CCLXXXIX. OBSERVATIONS ON TISSUE GLYCOLYSIS[1]

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(*Received 1 November 1938*)

THE substances whose action will be described are mainly: (1) the ammonium ion, (2) glutamic acid and (3) maleic acid. They affect tissue glycolysis in different ways: either, as with (1), by raising the aerobic glycolysis and at the same time depressing anaerobic glycolysis, or, as with (2), mainly by inhibiting anaerobic glycolysis, or, as with (3), by a slow and continuous rise of the aerobic glycolysis to the normal level of the anaerobic glycolysis. The observed effects only occur or are specially pronounced in brain tissue.

Methods

The experiments were carried out at 37·5° in the Warburg apparatus. Tissue slices, suspended in bicarbonate or phosphate Krebs-Ringer solution containing 0·2 % glucose (*p*H 7·4), were used. In most experiments respiration and glycolysis were simultaneously measured by Warburg's differential method [1930]. Additions to the suspension medium were present from the beginning in most cases, so that the first reading was taken 15–20 min. after the tissue came in contact with the solution. For brain, embryo and medulla of kidney R.Q. of 1, for testis, intestinal mucosa and spleen R.Q. = 0·9 and for Jensen sarcoma R.Q. = 0·8 were assumed [cf. Dickens & Šimer, 1930; 1931; Dickens & Weil-Malherbe, 1936].

Estimations of lactic acid were made according to Friedemann & Kendall [1929] after copper-lime treatment, estimations of NH_3 by steam distillation *in vacuo* and subsequent Nesslerization according to Parnas & Heller [1924].

I. THE EFFECT OF AMMONIUM IONS

Ashford & Dixon [1935] found that addition of $M/10$ KCl to the Ringer solution increases both respiration and aerobic glycolysis of rabbit brain slices, while inhibiting their anaerobic glycolysis. Later, Dixon & Holmes [1935] and Dickens & Greville [1935] showed that the same effect can be obtained with CsCl and RbCl. The effect is absent from tumour, kidney, testis and yolk sac of the rat [Dickens & Greville, 1935] and from chick embryo [Needham *et al.* 1937]. According to Dixon [1937] the effect on brain tissue is seen equally well with $M/20$ KCl, though it is only small at $M/100$.

NH_4Cl affects respiration and glycolysis of brain tissue in a very similar way: $M/30$ NH_4Cl causes a rise of respiration as well as of aerobic glycolysis up to the

[1] Most of the experimental work was carried out in spring and summer 1935, partly at the Biochemical Laboratory, Cambridge. The results were reported at the meeting of the Biochemical Society, December 1935 [see Weil-Malherbe, 1935].

normal level of the anaerobic glycolysis, while at the same time inhibiting the anaerobic glycolysis. As with KCl this effect is, with few exceptions, restricted to brain tissue (Table II). In spleen an increased aerobic glycolysis was found. An effect comparable with that in brain was also observed with intestinal mucous membrane in one experiment. But in another experiment with the same tissue this effect was very small and in a third experiment inhibition of both respiration and glycolysis occurred. The figures for the metabolism of the control were very similar to each other in these and other experiments.[1] With several other glycolysing tissues inhibition of anaerobic glycolysis by $M/30$ NH_4Cl was observed, but the effect on respiration and aerobic glycolysis was negligible or, at most, a slight suggestion of that found with brain.

The action of NH_4^+ on brain however differs in one respect from those of K^+, Cs^+ or Rb^+. Whereas in these cases concentrations $> M/100$ are apparently necessary to produce an effect, the effect of NH_4^+ is still recognizable at concentrations as small as $0{\cdot}3 \times 10^{-3}$ M (Table I), which still cause a marked increase of aerobic glycolysis and also a small stimulation of respiration. Low concentrations ($M/1000$ and less) sometimes seem to stimulate the anaerobic glycolysis as well; but in other experiments, reported in Table XI, inhibition was still observable at $M/1000$.

Table I. *The effect of NH_4^+ on brain metabolism. Slices of guinea-pig brain (grey matter) in bicarbonate-saline*

	Concentration 10^{-3} M NH_4Cl	Q_{O_2} 1st hr.	Q_{O_2} 2nd hr.	$Q_G^{O_2}$ 1st hr.	$Q_G^{O_2}$ 2nd hr.	$Q_G^{N_2}$ 1st hr.
(1)	33·3	−19·5	−10·2	19·2	12·1	4·4
	10	−11·6	− 9·0	8·0	6·1	6·0
	3·3	−11·8	− 8·6	11·2	8·8	19·6
	1	−10·6	− 8·7	9·2	7·2	26·0
	0	− 6·9	− 5·8	3·1	0·6	20·4
(2)	1	−10·8	− 8·8	10·2	7·6	16·0
	0·33	− 9·3	− 6·3	6·8	2·5	15·9
	0·1	− 7·0	− 6·3	4·0	1·0	17·8
	0	− 6·8	− 6·0	4·4	0·9	16·8

Dickens & Greville [1935] explained the K^+ effect as the result of a disturbance of the ionic balance of the medium leading to physical changes in the colloidal structure of the cell protoplasm and probably to increased permeability. The same may be true for NH_4^+ in high concentration, but with NH_4^+-concentrations of the order of 10^{-3} M a specific toxic effect seems more likely.

II. The effect of glutamic acid

(a) *Action on the anaerobic glycolysis*

During his studies on glutamine formation in nervous tissues Krebs [1935] discovered the inhibition of anaerobic lactic acid formation by glutamic acid. On his suggestion I investigated the phenomenon in greater detail.

l(+)Glutamic acid, in a concentration of $M/100$, inhibits the anaerobic glycolysis of brain slices by 30–70%. The inhibition increases with time and

[1] The figures reported here for the normal metabolism of rat intestinal mucous membrane differ from those found in the literature [e.g. Rosenthal & Lasnitzki, 1928]. We observed usually a high aerobic glycolysis of the same order as the respiration. Both activities showed a rather rapid fall.

Table II. *Metabolism of various glycolysing tissues with and without* M/30 NH_4Cl. *Bicarbonate-glucose-Ringer*

		With NH_4Cl						Control					
		Q_{O_2}		$Q_G^{O_2}$		$Q_G^{N_2}$		Q_{O_2}		$Q_G^{O_2}$		$Q_G^{N_2}$	
Species	Tissue	1st hr.	2nd hr.	1st hr.	2nd hr.	1st hr.	2nd hr.	1st hr.	2nd hr.	1st hr.	2nd hr.	1st hr.	2nd hr.
Guinea-pig	Medulla of kidney	−10·2	− 9·7	14·0	12·8	21·6	21·5	− 6·8	− 6·9	14·3	13·1	24·0	22·0
Guinea-pig	Retina	—	—	—	—	61·6	—	—	—	—	—	51·7	—
Rat	Testis	−11·3	− 5·1	3·7	2·1	6·0	4·9	− 7·5	− 7·9	2·9	1·8	7·4	6·5
Rat	Spleen (*a*)	−17·5	—	6·8	—	9·0	—	−10·0	—	4·4	—	11·7	—
	(*b*)	−15·2	−15·4	4·5	7·7	5·0	5·4	−12·9	−12·7	4·2	4·2	7·8	7·3
Rat	Diaphragm	—	—	—	—	3·8	—	—	—	—	—	4·1	—
Rat	Jensen sarcoma	−18·2	−14·7	25·6	19·2	30·9	24·2	−14·4	−12·4	22·8	21·9	29·0	21·6
Mouse	Yolk sac*	—	—	—	—	14·8	10·6	—	—	—	—	24·6	22·4
Mouse	Embryo†	− 8·9	− 8·1	2·9	1·7	9·5	7·7	−10·4	− 8·2	3·1	3·0	7·1	6·2
		1–10 min.	1–60 min.	1–10 min.	1–60 min.	1–10 min.	1–60 min.	1–10 min.	1–60 min.	1–10 min.	1–60 min.	1–10 min.	1–60 min.
Rat	Intestinal mucosa (*a*)	−47·5	−25·0	59·5	33·6	22·1	14·4	−18·7	−16·4	42·0	24·2	45·6	25·0
	(*b*)	−26·7	−18·1	28·4	18·5	23·2	14·8	−24·0	−15·4	26·8	15·5	38·4	20·4
	(*c*)	− 6·3	− 3·7	12·9	6·3	—	13·2	−27·3	−13·4	32·2	15·8	—	20·5

* Almost full term. † Average dry wt. 8 mg.

reaches its maximum value after about 60 min. (Table III). Variation of the glutamic acid concentration between $M/50$ and $M/1000$ does not greatly affect the inhibition.

