CXXX. GLUTATHIONE AND THE PASTEUR REACTION

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No completely satisfactory explanation has yet been offered for the mechanism by which respiration inhibits glycolysis (i.e. the Pasteur reaction). Lipmann [1933] believes that the inhibition of glycolysis in the oxygen-saturated cell is due to reversible oxidative inactivation of the glycolytic enzyme system.

The very striking observation that reduced glutathione at a concentration of 10^{-3} *M* specifically and reversibly inhibits the Pasteur reaction in the Jensen rat sarcoma and in rat brain was made by Bumm & Appel [1932]; their findings indicate that although reduced glutathione had no effect on either the respiration or the anaerobic glycolysis, it could increase the aerobic glycolysis to the anaerobic level. The disulphide form had no effect; the effect of the reduced form was reversible. They concluded from this [Bumm, 1933] that the glutathione system might serve *in vivo* as a regulator of the Pasteur-Meyerhof cycle. The significance of their results is far-reaching. Not only do their conclusions assign a very important role to glutathione, a compound which, because of its peculiar chemical nature and its widespread distribution in animal cells, seems to deserve an important place, but also they offer an explanation for the mechanism of the break in the Pasteur reaction which is so striking in tumour tissue.

In the course of work on another problem, some control experiments with glutathione and tumour tissue were made and, in view of the data reported by Bumm & Appel, it was surprising to find that glutathione did not stimulate aerobic glycolysis. For this reason, a systematic investigation of the effect of glutathione on the metabolism of tumours, brain tissue and a few other tissues which showed large anaerobic glycolysis was undertaken. No significant increase in the aerobic glycolysis in the presence of glutathione was ever observed in the Jensen rat sarcoma or in several other transplantable rat tumours, brain, testes and chick embryo.

METHODS

The metabolism of thin slices of tissue was studied in the presence and absence of glutathione in the differential manometers of Dixon & Keilin [1933], following the technique described by Elliott & Schroeder [1934]. In all experiments the bicarbonate medium of Krebs [1932] containing 0.24% glucose was used. Unless otherwise stated, the experimental period was 90 min. All experiments were conducted at 37°.

The tumours used were the Philadelphia No. 1 sarcoma [Waldschmidt-Leitz, McDonald *et al.* 1933], the Walker No. 256 carcinoma, the Flexner-Jobling sarcoma and the Jensen sarcoma. It was difficult to obtain good Jensen sarcoma tissue; the strain used here grows rather rapidly and quickly becomes soft and haemorrhagic. Quite good tissue could be obtained by subcutaneous inoculations in the leg. Sterile intraperitoneal implantations carried through several generations also gave improved quality in the intraperitoneal tumours.

In all cases precautions were taken to remove necrotic material and to obtain uniform samples of the tissue in all manometer vessels.

A period of 1.5–2 hr. elapsed between the killing of the animal and the starting of the last manometer. Until used, the tissue slices were stored in Ringer-Krebs solution through which a slow stream of O_2 — CO_2 bubbled continuously. (Tumour tissue was stored in Ringer-Krebs solution without glucose.)

The glutathione was obtained from three sources: for most experiments a very good preparation from Hoffmann-La Roche, showing theoretical reducing power, was used; a few experiments were done with a sample prepared by Pirie's method in the Biochemical Laboratory in Cambridge and kindly supplied by Dr K. A. C. Elliott, and some with a sample purified through cadmium precipitation by Dr Johnson at the United States Public Health Service Laboratories, obtained through the courtesy of Dr Mary E. Maver. The results obtained with all three samples were the same.

Glutathione solution of twelve times the required concentration was prepared and added to the manometer vessels immediately before placing the manometer in the bath. This precaution was necessary in order to shorten as much as possible the period of autoxidation in the Ringer-Krebs solution.

When estimations of glutathione were to be made, 2.5 ml. of the experimental solution (containing approximately 0.3 or 0.6 mg. glutathione per ml.) were pipetted into 1.5 ml. of 20% salicylsulphonic acid. This was then diluted to 15 ml. with distilled water and the estimation done on a 10 ml. aliquot by the method of Woodward & Fry [1932].

RESULTS AND DISCUSSION

The results obtained with tumours are listed in Table I. $Q_A^{O_2}$ and $Q_A^{N_2}$ represent the aerobic and the anaerobic glycolyses respectively. Otherwise, the conventional terminology is used.

