alanine, glycine and ethanol did not have this action, nor did the oxidized form of glutathione; cystine did not act but is only slightly soluble.

The seven substances of Table 1 had little effect on respiration in ordinary media, though this was not always evaluated accurately owing to the auto-oxidation of several of the thiols. When media containing thiols in the concentrations used in the present experiments were shaken in oxygen at 37°, oxygen was absorbed. This caused pressure changes of up to 12 mm. Hg/hr.; the rates of absorption decreased with time. If these changes were calculated as metabolic rates on the basis of an assumed 60 mg. of tissue, they corresponded to rates of 0–22 μ moles/g./hr. The addition of potassium chloride and 2:4-dinitrophenol did not affect the spontaneous oxidation. In Table 1, oxygen uptake in the presence of thiols and tissue is reported without correction for the pressure changes in the absence of tissue. This is done because cerebral tissues are known to protect some thiols from oxidation (Baker, 1937). Also, a major interest in the present respiratory measurements lay in the change brought about by the addition of potassium chloride. Rates of oxygen uptake were therefore obtained just before and after the

addition, as indicated by the interrupted lines in Fig. 1, and it is these rates which are recorded in Table 1.

Several of the thiols diminished the respiratory response of the tissue to 50 mM-potassium chloride (Table 1, Fig. 1). This was shown most clearly by cysteine (3 and 5 mM), homocysteine (8.3 mM), mercaptoacetic acid (5.5 and 8.3 mM) and by mercaptoethanol (21 mM), but not by glutathione, alanine, glycine or ethanol at comparable concentrations. The thiols had relatively little effect on tissue glycolysis in the presence of 50 mM-potassium chloride.

In the instances examined, thiols diminished also the increase in respiration normally brought about by 2:4-dinitrophenol. In experiments in phosphate media under the conditions of Table 1, 50 μ M-dinitrophenol increased the respiration of guinea-pig cerebral cortex from 63 to 140–160 μ moles/g./hr., but after incubation in 21 mM-mercaptoethanol the increase was only to 80 μ moles/g./hr., whereas in 5.5 mM-mercaptoacetic acid no increase occurred.

Table 1. *Effect of glutathione, cysteine and related compounds on respiration and lactic acid formation by cerebral cortex*

Guinea-pig cerebral cortical slices in glucose-phosphate media were employed, except as indicated below. The glutathione or related compound was present throughout the experiments in the vessels stated, but the KCl was added as a μ solution from a side arm after incubation for 40 min., so increasing the K⁺ ions of the medium from 6 to 53 mM. All tissues were shaken for 80 min. at 37° with an O₂ atmosphere. Values are the mean of results from two or three slices. Values with the following substances did not differ from controls: ethanol, 6, 30 and 60 mM; glycine, 11 and 2.2 mM; alanine, 11 and 2.2 mM.

Added compound (mM)	Uptake of O ₂ (μ moles/g. of tissue/hr.)		Formation of lactic acid (μ moles/g./hr.)	
	No KCl	With KCl	No KCl	With KCl
None	62, 65* 60,† 65†	149, 138* 135,† 155†	16, 18* 20†	40, 50* 41†
GSH, 33	72,* 80†	140†	41,* 36†	—
10	64,* 66†	130	30*	—
3.3	64*	115	22,* 25†	37†
1	65*	—	20*	—
GSSG, 10	58, 58†	126, 134†	18, 21†	50, 38†
3.3	57	130	—	—
1	59	131	—	—
L-Cysteine, 5	77*	72*	26†	36†
3	76,† 73	79,† 84,* 90	33,* 24†	76,* 32†
1	75,† 74*	110,† 103*	—	—
0.6	78,* 69	120,* 118	—	—
L-Cystine (sat.)	61,† 68*	145*	15,† 25*	43*
DL-Homocysteine 8.3	65	93	39	43
1.67	63	121	16	40
Mercaptoacetic acid, 8.3	62	93	40	71
5.5	56	70	28	53
1.67	59, 58†	121	21,† 15	60
2-Mercaptoethanol, 21	60, 62†	62, 60†	27, 33†	72, 39†
5	62, 57†	120, 121†	19, 21†	37, 38†
1.25	58†	119	20	—

* Tissue chopped.

† Medium buffered with glycylglycine.

‡ Rat cerebral cortex, chopped.

Tissue composition

The thiols that were studied brought about considerable changes in the phosphocreatine and inorganic phosphate of cerebral cortical tissues (Table 2). In concentrations which with the different compounds were between 1.7 and 33 mM, reduced glutathione, cysteine, mercaptoacetic acid and mercaptoethanol decreased the phosphocreatine content of the tissue and increased its inorganic phosphate.