Table III. *Inhibition of anaerobic glycolysis of brain slices by l(+)glutamic acid*

		$Q_G^{N_2}$						
		With glutamic acid		Control		Inhibition %		
Species	Conc.	1–10 min.	60–80 min.	1–10 min.	60–80 min.	1–10 min.	60–80 min.	Remarks
Rat	$M/100$	10·5	5·1	16·0	13·8	35	63	Mean of 4 exp.
Guinea-pig	$M/100$	12·2	6·0	21·0	18·4	42	67	Mean of 8 exp.
Rat	$M/1000$	13·5	5·4	15·6	12·1	13·5	55·5	
Guinea-pig	$M/1000$	18·2	8·5	18·9	14·8	4	42·5	Mean of 4 exp.

The effect seems to be specific for brain (Table IV). Even in retina it was found to be absent or, at the most, very feeble. Neither the anaerobic glycolysis of yeast cells, nor that of defibrinated blood (guinea-pig), nor the acid production from starch in a dialysed muscle extract (rabbit) was inhibited by glutamic acid.

Table IV. *Effect of l(+)glutamic acid on the anaerobic glycolysis of various glycolysing tissues*

		$Q_G^{N_2}$ (1st hr.)	
Species	Tissue	With $M/100$ glutamate	Control
Guinea-pig	Retina	48·7	51·8
Pigeon	Retina	76·0	81·0
Guinea-pig	Medulla of kidney	27·6	29·0
Rat	Intestinal mucosa	16·3	15·5
Guinea-pig	Intestine (whole wall)	7·5	6·9
Guinea-pig	Spleen	5·3	5·9
Rat	Testis	7·2	7·5
Rat	Diaphragm	6·1	5·9
Rat	Jensen sarcoma	36·9	39·2
Rat	Yolk sac	13·1	12·9
Guinea-pig	Heart	7·2	6·5

Specificity of the effect. A few closely related substances cause similar inhibition of the anaerobic glycolysis of brain tissue. They are: (1) the non-natural isomeride, *d*(−)glutamic acid; (2) glutamine; (3) *dl*-β-hydroxyglutamic acid[1] (Table V and VI). *d*(−)Glutamic acid is slightly less efficient than *l*(+)glutamic acid: at a concentration of $M/3000$ where the *l*-acid is still seen to cause some inhibition even a stimulating effect appears. The effects of the two stereoisomerides in different concentrations are compared in the same experiment (Table V). The actions of glutamine and hydroxyglutamic acid are shown in Table VI. Oxidized and reduced glutathione are also included, although they did not affect the anaerobic glycolysis; there was perhaps an initial activation by the reduced glutathione. The observation of Geiger [1935] that oxidized glutathione inhibits the anaerobic glycolysis of chopped brain could not be confirmed with brain slices. Geiger's results have been severely criticized by Dixon [1937].

Apart from the substances mentioned no other amino-acid of 13 tested[2] (*l*(+)alanine, *l*(+)valine, *l*(−)leucine, *l*(−)methionine, *l*(−)proline, *l*(−)hydroxy-

[1] Gift from Prof. Harington to Dr Krebs.

[2] Concentrations $M/100$. Retention was allowed for where necessary.

Table V. *Inhibition of anaerobic brain glycolysis by various concentrations of* l(+)- *and* d(−)*glutamic acids*

	With *l*(+)glutamic acid					With *d*(−)glutamic acid				
		$Q_G^{N_2}$		% Inhibition			$Q_G^{N_2}$		% Inhibition	
Species	Conc. 10^{-3} *M*	1–10 min.	60–80 min.	1–10 min.	60–80 min.	Conc. 10^{-3} *M*	1–10 min.	60–80 min.	1–10 min.	60–80 min.
Rat	0	15·6	12·1	—	—	0	15·6	12·1	—	—
	10	9·7	3·6	38	70	10	13·2	5·9	15	51
	3	10·9	3·9	30	68	3	11·9	5·8	23·5	52
	1	13·5	5·4	13·5	55	1	13·6	6·9	13	43
	0·3	16·9	9·4	0	22	0·3	20·1	12·7	0	0
Guinea-pig	0	19·6	15·4	—	—	0	19·6	15·4	—	—
	10	14·5	6·7	26	56·5	10	16·3	7·2	17	53
	3	15·2	7·6	22·5	51	3	16·9	11·5	14	25
	1	16·8	6·0	14	61	1	22·6	12·5	0	19
	0·3	17·0	12·2	13	21	0·3	23·5	18·9	0	0

Table VI. *Specificity of the inhibition of anaerobic brain glycolysis by glutamic acid and related substances*

		$Q_G^{N_2}$		% Inhibition		
Species	Addition	1–10 min.	60–80 min.	1–10 min.	60–80 min.	Remarks
Rat	0	16·7	14·8	—	—	Mean of 2 exp.
	M/100 *d*(−)glutamic acid	12·8	8·8	23	41	
Guinea-pig	0	21·1	17·9	—	—	Mean of 5 exp.
	M/100 *d*(−)glutamic acid	16·6	8·5	21	53	
Guinea-pig	0	19·3	17·7	—	—	
	M/100 *l*(+)glutamic acid	10·5	5·5	45	69	
	M/100 *dl*-β-hydroxyglutamic acid	13·6	6·8	30	62	
	M/100 glutamine	14·8	8·1	23	54	
	M/500 glutamine	18·8	14·1	3	20	
Rabbit	0	17·5	12·8	—	—	
	M/100 *d*(−)glutamic acid	12·0	7·1	31	45	
	M/100 glutamine	10·1	4·8	42	62	
Guinea-pig	0	18·6	17·0	—	—	
	M/100 glutathione (GSSG)	18·1	16·8	—	—	
Guinea-pig	0	14·0	10·1	—	—	Gas mixture freed from O_2 over hot copper
	M/100 glutathione (GSH)	19·7	11·8	—	—	

proline, *dl*-serine, *l*(−)aspartic acid, *l*(−)pyrrolidonecarboxylic acid, *l*(+)ornithine, *l*(+)arginine, *l*(−)histidine, *l*(−)tryptophan) affected the anaerobic glycolysis of brain slices. In addition a large number of mono-, di- and tri-basic organic acids (for some of them see Table XVI) were tested. Except for a slight unspecific inhibition in a few cases, no effect was found. The effect of the glutamic acid group is therefore quite specific.

(*b*) *Augmentation of the glutamic acid effect on anaerobic glycolysis*

Meyerhof & Lohmann [1926, 1, 2] found that lactic acid in *M*/100–*M*/50 concentration inhibited anaerobic glycolysis of rat brain by about 50%. As in the case of glutamic acid they observed a similar effect of the natural and unnatural isomerides. Dickens & Greville [1933] who reinvestigated this effect found a similar inhibition of anaerobic brain glycolysis only with higher concentrations (*M*/10–*M*/20); they also found *d*(−)lactate less efficient than *l*(+)lactate.