In none of the experiments with tumour tissue in the presence of glutathione was any significant increase in the aerobic glycolysis found. The slight variations observed can easily be attributed to experimental error and the difficulty of choosing exactly reproducible slices of tumour tissue. In almost all experiments there is an increased O_2 uptake which can be accounted for by the oxidation of the glutathione.

Estimations of glutathione content of the medium at the beginning and at the end of the experimental period were made in separate experiments with tumour slices treated (with gas, shaken etc.) in exactly the same way as those in the manometer vessels. A few typical values obtained for the Philadelphia No. 1 sarcoma and the Walker No. 256 carcinoma are listed in Table II.

Using Hoffmann-La Roche glutathione at a concentration of $10^{-3} M$ in the presence and absence of tumour tissue (Philadelphia No. 1) measurements were made over a period of 110 min. of the content of reduced glutathione in aliquots removed at various intervals. These values are plotted against time in Fig. 1.

It is obvious that the glutathione is being rapidly oxidized in both the control and in the experiment containing tumour. This rapid autoxidation in the control can probably be accounted for by the slightly alkaline reaction of the Ringer-Krebs solution, since it is characteristic of glutathione that its autoxidation occurs with much greater speed at an alkaline reaction. In the case of brain the tissue seems to protect the glutathione against oxidation, an effect which has been observed for various tissues by other investigators. In the presence of tumour tissue the oxidation of glutathione occurs with slightly

Table I. *Effect of glutathione on the metabolism of tumour tissue*

Strain of tumour	Age of tumour days	Source of glutathione	Concentration of glutathione	Q_{O_2}	$Q_A^{O_2}$	$Q_A^{N_2}$
			M			
Philadelphia No. 1 sarcoma	—	H. R.*†	0	—	—	+32.6
			10^{-3}	—	—	+31.9
	—	H. R.†	0	9.9	+22.9	+30.3
			10^{-3}	10.2	+19.6	—
	18	Cambridge†	0	8.0	+22.7	—
			10^{-3}	9.9	+24.1	—
	24	H. R.	0	8.1	+20.3	—
			2×10^{-3}	9.3	+18.2	—
	13	U. S. P. H.‡	0	10.4	+26.6	+40.9
			2×10^{-3}	11.8	+24.9	—
Walker No. 256 carcinoma	15	Cambridge†	0	8.5	+22.6	—
			10^{-3}	9.5	+24.2	—
	18	H. R.	0	8.5	+24.5	—
			10^{-3}	8.9	+26.0	—
	15	H. R.	0	8.3	+19.9	+31.7
			10^{-3}	9.3	+20.0	—
	13	H. R.	0	8.5	+30.4	—
			2×10^{-3}	9.0	+25.9	—
	14	U. S. P. H.	0	9.3	+25.2	+33.5
			10^{-3}	9.2	+24.4	—
Jensen sarcoma	15	U. S. P. H.	0	10.5	+25.4	+40.7
			2×10^{-3}	11.7	+27.0	—
	12	H. R.	0	12.3	+17.1	—
			10^{-3}	14.2	+15.7	—
	14	H. R.	0	12.3	+12.2	+33.9
			10^{-3}	15.1	+15.1	—
	—	H. R.	0	13.6	+13.6	+35.5
			2×10^{-3}	13.1	+11.4	+31.4
	21	U. S. P. H.	0	10.8	+13.3	+33.1
			2×10^{-3}	13.8	+13.4	—
Flexner-Jobling carcinoma	28	H. R.	0	9.0	+24.1	+26.9
			10^{-3}	10.7	+23.5	—
	30	H. R.	0	7.6	+24.6	+28.1
			10^{-3}	9.9	+24.5	—

* Hoffmann-La Roche. † 60-min. experimental period.

‡ United States Public Health Service.

Table II. *Disappearance of reduced glutathione in presence of tumour*

	Reduced glutathione		Dry wt. of tissue mg.
	Initial mg./ml.	Final mg./ml.	
Control	0.31	0.09	—
Walker No. 256	0.31	0.04	24.3
Control	0.32	0.07	—
Philadelphia No. 1	0.32	0.08	22.9
Walker No. 256	0.32	0.04	22.8
Walker No. 256	0.32	0.02	24.2

greater speed than in the control. In spite of this rapid disappearance of reduced glutathione from the medium, it seems quite evident from the curve that enough was present over most of the experimental period to produce increased glycolysis if this effect were to occur. It might be of interest to point out that when the weights and the R.Q. of tissue in the control and experimental manometers are

Fig. 1. A, control; B, plus brain; C, plus tumour.

approximately the same, any increase in glycolysis during any part of the experimental period can be detected from the slopes of the curves obtained by taking readings on the Dixon-Keilin manometers during the course of the experiment. No such increase was observed even during the first half-hour when the concentration of reduced glutathione was highest. Doubling the concentration of glutathione, thus making the concentration of reduced glutathione greater throughout the entire experimental period also had no effect. Therefore the lack of stimulation cannot be attributed to a too low concentration of glutathione.