Moreover, these changes were correlated with actions of the thiols on carbohydrate metabolism. The concentrations of added compounds which decreased phosphocreatine to below $0.7 \mu\text{mole/g.}$ of tissue were those which increased aerobic glycolysis

and diminished respiratory response to potassium salts. When (Fig. 2) all the values for phosphocreatine in Table 2 are plotted against the corresponding values for aerobic glycolysis, correlation is definite though not close (correlation coefficient 0.59, 19 pairs of values). When, however, results with different concentrations of a given compound are compared, decrease in phosphocreatine is always associated with an increase in aerobic glycolysis. Similarly, decrease in phosphocreatine below $0.7 \mu\text{mole/g.}$ always diminished the respiration observed after adding 50 mM-potassium chloride. Oxidized glutathione, cystine, alanine and glycine at concentrations up to 10 mM were without appreciable effect on the tissue content of inorganic or creatine phosphate.

The diminution in phosphocreatine brought about by potassium chloride (see McIlwain, 1952b) is reproduced in these experiments. Usually an increase occurred in the tissue's inorganic phosphate when its phosphocreatine decreased.

The glutathione of cerebral tissues, in contrast with their phosphocreatine, proved relatively stable in circumstances which greatly altered their carbohydrate metabolism (Table 3). Thus anaerobic conditions or the addition of potassium salts, which increase lactic acid formation, did not alter greatly the balance between oxidized and reduced glutathione. It is notable, however, that cystine increased the oxidized glutathione; it will be

Fig. 1. Oxygen uptake in vessels containing chopped rat cerebral cortex in glucose-phosphate media, with and without the further addition of cysteine or cystine. Results in the presence of cysteine (3 mM) are given by the upper two curves, of which all ordinates have been increased by $50 \mu\text{moles/g.}$ for clarity in the diagram. Results in the presence of cystine (sat. solution) in the same experiment were indistinguishable from the lower two curves, which were obtained in the medium alone in the absence of cystine. Arrows indicate the time at which KCl (to 53 mM) was added in the vessels in which the oxygen uptake is represented by ●; uptake without added KCl is represented by O. The interrupted lines are tangents to two of the curves, drawn at points just before and just after the addition of the KCl.

Fig. 2. Comparison of the effects of added substances on the glycolysis and on the phosphocreatine content of guinea-pig cerebral cortical slices. Media and other conditions of the experiments were as described in Table 2, to which the numbers refer. Lines join points quoting results with media which differed only in their content of a particular added thiol.

recalled that cystine was without appreciable effect on respiration or glycolysis (Table 1). Cysteine and ascorbate also increased the proportion of oxidized glutathione, to an extent which increased with time and is attributed to the oxidation of cysteine and ascorbate during the experiment.

DISCUSSION

The limitation normally imposed on respiration and aerobic glycolysis in cerebral tissues is well displayed in the present experiments. Respiration was increased from 60 to 150 μ moles/g./hr., and lactic acid formation from 20 to 70 μ moles/g./hr.,

Table 2. *Effect of thiols and related compounds on tissue phosphates*

Guinea-pig cerebral cortical slices were employed, after respiration in glycylglycine-buffered media under the conditions described in Table 1. Values are the means of results with two or three slices which agreed within 10%, except when the s.e.m. and number of determinations are quoted. Values with 2 and 5 mM-L-alanine and 11 mM-glycine did not differ from controls.

Expt. no. (see Fig. 2)	Added compounds (mM)	Creatine phosphate (μ moles/g.)	Inorganic phosphate (μ moles/g.)
1	None	1.24 $\pm$ 0.07 (5)	2.79 $\pm$ 0.13 (5)
2	KCl, 50	0.31	3.80
3	GSH, 33	0.73	3.07
4	GSH, 10	0.9	2.88
5	GSH, 5	0.87	3.17
6	GSH, 5; KCl, 50	0.33	4.25
7	GSSG, 10	1.20	2.84
8	Cysteine, 5	0.21	3.49
9	Cysteine, 5; KCl, 50	0.17	3.64
10	Cysteine, 3	0.38	2.76
11	Cysteine, 3; KCl, 50	0.27	3.05
12	Cystine, sat.	0.95	2.24
13	Mercaptoacetic acid, 5.5	0.58	3.36
14	Mercaptoacetic acid, 5.5; KCl, 50	0.32	4.54
15	Mercaptoacetic acid, 1.67	0.76	2.78
16	Mercaptoethanol, 21	0.23	3.74
17	Mercaptoethanol, 5	0.75	2.77
18	Mercaptoethanol, 5; KCl, 50	0.50	3.19
19	Mercaptoethanol, 1.25	1.10	2.64