M/100 *dl*-lactate did not inhibit the anaerobic glycolysis of guinea-pig brain in our experiments; in an experiment with rat brain however some inhibition was observed. M/100 lactate however always enhanced the inhibition caused by glutamic acid. This effect is especially large, when small concentrations of glutamic acid are used (Table VII).

Table VII. *Increase of the glutamic acid effect on anaerobic brain glycolysis*

Species	Addition	$Q_G^{N_2}$ 1–10 min.	$Q_G^{N_2}$ 60–80 min.	% inhibition 1–10 min.	% inhibition 60–80 min.	Remarks
Guinea-pig	0	19·9	17·5	—	—	
	M/100 *dl*-lactate	18·9	16·3	5	7	
	M/100 $l(+)$glutamate	13·1	7·3	34	58	
	M/100 *dl*-lactate + M/100 $l(+)$glutamate	9·6	3·7	52	79	
Rat	0	18·3	10·3	—	—	
	M/100 *dl*-lactate	14·4	6·2	21	40	
	M/1000 $l(+)$glutamate	12·4	6·6	32	36	
	M/100 *dl*-lactate + M/1000 $l(+)$glutamate	7·8	3·1	57	70	
Guinea-pig	0	23·1	20·0	—	—	Mean of 4 exp.
	M/100 succinate	22·5	19·1	3	4	
	M/100 $l(+)$glutamate	14·8	7·0	36	65	
	M/100 succinate + M/100 $l(+)$glutamate	8·3	3·5	64	82·5	
Guinea-pig	0	24·2	21·2	—	—	
	M/1600 $l(+)$glutamate	21·6	14·7	11	31	
	M/100 succinate + M/1600 $l(+)$glutamate	19·6	9·6	19	55	
		1st hr.	2nd hr.	1st hr.	2nd hr.	
Guinea-pig	0	17·4	17·1	—	—	
	M/100 *dl*-lactate	19·8	16·3	0	5	
	M/100 succinate	16·8	15·6	3	9	
	M/100 $l(+)$glutamate	9·1	4·9	48	71	
	M/100 *dl*-lactate + M/100 $l(+)$glutamate	7·9	4·2	55	75·5	
	M/100 succinate + M/100 $l(+)$glutamate	7·2	2·6	59	85	

Another substance which unexpectedly increased the glutamic acid effect was found to be succinic acid. M/100 succinic acid alone never affected the anaerobic glycolysis. In combination with glutamic acid however the residue of the anaerobic glycolysis observed in presence of glutamic acid alone was again reduced by nearly 50 %. The effect can again best be demonstrated with small concentrations of glutamic acid (Table VII, Fig. 1).

Many other substances which were tried in combination with glutamic acid did not influence its action.

(c) *Reversibility of the inhibition of anaerobic glycolysis*

The glutamic acid inhibition of anaerobic glycolysis of brain slices can be reversed by pyruvic acid (Table VIII, Fig. 2). Variation of the pyruvic acid concentration between M/100 and M/1000 does not change the result. The fall of anaerobic glycolysis is not entirely prevented, but only delayed. After some time inhibition again prevails. Pyruvic acid also relieves the inhibition caused by $d(-)$glutamic acid and by the combination of glutamic + succinic acids. The

action of pyruvic acid is less efficient if it is added after the first 20 min. of the experiment.

A similar effect, though to a smaller extent, is displayed by α-ketoglutaric acid (Table VIII).

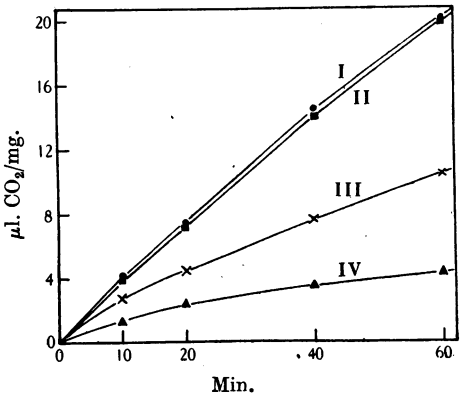

Fig. 1.

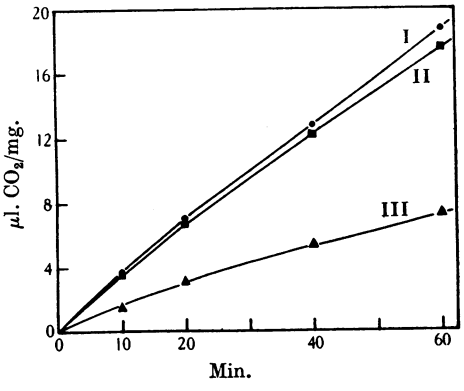

Fig. 2.

Fig. 1. Anaerobic glycolysis of guinea-pig brain slices in bicarbonate-glucose-Ringer. I, no addition; II, with $M/100$ succinate; III, with $M/100$ $l(+)$glutamate; IV, with $M/100$ succinate + $M/100$ $l(+)$glutamate.

Fig. 2. Anaerobic glycolysis of rat brain slices in bicarbonate-glucose-Ringer. I, with $M/100$ pyruvate; II, with $M/100$ pyruvate + $M/100$ $l(+)$glutamate; III, with $M/100$ $l(+)$glutamate.

Table VIII. *Reversal of the glutamic acid effect on anaerobic brain glycolysis*

Species	Addition	$Q_G^{N_2}$ 1–10 min.	$Q_G^{N_2}$ 60–80 min.	% Inhibition 1–10 min.	% Inhibition 60–80 min.
Rat	0	16·7	14·9	—	—
	$M/500$ pyruvate	21·7	17·8	—	—
	$M/100$ $l(+)$glutamate	9·1	6·0	45·5	60
	$M/100$ $l(+)$glutamate + $M/500$ pyruvate	21·6	15·3	0	0
Guinea-pig	0	23·2	19·4	—	—
	$M/100$ pyruvate	22·8	20·3	—	—
	$M/100$ $l(+)$glutamate	15·3	8·4	34·0	56·7
	$M/100$ $l(+)$glutamate + $M/100$ pyruvate	22·0	13·6	5	30
Guinea-pig	0	21·2	18·7	—	—
	$M/100$ $l(+)$glutamate + $M/100$ succinate	10·3	4·9	51·5	74
	$M/100$ $l(+)$glutamate + $M/100$ succinate + $M/100$ pyruvate	19·6	13·7	7·5	26·6
Guinea-pig	0	20·6	17·7	—	—
	$M/100$ $l(+)$glutamate + $M/100$ succinate + $M/100$ pyruvate	17·5	13·4	15	24
	$M/100$ $l(+)$glutamate + $M/100$ succinate + $M/300$ pyruvate	16·7	11·9	19	34
	$M/100$ $l(+)$glutamate + $M/100$ succinate + $M/1000$ pyruvate	19·8	15·2	4	14
Guinea-pig	0	16·9	11·9	—	—
	$M/100$ $d(-)$glutamate	16·0	7·8	5·5	34·5
	$M/100$ $d(-)$glutamate + $M/100$ pyruvate	21·0	10·9	0	8
Guinea-pig	0	18·7	17·0	—	—
	$M/100$ α-ketoglutarate	23·2	21·0	—	—
	$M/100$ $l(+)$glutamate	14·3	7·4	23·5	56·5
	$M/100$ α-ketoglutarate + $M/100$ $l(+)$glutamate	18·2	11·4	3	33

As Mendel *et al.* [1931] have shown, pyruvic acid also counteracts the inhibition of anaerobic glycolysis of tumour tissue caused by glyceraldehyde. The same is true for embryonic tissue [Needham & Nowiński, 1937]. In brain however pyruvic acid cannot prevent the inhibition of anaerobic glycolysis by glyceraldehyde [Holmes, 1934; Baker, 1938].