With brain tissue small increases in aerobic glycolysis were observed in the presence of glutathione (Table III). Compared with the normal aerobic glycolysis this increase is large, but considering the magnitude of the anaerobic glycolysis it is questionable how significant this increase really is. It must be emphasized here that brain differs from tumour in that glutathione does not increase the Q_{O_2} value. This can readily be explained by the fact that in the presence of brain there is only a very slight autooxidation of glutathione. Either the normal oxidation is inhibited or the oxidized glutathione is again reduced by the brain tissue. The results obtained by estimating initial and final glutathione contents with and without brain tissue are shown in Table IV. The inhibition of the normal autooxidation is evident from the curve in Fig. 1.

In a few cases where increased glycolysis was observed manometrically, lactic acid estimations were made by the method of Friedemann & Graesser [1933] on an aliquot of the contents of the manometer vessel, quantitatively washed out, as described by Elliott & Schroeder [1934]. There is fairly good

Table III. *Effect of glutathione on the metabolism of brain tissue*

Source of glutathione	Concentration of glutathione <i>M</i>	Q_{O_2}	$Q_A^{O_2}$	$Q_A^{N_2}$
H. R.	0	15.0	+0.8	—
	10^{-3}	15.3	+2.2	—
H. R.	0	14.2	+2.5	+15.5
	10^{-3}	14.8	+5.3	—
H. R.	0	—	0.0	+19.4
	2×10^{-3}	—	+4.9	—
H. R.	0	9.5	+2.2	+11.6
	2×10^{-3}	10.4	+3.6	+12.1
U. S. P. H.	0	14.6	+0.6	+15.0
	10^{-3}	14.4	+6.2	—
U. S. P. H.	0	14.2	+2.5	+15.5
	10^{-3}	13.8	+4.3	—
U. S. P. H.	0	14.6	+1.6	+13.0
	10^{-3}	14.1	+5.3	—
H. R.*	0	10.3	+1.9	+23.3
	10^{-3}	10.5	+2.9	—

* Rabbit brain.

Table IV. *Disappearance of reduced glutathione in presence of brain*

	Reduced glutathione		Dry wt. of tissue mg.
	Initial mg./ml.	Final mg./ml.	
Control	0.341	0.117	—
Rat brain	0.341	0.250	24.5
"	0.341	0.272	28.6
"	0.341	0.246	21.1
Control	0.320	0.074	—
Rat brain	0.320	0.222	13.6
"	0.320	0.223	14.4
"	0.320	0.253	17.1
Control	0.325	0.089	—
Rabbit brain	0.325	0.218	14.9

Table V. *Lactic acid production in brain*

	$Q_A^{O_2}$ Determined		$Q_A^{N_2}$ Determined	
	Manometrically	Chemically	Manometrically	Chemically
Control	+1.7	+2.2	+13.0	+14.0
+ 10^{-3} <i>M</i> glutathione	+5.3	+6.4	—	—
Control	+2.5	+3.0	+15.5	+16.0
+ 10^{-3} <i>M</i> glutathione*	+4.3	+4.5	—	—
+ 10^{-3} <i>M</i> glutathione†	+5.3	+4.0	—	—

* United States Public Health Service.

† Hoffmann-La Roche.

agreement between the values for glycolysis found manometrically and analytically as may be seen from Table V.

A few experiments have been done with testis and chick embryo. No effect of glutathione on the glycolyses of these tissues has been observed. The data obtained are listed in Table VI.

Table VI. *Effect of glutathione* on the metabolism of testis and chick embryo*

Tissue	Concentration of glutathione	Q_{O_2}	$Q_A^{1/2}$	$Q_A^{N_2}$
	M			
Testis	0	12.0	+8.1	+8.0
	10^{-3}	9.3	+7.0	+10.8
	0	12.0	+8.5	+13.5
	2×10^{-3}	11.4	+9.0	—
Chick embryo†	0	14.9	+5.1	+23.0
	10^{-3}	17.5	+6.7	—
	0	13.3	+6.7	+23.2
	2×10^{-3}	16.2	+5.6	—

* Hoffmann-La Roche.