Table 3. *Proportion of oxidized glutathione in guinea-pig cerebral cortical slices after incubation in phosphate saline*

Slices of 80–100 mg. were shaken in 3.5 ml. of medium for the time indicated, and quickly rinsed in medium without the specified additions and ground in ice-cold sulphosalicylic acid. Glutathione was determined by glyoxalase and the values quoted are the mean of results with two or three slices which agreed to within 7%, except when the s.e.m. and number of values are indicated. The total glutathione in the different experiments and at the different times was between 1 and 1.6 μ moles/g.

Gas	Additions (mM)	Incubation time (min.)		
		30	60	120
		Oxidized glutathione (% of total)		
O ₂	Glucose, 10	14	12 $\pm$ 4 (7)	20
O ₂	Malate, 30	19	16	22
O ₂	Ascorbate, 30	15	17	57*
O ₂	Glucose, 10; KCl, 40	16	14	—
O ₂	Glucose, 10; cysteine, 7	—	28	—
O ₂	Glucose, 10; cysteine, 30	27	47	—
O ₂	Glucose, 10; cysteine, sat.	56	52	—
N ₂	Glucose, 10	12 $\pm$ 3 (4)	—	—
N ₂	Glucose, 10; cysteine, 7	28	—	—
N ₂	Glucose, 10; cysteine, 30	14	—	—
N ₂	Glucose, 10; cysteine, sat.	52	—	—

* At this time the oxygen uptake of tissues with ascorbate had fallen to 35 μ moles of O₂/g./hr. That of others in oxygen remained within the normal values quoted in Table 1.

by appropriate stimulation. For such changes to be mediated by glutathione, appreciable concomitant alteration would be expected in the tissue content of the oxidized or reduced forms of this compound. Such alteration was not found. The reduced form of glutathione preponderated even aerobically, which presumably reflects the action of the potent glutathione reductase of the tissue (McIlwain & Tresize, 1957; McIlwain, 1959*b*). However, cystine could increase oxidized glutathione: but this change did not bring about much alteration in glucose metabolism.

On the other hand, the evidence connecting the concentration of phosphates in cerebral tissues with their rates of respiration and glycolysis (McIlwain, 1952*a, b*, 1959*a*) has been extended. Increase in aerobic glycolysis by glutathione, cysteine, glutathione, mercaptoacetate or mercaptoethanol was accompanied by increased inorganic phosphate and decreased creatine phosphate. Comparable concentrations of non-thiol analogues were without such actions. Glutamic acid has a specific action in lowering creatine phosphate in cerebral tissues (McIlwain, 1952*b*) and also increases respiration and glycolysis when added to glucose-containing media (Weil-Malherbe, 1938; McIlwain, 1959*a*); but the glutamic acid moiety does not appear likely to be responsible for the effects of glutathione, because oxidized glutathione did not have comparable action on creatine phosphate and glycolysis.

In other circumstances in which increased glycolysis and decreased respiratory response have been associated with loss of labile phosphates from cerebral tissues, the changes in respiration and glycolysis have been regarded as resulting from the changes in phosphates (McIlwain, 1952*a, b*, 1959*a*). This is feasible, as inorganic phosphate and phosphate acceptors are necessary for normal respiration and glycolysis and their quantities in the tissue are limited. The present findings are encompassed within this general picture, which, however, leaves unknown the means by which the thiols decrease the amount of creatine phosphate.

SUMMARY

1. Increase in aerobic glycolysis of cerebral cortical tissues by 10–30 mM-glutathione is paral-

leled by 3–20 mM-cysteine, homocysteine, mercaptoacetic acid and mercaptoethanol.

2. Most of these substances diminished the respiration of cerebral tissues in media containing 50 mM-potassium chloride. Specificity in both actions was shown by examining structurally related compounds.

3. The changes in respiration and glycolysis could occur without appreciable change in the balance of oxidized and reduced glutathione in the tissue. Conversely, this balance could be disturbed without marked effect on glucose metabolism.

4. The changes in respiration and glycolysis brought about by the thiols and other agents were associated with change in the tissue's inorganic and creatine phosphates; tissue phosphates are regarded as the main factor in regulating the tissue's glucose metabolism.

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