The increased CO_2 evolution in presence of pyruvic acid is not due to an increased decarboxylation of pyruvic acid. The rate of anaerobic disappearance of pyruvic acid was determined by the carboxylase method after incubation with brain slices in presence and in absence of glucose and of *l*(+)glutamic acid. It was found to be independent of these (Table IX). A similar result has recently

Table IX. *Anaerobic disappearance of pyruvic acid after incubation with guinea-pig brain slices*

Initial concentration of pyruvic acid: *M*/200. Duration of exp.: 90 min.

Addition	$Q_{pyruvate}$ Exp. 1	$Q_{pyruvate}$ Exp. 2
0	−3·8	−3·5
0·2% glucose	−4·4	−3·0
0·2% glucose + *M*/100 *l*(+)glutamate	−4·3	−3·2

been reported by Kritzmann [1938] who found that the disappearance of pyruvic acid from minced brain is not increased by the presence of *l*(+)glutamic acid. From a *Q*-value = 3–4 for pyruvic acid disappearance an extra CO_2 production of 1·5–2 (Q_{CO_2}) can be deduced [cf. Weil-Malherbe, 1937]. This is too small to account for the restoration of the acid production after inhibition by glutamic acid.

(*d*) *Mechanism of the glutamic acid effect*

(1) Since it is known that brain tissue can convert *l*(+)glutamic acid into α-ketoglutaric acid by oxidative deamination [Weil-Malherbe, 1936] the described effects might have been due to a liberation of NH_3 which indeed inhibits the anaerobic glycolysis of brain in fairly low concentrations, as has been shown in the first section of this paper. There are however several objections to this view: (*a*) the deamination is an oxidative process and does not occur anaerobically, at any rate not to any significant extent. (*b*) During the deamination of *l*(+)glutamic acid, owing to secondary reactions, the concentration of free NH_3 in the suspension medium does not rise. This has been well established for brain tissue under aerobic conditions [Krebs, 1935; Weil-Malherbe, 1936]. Table X

Table X. *Anaerobic ammonia formation by guinea-pig brain slices*

Addition (0·2% glucose in all exp.)	$Q_G^{N_3}$ 1st hr.	$Q_{NH_3}^{N_3}$ 1st hr.
0	17·3	0·15
M/100 *l*(+)glutamate	11·4	0·11
M/100 *d*(−)glutamate	11·4	0·23
M/100 pyruvate	15·9	0·18
M/100 *l*(+)glutamate + *M*/100 pyruvate	15·9	0·21
M/100 *d*(−)glutamate + *M*/100 pyruvate	14·3	0·23

shows that there is no increase of free NH_3 in presence of *l*(+)- or *d*(−)glutamic acid under anaerobic conditions either. (*c*) *M*/1000 *l*(+)glutamic acid sometimes has a greater inhibitory effect than *M*/1000 NH_4Cl (Table XI).

Table XI. *Comparison of the inhibitions of anaerobic glycolysis of guinea-pig brain slices by* M/*1000* l(+)*glutamate and* M/*1000* NH_4Cl

Addition	$Q_G^{N_2}$ (1st hr.) Exp. 1	Exp. 2	Exp. 3
0	13·3	15·4	16·8
M/1000 *l*(+)glutamate	10·2	9·5	13·7
M/1000 NH_4Cl	9·3	9·5	16·0

In spite of these facts it could be argued that a transitory liberation of a very small amount of NH_3 inside the cell may suffice to produce the same effects as a higher concentration in the surrounding medium. However, though a very slight deamination of *l*(+)glutamic acid cannot be excluded, this explanation cannot account for the effects of *d*(−)glutamic and β-hydroxyglutamic acids which are, as far as we know, not deaminated by brain tissue. The sample of *d*(−)glutamic acid used was probably optically pure. It was a gift from Dr Krebs and was prepared from the racemic acid by yeast fermentation. Analytical figures were published by Krebs [1935].

(2) Braunstein & Kritzmann [1937] suggested that the intermolecular transfer of amino groups, discovered by them, might, in presence of glutamic acid, lead to the formation of alanine instead of lactic acid from glucose and that this process might explain the inhibition of lactic acid formation by glutamic acid. This reaction would however involve the formation of an equivalent amount of α-ketoglutaric acid from glutamic acid, so that the rate of acid formation would remain the same and no inhibition would result in manometric experiments. It is of course possible that the "trapping" of the pyruvic acid arising during glycolysis deprives the glycolytic enzyme system of its natural hydrogen acceptor and that its replacement by the ketoglutaric acid formed from glutamic acid does not restore the original activity. The reversing effect of added pyruvate supports such an interpretation. But against it the following facts must be considered: (1) there is no increased consumption of pyruvate in presence of glutamic acid, at least as far as can be concluded from carboxylase estimations, (2) since *d*(−)glutamic and β-hydroxyglutamic acids act similarly to *l*(+)glutamic acid, one must assume that they can replace it in the process of amino transfer.

(3) *l*(+)Glutamic, *d*(−)glutamic and *dl*-β-hydroxyglutamic acids were the only substances found by Krebs [1935] to react with the enzyme concerned with the synthesis and hydrolysis of glutamine, the first as a natural substrate, the latter two causing competitive inhibition. Though there is as yet no other evidence for it, it is not impossible that the state of this enzyme, whether combined or free, has something to do with the control of glycolysis in nervous tissues. This would explain why the glutamic acid effect is specific for nervous tissue which is the only one of the highly glycolysing tissues where a synthesis of glutamine occurs.

(e) *Action on aerobic glycolysis*

Whereas the substances of the glutamic acid group depress the glycolysis of brain tissue under anaerobic conditions, they provoke on the other hand an appreciable aerobic lactic acid formation. Freshly cut brain slices, suspended in bicarbonate-glucose-Ringer solution have a fairly high aerobic glycolysis during the first 10–20 min. of the experiment ($Q_G^{O_2}$=4–6). Normally this aerobic glycolysis soon disappears entirely in the further course of the experiment. In the presence of the substances of the glutamic acid group (i.e. *l*(+)- and *d*(−)glutamic acids, glutamine and *dl*-β-hydroxyglutamic acid) it remains at

this high level. The respiration is unchanged or even accelerated, especially with *l*(+)glutamic acid. Reduced glutathione which differs from these substances in its action on anaerobic glycolysis has the same effect upon aerobic glycolysis of brain slices [see also Baker, 1937]. I confirmed Baker's finding that the autoxidation of reduced glutathione becomes quite negligible after a short incubation with brain slices. Nevertheless, only the results of the chemical lactic acid analysis are reproduced in Table XII.

Table XII. *Effect of glutamic acids on aerobic glycolysis of guinea-pig brain slices*

Addition	Q_{O_2} 1st hr.	$Q_G^{O_2}$ 1–10 min.	$Q_G^{O_2}$ 60–80 min.	Remarks
0	−10·3	3·7	1·0	Mean of 6 exp.
M/100 *l*(+)glutamate	−13·7	5·6	4·8	
0	−10·7	3·8	1·3	Mean of 4 exp.
M/100 *d*(−)glutamate	− 9·2	8·7	6·3	
0	− 9·9	4·5	1·7	
M/100 *l*-glutamine	−13·8	7·3	6·1	
0	−10·3	3·0	0·6	
M/100 *dl*-β-hydroxyglutamate	−10·1	5·2	4·1	

	$Q_G^{O_2}$ (2 hr.) Manometrically	Chemically
0	0·8	1·9
M/100 *l*(+)glutamate	4·6	5·5
M/100 *d*(−)glutamate	5·2	6·0
M/100 glutathione (GSH)	—	5·3

The rise of aerobic glycolysis is usually greater with the non-natural than with the natural glutamic acid.

In no other glycolysing tissue was an increase of aerobic glycolysis observed in presence of *l*(+)glutamic acid (Table XIII).