† 4-day-old whole chick embryos.

Chick embryo also inhibits the autoxidation of glutathione, although to a less extent than brain.

The experiments of Bumm & Appel were done entirely on the Jensen rat sarcoma and brain. If the results they obtained were real and if the function they assigned to glutathione be real, then glutathione should increase the aerobic glycolysis of all tumours. We could not reproduce their results either with the Jensen tumour or with any of the other tumours investigated. It was thought possible that perhaps the difference between the results reported here and those of Bumm & Appel might be due to a profound difference in tumour strain. However, the fact that we could not reproduce their results with brain tissue suggested that there must be some other reason for the disagreement.

We have repeated experiments reported by Dickens [1934] who obtained an inhibition of the Pasteur reaction in the Jensen rat sarcoma by pre-treatment of the slices with phenylhydrazine. The procedures recommended by Dickens for these experiments were carefully observed, the phenylhydrazine hydrochloride being recrystallized three times from alcohol, dissolved in glucose-containing Ringer-Krebs solution and neutralized with sodium bicarbonate-saturated Ringer-Krebs solution just before using. Carefully selected slices of tumour from which all necrotic and haemorrhagic portions had been cut away were chosen for treatment with phenylhydrazine. The concentration varied from 2.5×10^{-3} to $5.0 \times 10^{-3} M$ and the period of pre-treatment was either 15 or

Table VII. *Effect of pre-treatment with phenylhydrazine on tumour metabolism*

Tumour	Concentration of phenylhydrazine	Time of pretreat- ment	Q_{O_2}	$Q_A^{1/2}$	$Q_A^{N_2}$
	M	min.			
Philadelphia No. 1	0	15	10.8	+22.7	+39.0
	2.5×10^{-3}	15	8.7	+24.5	+32.2
	0	15	9.1	+22.7	+39.5
	3.75×10^{-3}	15	5.8	+30.8	—
Walker No. 256	0	20	9.3	+25.2	+33.5
	2.5×10^{-3}	20	6.8	+28.4	—
	0	20	10.5	+25.4	+40.7
	5.0×10^{-3}	20	2.9	+40.4	—
Jensen rat sarcoma	0	20	13.3	+12.2	+33.9
	2.5×10^{-3}	20	12.8	+22.5	—
	0	20	10.8	+13.3	+33.1
	3.75×10^{-3}	20	5.8	+22.5	—

20 min. After exposure to the phenylhydrazine solution, the tissue was very thoroughly washed, then placed in the manometer vessels and the experiments conducted as usual. The results obtained are shown in Table VII.

Although not many experiments have been done with phenylhydrazine, enough are shown here to illustrate the fact that there is definitely a difference between tumours of different types in their response to the action of this foreign substance. With the Philadelphia No. 1 and Walker 256 tumours large increases in aerobic glycolysis occurred only when respiration was strongly inhibited. Using the Jensen sarcoma, qualitatively the same type of result could be obtained as that reported by Dickens. Usually, however, an inhibition of respiration accompanied this increase in glycolysis. No such confirmation of the effect reported by Bumm & Appel has been observed with the Jensen sarcoma.

Bumm & Appel have indicated in their paper that they believe their results are particularly pertinent because tumour tissue is characterized by an unusually high content of glutathione, and that this probably accounts for the great aerobic glycolysis which normally occurs in tumour tissue. This suggestion is, however, not convincing in view of the considerable disagreement on the question of the glutathione content of tumour tissue [Voegtlin & Thompson, 1926; Yaoi & Nakahara, 1926; Holmes, 1926; Bierich *et al.* 1927; Kennaway & Hieger, 1927; Lecloux *et al.* 1927; Heinlein, 1932; Woodward, 1935].

SUMMARY

Glutathione has no significant effect on the aerobic glycolyses of tumour, brain, testes and embryo, as measured in the Dixon-Keilin manometers. The results obtained in these experiments do not indicate that glutathione participates in the Pasteur reaction.

The specific effect of phenylhydrazine on the Pasteur reaction observed by Dickens in Jensen sarcoma was not found in other tumours. The increased aerobic glycolysis usually was paralleled by inhibited respiration.

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