Table XIII. *Aerobic metabolism of various glycolysing tissues with glutamic acid*

Species	Tissue	With *M*/100 *l*(+)glutamate Q_{O_2}	With *M*/100 *l*(+)glutamate $Q_G^{O_2}$	Control Q_{O_2}	Control $Q_G^{O_2}$
Rat	Testis	− 9·9	3·1	− 7·5	3·8
	Spleen	−10·4	2·8	− 9·3	2·6
	Intestine (whole wall)	− 8·0	4·6	− 7·7	4·7
	Jensen sarcoma (*a*)	−10·5	23·3	− 9·7	20·9
	(*b*)	−12·7	26·1	−12·3	30·8
	Yolk sac	− 4·4	1·9	− 2·5	3·9
Guinea-pig	Medulla of kidney	−10·7	14·2	−11·3	13·8

Pasteur-Meyerhof quotient: respiration, aerobic and anaerobic glycolyses of brain slices were simultaneously determined in several cases with both *l*(+)- and *d*(−)glutamic acids. Owing to the inhibition of the anaerobic glycolysis the aerobic glycolysis almost reaches the level of the anaerobic glycolysis. Thus the Pasteur-Meyerhof quotient $\left(\text{P.M.Q.} = \frac{Q_G^{N_2} - Q_G^{O_2}}{Q_{O_2}}\right)$ which was 1·8–2·0 in the controls fell to 0·2–0·6 in presence of glutamic acid. This is usually interpreted as an

indication that the Pasteur reaction is inhibited. But it is doubtful whether much significance can be attributed to these figures. We do not know whether or not the reactions which lead to an inhibition of glycolysis under anaerobic conditions also operate under aerobic conditions. Indeed, in the presence of high concentrations of NH_4^+ or K^+, the aerobic glycolysis rises to the level of the normal anaerobic glycolysis, while the anaerobic glycolysis is strongly inhibited in the same medium. This is obviously a case where aerobic and anaerobic conditions cannot be compared and where the P.M.Q. becomes meaningless. The same may be true for the effects of glutamic acid. As long as the aerobic glycolysis does not reach the level of the *normal* anaerobic glycolysis it is not safe to assume a breakdown of the Pasteur mechanism though, as in this case, the P.M.Q. may be practically zero.

III. The effect of maleic acid

Preliminary notes

(1) In several experiments solutions of maleic acid were prepared from freshly distilled maleic anhydride. The results differed in no way from those obtained with a sample of commercial maleic acid.

(2) Since the effects observed often change so quickly with time, the usual method of calculating average figures for respiration and glycolysis over periods of hours does not give an adequate impression of the real phenomena. The Q-values were therefore calculated for periods of 20 min. and the figures plotted at the midpoint of the corresponding period. Of course only a limited proportion of the experiments which were actually done can thus be communicated.

It will be noticed that $Q_{O_2}^{O_2}$ often assumes negative values in the control experiment after some time. This is probably due to oxidative disappearance of acid initially formed.

(3) *Retention*: Solutions of $M/50$ maleic acid in bicarbonate-Ringer at pH 7·4 have a retention of about 9 %. In the early experiments no allowance for this was made, since it was believed that the smallness of the retention could not greatly influence the results and did not warrant the rather complicated extra analyses and calculations involved. Later however the retention was allowed for, using a new simplified principle developed by Dickens [unpublished]. It appeared that, if no allowance for retention were made, the curve of the aerobic glycolysis was quite similar to, and only slightly lower than the true glycolysis, corresponding to the proportion of the retention. The respiration, too, was hardly affected as long as the aerobic glycolysis was low. When the aerobic glycolysis was high, however, the errors became very serious indeed the respiration appearing too high by 100–150 %. Thus a secondary rise of respiration following the rise of aerobic glycolysis was revealed as spurious. With $M/50$ mixtures of glutamic and maleic acids or citric and maleic acids which have a retention of 12–14 % the errors amounted to several hundred per cent.

(*a*) *The effect on brain metabolism*

The action of maleic acid as a respiratory poison is well known [Thunberg, 1920; Grönvall, 1924; Gözsy & Szent-Györgyi, 1934]. I find that the inhibition of brain respiration depends (1) on the substrate present, (2) on the medium used: it is higher in phosphate than in bicarbonate medium.

(1) The inhibition in phosphate-Ringer solution is largest with glucose as substrate and only slightly less with lactate. It is on the other hand comparatively small with succinate and pyruvate, though it increases with time (Table XIV).

Table XIV. *Inhibition of brain respiration (guinea-pig) in phosphate-Ringer by M/50 maleate in presence of various substrates*

	Q_{O_2}						% Inhibition		
	Without maleate			With maleate					
Substrate added	1st hr.	2nd hr.	3rd hr.	1st hr.	2nd hr.	3rd hr.	1st hr.	2nd hr.	3rd hr.
0·2% glucose	− 9·8	−10·0	−10·3	− 4·3	−1·2	−0·8	56	88	92
M/50 lactate	−10·9	−10·7	−10·8	− 6·3	−1·6	−0·8	42	85	92
M/50 pyruvate	− 9·9	− 9·3	− 9·3	− 8·9	−5·9	−3·5	10	36	62
M/50 ketoglutarate	− 9·2	− 7·9	− 7·0	− 7·2	−3·0	−1·3	22	62	81
M/50 succinate	−12·0	−10·5	− 8·3	−11·3	−6·9	−4·2	6	34	49
M/50 fumarate	− 9·1	− 7·1	− 4·6	− 7·5	−3·3	−1·5	18	54	78

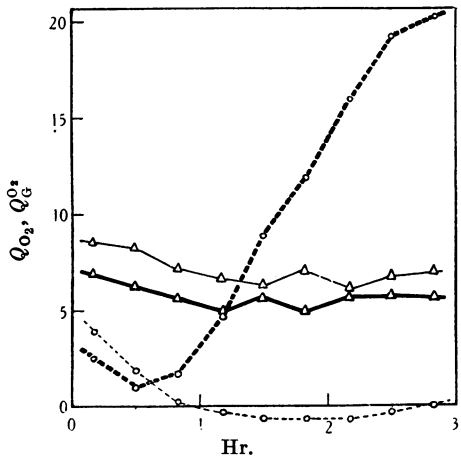

Fig. 3. Respiration and aerobic glycolysis of guinea-pig brain slices. Thick line: with *M*/50 maleate. Retention allowed for. Thin line: control in bicarbonate-glucose-Ringer. △——△ respiration. o- - -o aerobic glycolysis.

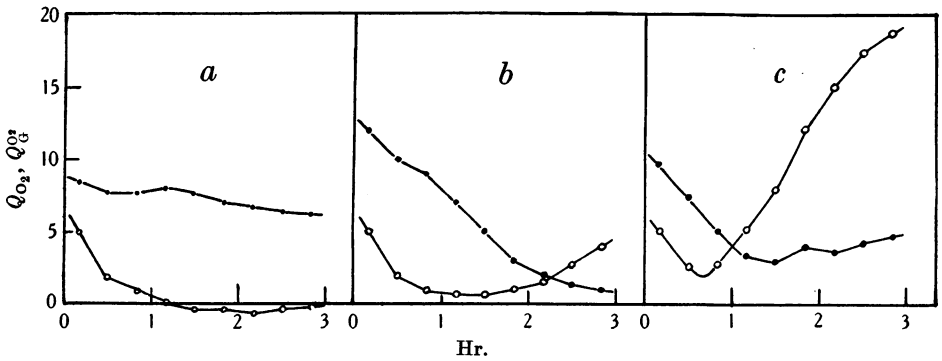

Fig. 4. Respiration and aerobic glycolysis of guinea-pig brain slices. (*a*) control; (*b*) with *M*/100 maleate; (*c*) with *M*/50 maleate. Retention allowed for. •——• respiration. o——o aerobic glycolysis.

(2) In bicarbonate-Ringer solution with glucose as substrate the following observations were made: in presence of *M*/100 maleate there is a slow and steady fall of respiration which at the end of the 3rd hr. reaches almost zero. The aerobic glycolysis does not differ much from the control; a slow rise is sometimes

observed in the 3rd hr. (Fig. 4). If the maleate concentration is increased to $M/50$, the fall of respiration is much less severe. In one experiment (Fig. 4) respiration fell to about 40–50 % of the control within the first 20 min. and remained at this level. In another experiment (Fig. 3) there was only about 20–30 % inhibition throughout. On the other hand there was a steady rise of the aerobic glycolysis starting after 30–60 min. and reaching the level of the normal anaerobic glycolysis towards the end of the 3rd hr. Values of $Q_G^O = 24$ have been observed (see Fig. 7). It seems that the onset of the strong aerobic glycolysis prevents the complete breakdown of respiration in presence of $M/50$ maleate. A concentration of $M/100$ maleate is too small to bring about an early and sufficiently large rise of aerobic glycolysis and therefore the paradoxical result is obtained that in this case the respiration is finally inhibited more strongly than with $M/50$ maleate.

In absence of glucose no acid formation occurs. Chemical analysis shows that the acid formed from glucose is lactic acid (Table XV).

Table XV. *Lactic acid formation (by chemical estimation) in presence of maleate. Guinea-pig brain*

Addition	$Q_{lactate}$ mean of 3 hr.
0	2·5
$M/100$ maleate	5·3
$M/100$ maleate + $M/100$ glutamate	8·2
$M/100$ maleate + $M/100$ glutathione	9·6
0	2·7
$M/50$ maleate	7·2
$M/50$ maleate + $M/50$ glutamate	7·8
$M/50$ maleate + $M/50$ ketoglutarate	6·8

Specificity of the maleic acid effect. Though the action of maleic acid is not entirely a specific inhibition of the Pasteur reaction, it is of considerable interest for several reasons: (1) the inhibition of respiration is comparatively small; (2) maleic acid is a simple organic compound closely related to normal metabolites of the cell. Although there are several di- and tri-basic acids which are known to have toxic actions on cell metabolism, none of these caused such a drastic increase of aerobic glycolysis. The following acids have been tested: malonic, hydroxymalonic, hydroxymaleic, tartaric, racemic, dihydroxytartaric, oxalic, itaconic and citraconic acids (all in $M/50$ concentration). Oxalic acid, for instance, inhibited both respiration and glycolysis. Others, like hydroxymalonic acid, had an inhibitory action on brain respiration which was certainly not smaller than that of maleic acid. But although there was no inhibition of the anaerobic glycolysis,[1] the aerobic glycolysis was not significantly increased. $M/50$ maleic acid did not inhibit the anaerobic glycolysis of brain slices (Table XVI). This is in contrast to the experiments of Morgan & Friedmann [1938, 2] who, it is true, worked under different conditions since they used a concentration of $M/12·5$ and minced brain suspended in phosphate buffer.

It may be mentioned in this connexion that $M/50$ malonate does not affect the respiration of brain slices in bicarbonate-glucose-Ringer solution. In phosphate-glucose-Ringer there is an inhibition of 40–50 % (Table XVI).

[1] Jowett & Quastel [1937] found an inhibition of anaerobic brain glycolysis with $M/14$ hydroxymalonate.

Table XVI. *Metabolism of guinea-pig brain slices in presence of dibasic acids (non-metabolites)*

B = Bicarbonate-glucose-Ringer; P = phosphate-glucose-Ringer.

Addition	Medium	Q_{O_2} 1st hr.	Q_{O_2} 2nd hr.	Q_{O_2} 3rd hr.	$Q_G^{O_2}$ 1st hr.	$Q_G^{O_2}$ 2nd hr.	$Q_G^{O_2}$ 3rd hr.	$Q_G^{N_2}$ 1st hr.	$Q_G^{N_2}$ 2nd hr.	$Q_G^{N_2}$ 3rd hr.
0	B	− 7·8	− 7·0	− 7·0	3·7	0·5	0	17·9	14·6	—
M/60 oxalate*		− 15·0	− 7·1	− 3·7	6·0	2·6	1·9	5·1	3·9	—
M/50 malonate		− 8·4	− 7·0	− 6·9	1·9	0·9	1·6	12·8	10·0	—
M/50 hydroxymalonate		− 7·8	− 4·9	− 5·4	0·2	1·5	1·1	17·7	14·5	—
0	B	− 7·8	− 7·1	—	1·7	− 1·0	—	19·9	18·4	—
M/50 malonate		− 9·4	− 7·8	—	1·6	− 0·7	—	19·5	18·3	—
0	P	− 8·5	− 8·6	—	—	—	—	—	—	—
M/50 malonate		− 5·2	− 4·1	—	—	—	—	—	—	—
0	B	—	—	—	—	—	—	20·5	18·6	—
M/50 maleate		—	—	—	—	—	—	19·9	18·4	—
M/50 hydroxymalonate		—	—	—	—	—	—	20·0	18·9	—
0	B	—	—	—	—	—	—	19·1	16·2	15·7
M/50 maleate		—	—	—	—	—	—	19·2	15·7	15·0

* Ca^{++}-content of Ringer solution allowed for.

Tale XVII. *Metabolism of various glycolysing tissues in presence of* M/*50 maleic acid*

		With *M*/50 maleate								Control							
		Q_{O_2}			$Q_G^{O_2}$			$Q_G^{N_2}$		Q_{O_2}			$Q_G^{O_2}$			$Q_G^{N_2}$	
Species	Tissue	1st hr.	2nd hr.	3rd hr.	1st hr.	2nd hr.	3rd hr.	1st hr.	2nd hr.	1st hr.	2nd hr.	3rd hr.	1st hr.	2nd hr.	3rd hr.	1st hr.	2nd hr.
Guinea-pig	Medulla of kidney (*a*)	− 5·6	− 6·3	− 2·9	10·2	11·5	10·7	24·0	23·0	− 11·8	− 11·0	− 13·7	11·2	9·6	8·9	27·4	26·5
	(*b*)	− 5·4	− 5·5	− 7·3	9·2	10·2	11·5	—	—	− 10·8	− 9·7	− 8·5	12·2	11·1	9·7	—	—
Guinea-pig	Heart	− 4·1	− 1·8	—	4·5	5·2	—	4·4	3·2	− 5·3	− 2·9	—	2·4	1·4	—	6·5	4·4
Rat	Intestinal mucosa	− 7·0	—	—	16·2	—	—	—	—	− 13·3	—	—	17·5	—	—	—	—
Rat	Testis	− 7·5	− 4·5	—	2·1	1·5	—	—	—	− 9·2	− 7·1	—	2·0	0·4	—	—	—
Rat	Spleen (*a*)	− 6·9	− 7·1	—	5·4	8·8	—	—	—	− 14·3	− 13·7	—	3·3	4·8	—	—	—
	(*b*)	− 7·7	− 4·6	—	6·0	9·6	—	7·2	7·0	− 12·9	− 12·8	—	4·2	4·2	—	7·8	7·3
Rat	Jensen sarcoma (*a*)	− 3·5	− 0	—	32·1	27·7	—	30·4	21·9	− 14·4	− 12·4	—	19·9	19·4	—	29·0	21·6
	(*b*)	− 9·0	− 3·5	—	23·9	22·7	—	35·2	30·9	− 7·1	− 4·8	—	23·8	22·9	—	37·0	32·0
Rat	Embryo*	− 8·9	− 5·3	—	9·4	12·7	—	16·5	15·2	− 11·1	− 10·4	—	5·0	4·6	—	19·5	18·6

* Average dry wt. 5 mg.

Influence of metabolites on the maleic acid effect

Certain substrates seem to increase the toxicity of maleic acid. With $M/50$ $l(+)$glutamic acid there is an almost immediate rise of the aerobic glycolysis to the normal anaerobic level followed by a rapid fall. The respiration falls sharply from the beginning (Fig. 5*a*). $d(-)$Glutamic acid has the same effect. With $M/50$ citrate there is a similar fall of respiration. The aerobic glycolysis reaches its peak after 90 min. and declines later (Fig. 5*b*). The presence of $M/50$ pyruvate on the other hand not only keeps the respiration intact, but also abolishes the effect on aerobic glycolysis to a large extent (Fig. 5*c*).

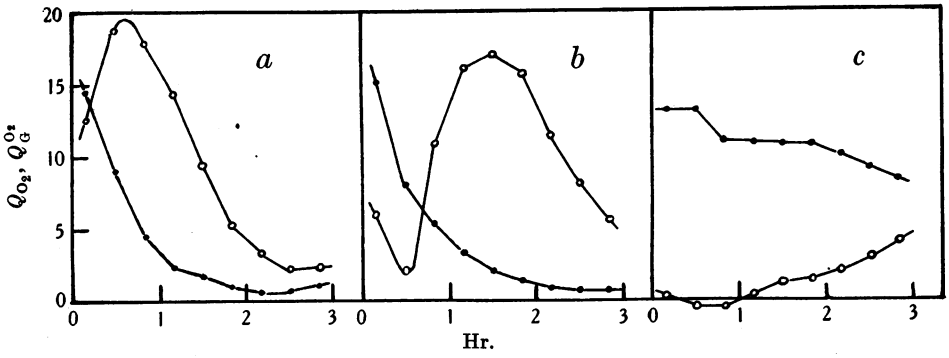

Fig. 5. Respiration and aerobic glycolysis of guinea-pig brain slices. (*a*) with $M/50$ maleate + $M/50$ $l(+)$glutamate; (*b*) with $M/50$ maleate + $M/50$ citrate; (*c*) with $M/50$ maleate + $M/50$ pyruvate. Retention allowed for in all experiments. •——• respiration. o——o aerobic glycolysis.

In Fig. 6 some of the older experiments are reproduced. Since no allowance for retention was then made, only the curves of the aerobic glycolysis which is only slightly affected by the retention are given. $M/50$ ketoglutarate has an effect similar to that of citrate. With $M/50$ fumarate the aerobic glycolysis is diminished.

Although the aerobic glycolysis is hardly increased by $M/100$ maleate addition of $M/100$ glutamate raises it strongly, especially during the 2nd hr. $M/100$ reduced glutathione has a very similar effect (see also Table XV). This is particularly interesting in view of the reaction of maleic acid with thiol compounds discovered by Morgan & Friedmann [1938, 1]. If the effects of maleic acid were due to a destruction of the glutathione of the tissue, the addition of an equivalent amount of glutathione together with the maleic acid should neutralize the effect. What happens is actually the reverse: the effect of maleic acid is accentuated, as is the case with glutamic acid.

Another fact may here be commented upon: the anaerobic glycolysis of brain slices is inhibited by a combination of $M/100$ glutamic acid + $M/100$ maleic acid to approximately the same extent as by $M/100$ glutamic acid alone. Yet under aerobic conditions a strong glycolysis occurs which may be double the glycolysis occurring in the same medium under anaerobic conditions. As pointed out before, the P.M.Q. is here meaningless.

Reversibility: the effect of maleic acid can to some extent be reversed by washing (Fig. 7). After 2 hr. incubation with $M/50$ maleate the slices were removed from the vessels and rinsed several times with Ringer solution; the manometric measurements were then resumed without maleate. In another pair of vessels the observation of the metabolism in presence of $M/50$ maleate was

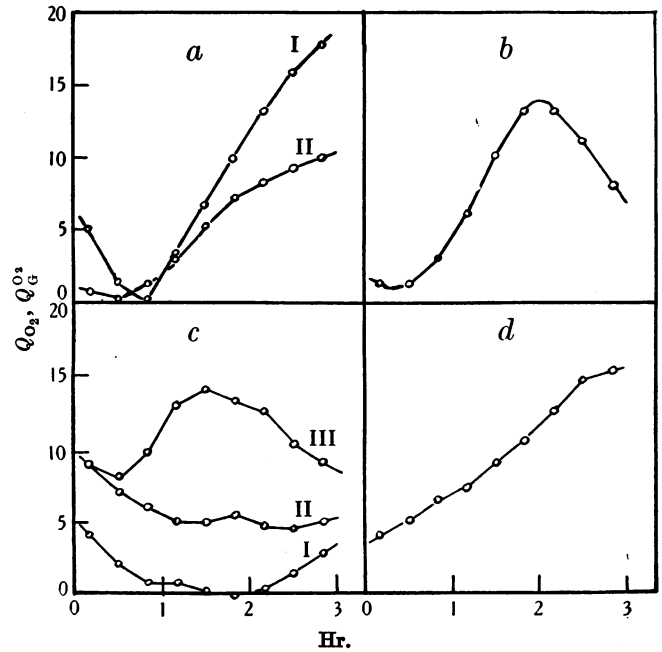

Fig. 6. Aerobic glycolysis of guinea-pig brain slices. (*a*) I, with *M*/50 maleate; II, with *M*/50 maleate + *M*/50 fumarate. (*b*) Same exp. *M*/50 maleate + *M*/50 α-ketoglutarate. (*c*) I, with *M*/100 maleate; II, with *M*/100 *l*(+)glutamate; III, with *M*/100 maleate + *M*/100 *l*(+)glutamate. (*d*) Same exp. *M*/100 maleate + *M*/100 GSH.

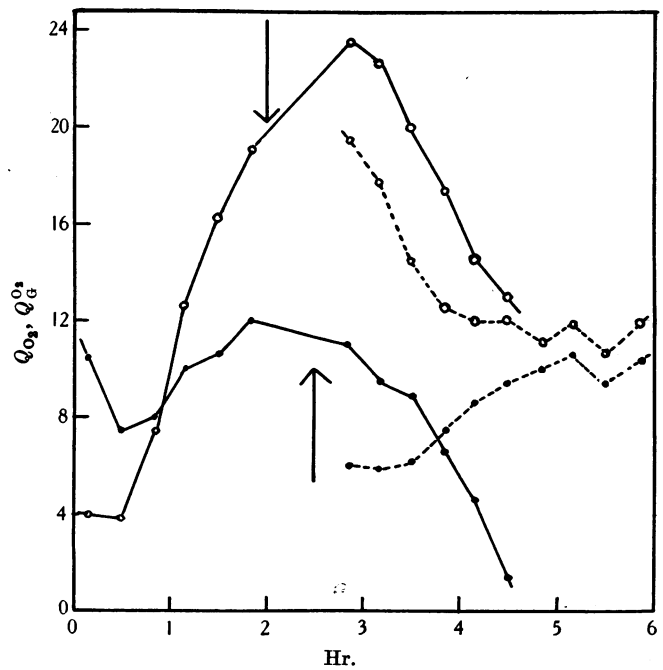

Fig. 7. Reversibility of the maleic acid effect by washing. Guinea-pig brain slices. Downward arrow: slices removed from *M*/50 maleate sol. Upward arrow: slices put back in thermostat after washing. Continuous line: metabolism in presence of *M*/50 maleate. The rise of respiration in the 2nd hr. is probably spurious, since no allowance for retention was made. Broken line: metabolism in bicarbonate-glucose-Ringer after washing. •——• respiration. o——o aerobic glycolysis.

continued until its final destruction. There was, after the washing, some fall of aerobic glycolysis, but not to the normal level, while the respiration recovered almost completely. Another experiment gave a similar result.

(b) The effect on other tissues

The effect of maleic acid is not restricted to brain, but was also observed in other tissues, though to a less marked degree. The inhibition of respiration is usually higher in these tissues (Table XVII). A rise of aerobic glycolysis occurred for instance in embryo, heart and spleen, also in one experiment with Jensen sarcoma, where the respiration was strongly inhibited. Retention was not allowed for in these experiments, but since the aerobic glycolysis was in most cases small or moderate, the errors of respiration values will be small.

(c) The mechanism of the maleic acid effect

(1) The slow development of the maleic acid effect might have been due to poisoning of the "ammonia-binding mechanism" of brain [Weil-Malherbe, 1936] which would lead to an increase of the concentration of NH_4^+ in the tissue. This would also have explained why the effect was so much accelerated by the presence of glutamic acid which would have acted as NH_3 donator. Estimations of NH_3 however showed that no increase occurred after 3 hr. incubation of brain slices with $M/50$ maleate or with $M/50$ maleate + $M/50$ glutamate.

(2) It is of course tempting to seek a connexion between the biological action of maleic acid and the recently discovered reaction with thiol groups [Morgan & Friedmann, 1938, 1, 2]. Indeed Lehmann & Needham [1938] quote the results of Morgan & Friedmann as supporting the theory that the glycolysis of brain needs glutathione as coenzyme. Yet, as has been shown, glutathione does not reverse the action of maleic acid, but, on the contrary, enhances it.

Another compound which is known to react with thiol compounds is iodoacetic acid [Dickens, 1933]. But whereas maleic acid acted as a stimulant of glycolysis in the experiments described, iodoacetic acid is a very potent inhibitor of glycolysis. This action of iodoacetic acid too could not be correlated with the destruction of glutathione [Schroeder *et al.* 1933; cf. also Smythe, 1936]. Needham & Lehmann [1937] could not reverse the inhibition of glycolysis of embryonic tissue caused by iodoacetic acid by the addition of glutathione.

IV. Relevance of the observed phenomena to the theories of the Pasteur reaction

Even if the action of a substance on isolated enzyme systems has been carefully studied, it may be dangerous to apply this knowledge directly to observations made on the intact cell, where its action may be quite different. As for maleic acid little is known about its action on isolated enzymes and nothing that could explain the observed effects. But that they are due to reactions with enzymes seems to be a justifiable conclusion in view of the inactivity of other substances with similar physical properties and a similar or even greater toxicity. This strengthens the case for an enzymic rather than a physico-chemical control of the Pasteur reaction.

Since *l*-glyceraldehyde is in all probability not an intermediate of animal metabolism [Needham & Lehmann, 1937], *l*(+)glutamic, *l*(+)lactic and succinic acids are the only naturally occurring substances which have been shown to inhibit anaerobic glycolysis. Their close connexion with the various

cycles of carbohydrate oxidation may suggest that the degree of saturation of certain dehydrogenases which are parts of these is a factor in the control of the Pasteur mechanism.

SUMMARY

1. NH_4^+ affects the metabolism of brain slices in a way similar to K^+, Rb^+ and Cs^+. $M/30$ NH_4Cl causes increase of respiration, increase of aerobic glycolysis to the anaerobic level and inhibition of anaerobic glycolysis. Some increase of aerobic glycolysis is still seen with concentrations of $M/1000$–$M/3000$ NH_4Cl. The only other tissue where a similar effect of $M/30$ NH_4Cl was observed was spleen. In intestinal mucosa the effect was observed once, but the observation could not be repeated.

2. $M/100$–$M/1000$ $l(+)$glutamic acid inhibits the anaerobic glycolysis of brain slices by 30–70%. $d(-)$Glutamic acid, l-glutamine and dl-β-hydroxyglutamic acid act similarly. The effect is only observed in brain.

3. The effect of glutamic acid is increased by the addition of $M/100$ lactate or $M/100$ succinate.

4. The effect of glutamic acid is largely reversed by $M/100$–$M/1000$ pyruvate.

5. The substances of the glutamic acid group which inhibit the anaerobic glycolysis of brain increase the aerobic glycolysis to values of $Q_G^{O_2} = 5$–8. This effect, too, only occurs in brain.

6. $M/50$ maleic acid inhibits the respiration of brain slices in bicarbonate-glucose-Ringer to a degree varying in different experiments from 10 to 50%. Aerobic glycolysis rises slowly and reaches the anaerobic level during the 3rd hr. of the experiment. No other simple organic acid has a similar effect.

7. $M/50$ glutamate and $M/50$ citrate accelerate the effect of maleate; $M/50$ pyruvate abolishes it to a large extent.

8. Reduced glutathione does not reverse the effect of maleic acid, but like glutamic acid enhances it.

9. The effect of maleic acid can be partly reversed by washing.

10. In embryo, heart and spleen maleic acid causes a rise of aerobic glycolysis, which in some cases reaches the level of the anaerobic glycolysis.

REFERENCES

Ashford & Dixon (1935). *Biochem. J.* **29**, 157.
Baker (1937). *Biochem. J.* **31**, 980.
—— (1938). *Biochem. J.* **32**, 332.
Braunstein & Kritzmann (1937). *Enzymologia*, **2**, 129.
Dickens (1933). *Biochem. J.* **27**, 1141.
—— & Greville (1933). *Biochem. J.* **27**, 1134.
—— —— (1935). *Biochem. J.* **29**, 1468.
—— & Šimer (1930). *Biochem. J.* **24**, 1301.
—— —— (1931). *Biochem. J.* **25**, 985.
—— & Weil-Malherbe (1936). *Biochem. J.* **30**, 659.
Dixon (1937). *Biol. Rev.* **12**, 431.
—— & Holmes (1935). *Nature, Lond.*, **135**, 995.
Friedemann & Kendall (1929). *J. biol. Chem.* **82**, 23.
Geiger (1935). *Biochem. J.* **29**, 811.
Gözsy & Szent-Györgyi (1934). *Hoppe-Seyl. Z.* **224**, 1.
Grönvall (1924). *Skand. Arch. Physiol.* **45**, 303.
Holmes (1934). *Ann. Rev. Biochem.* **3**, 395.

Jowett & Quastel (1937). *Biochem. J.* **31**, 275.
Krebs (1935). *Biochem. J.* **29**, 1951.
Kritzmann (1938). *Enzymologia*, **5**, 44.
Lehmann & Needham (1938). *Enzymologia*, **5**, 95.
Mendel, Bauch & Strelitz (1931). *Klin. Wschr.* **10**, 118.
Meyerhof & Lohmann (1926, 1). *Biochem. Z.* **171**, 381.
—— —— (1926, 2). Biochem. Z. **171**, 421.
Morgan & Friedmann (1938, 1). *Biochem. J.* **32**, 733.
—— —— (1938, 2). *Biochem. J.* **32**, 862.
Needham & Lehmann (1937). *Biochem. J.* **31**, 1913.
—— & Nowiński (1937). *Biochem. J.* **31**, 1165.
—— —— Dixon & Cook (1937). *Biochem. J.* **31**, 1185.
Parnas & Heller (1924). *Biochem. Z.* **152**, 1.
Rosenthal & Lasnitzki (1928). *Biochem. Z.* **196**, 340.
Schroeder, Woodward & Platt (1933). *J. biol. Chem.* **101**, 133.
Smythe (1936). *J. biol. Chem.* **114**, 601.
Thunberg (1920). *Skand. Arch. Physiol.* **40**, 1.
Warburg (1930). Metabolism of Tumours. Constable, London.
Weil-Malherbe (1935). *J. Soc. chem. Ind., Lond.*, **54**, 1115.
—— (1936). *Biochem. J.* **30**, 665.
—— (1937). *Biochem. J.* **31**, 2202.