Biochemical Pharmacology, Vol. 17, pp. 2281–2290. Pergamon Press. 1968. Printed in Great Britain

RESTORATION OF GLYCOLYSIS AND RESPIRATION IN EHRLICH ASCITES TUMOR CELLS INHIBITED BY 2,2′-DITHIODIPYRIDINE*

D. R. GRASSETTI and J. F. MURRAY, JR.

Arequipa Foundation, Russ Building, San Francisco, Calif., U.S.A.

(*Received* 23 *February* 1968; *accepted* 31 *May* 1968)

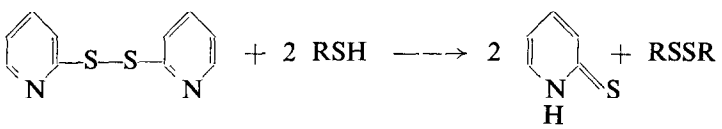
Abstract—Glycolysis and respiration of Ehrlich ascites cells are strongly inhibited by 2,2′-dithiodipyridine (2-PDS). If such inhibited cells are treated with dithiothreitol (DTT) + $NADP^+$ + ATP, with glucose as substrate, the anaerobic production of CO_2 is restored to an extent about 50 per cent that of untreated cells, and lactate production to an extent of 30 per cent. The excess CO_2 over lactate production is due to hydrolysis of the exogenous ATP, presumably by action of ATPase. DTT alone causes a reversal of glycolysis close to 20 per cent.

Studies with ^{14}C-labeled glucose have shown that the action of DTT + $NADP^+$ + ATP on metabolically inert 2-PDS-treated cells restores the production of CO_2 from the C_1 of glucose at a rate almost double that of untreated cells, both aerobically and anaerobically. The same compounds cause 2-PDS-inhibited cells to produce amounts of CO_2 from the C_6 of glucose equal to about 40 per cent that of untreated cells (aerobically).

A mixture of DTT + $NADP^+$ + ATP, when acting on previously untreated cells, stimulates about threefold the production of CO_2 from the C_1 of glucose, both aerobically and anaerobically. The same mixture inhibits the aerobic production of CO_2 from the C_6 of glucose about 55 per cent.

On treatment of Ehrlich ascites cells with 2-PDS, a rapid evolution of CO_2 from the C_1 of glucose occurs, and ceases abruptly after about 5 min. This is consistent with our previous finding that 2-PDS mediates the direct oxidation of glucose 6-phosphate.

IT WAS recently found in this laboratory that 2,2′-dithiodipyridine† is a strong inhibitor of glycolysis and of respiration of Ehrlich ascites tumor cells.[1] Further study has shown that this compound reacts with thiols[2] as follows:



The progress of the reaction can be followed spectrophotometrically.[2] Since 2-thiopyridone is almost exclusively in the thione form, the reaction is driven to completion.

We have shown that the inhibition of respiration of Ehrlich ascites cells is accompanied by the formation of 2-TP, presumably due to the reaction of 2-PDS with thiols

* This investigation was supported by Public Health Service Grant CA 08538-02 from the National Cancer Institute, National Institutes of Health, Bethesda, Md.

† Abbreviations used in this paper: 2-PDS = 2,2′dithiodipyridine; 2-TP = 2-thiopyridone; $NADP^+$, NADPH = oxidized and reduced forms of nicotinamide adenine dinucleotide phosphate; GSH = glutathione; GSSG = oxidized glutathione; ATP = adenosine triphosphate; DTT = dithiothreitol; HMP = hexose monophosphate.

of the living cell.[3] The ability of 2-PDS to oxidize reduced glutathione may lead to the oxidation of nonsulfhydryl cell components, such as glucose 6-phosphate. We studied *in vitro* the system depicted in Fig. 1 and have shown[3,4] that the rapid nonenzymatic oxidation of GSH by 2-PDS (reaction 3) causes continuous regeneration of GSSG which, through reaction 2 (catalyzed by glutathione reductase) oxidizes NADPH.

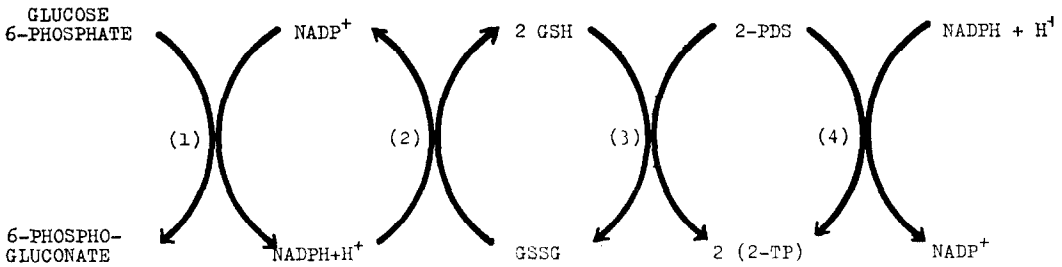


FIG. 1. Reaction 1 is catalyzed by glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate:$NADP^+$ oxidoreductase, EC 1.1.1.49); reactions 2 and 4 are catalyzed by glutathione reductase (NADPH [NADH]:GSSG oxidoreductase, EC 1.6.4.2); reaction 3 is nonenzymatic.

When glucose 6-phosphate and glucose 6-phosphate dehydrogenase are present, $NADP^+$ will oxidize the glucose 6-phosphate to 6-phosphogluconate (reaction 1). The overall reaction can be described as the oxidation of glucose 6-phosphate to 6-phosphogluconate by 2-PDS.

In the present paper we present evidence that the reactions of Fig. 1 actually take place in intact Ehrlich ascites cells upon treatment with 2-PDS. We further report our finding that the inhibition of lactate production brought about by 2-PDS can be reversed by treating the inhibited cells with a thiol.

MATERIALS AND METHODS

Materials. 2,2′-Dithiodipyridine was prepared by the procedure of Marckwald *et al.*[5] and its purity was determined as reported previously.[2] ATP, $NADP^+$ and yeast lactate dehydrogenase (L-lactate: cytochrome C oxidoreductase, EC 1.1.2.3) were purchased from Sigma Chemical Co. Radioactive glucose was obtained from New England Nuclear Corp. Dithiothreitol was purchase from Calbiochem. Ehrlich ascites cells were harvested from 7- to 10-day transplants in Swiss female mice (18–20 g) and washed free of blood as reported previously.[3]

Manometric experiments. These were performed using a Warburg respirometer at 37° as follows. Each flask contained 30 μmole glucose in 2·5 ml Krebs–Ringer bicarbonate buffer, pH 7·4.[6] The buffer was gassed immediately before use with N_2–CO_2 (95:5) for 10 min. The flasks for inhibition and reversal studies contained also 3·0 μmole 2-PDS. Washed ascites cells (0·2 ml, 80 × 10^6 cells) were added and the flask was gassed for 10 min with N_2–CO_2 (95:5). Manometric readings were made 10 min after gassing in order to make sure that essentially complete inhibition by 2-PDS had taken place. At this time 0·3 ml of a solution of the compound or mixture under study (DTT, $NADP^+$, ATP) in the same buffer was tipped into the main compartment from the sidearm. Readings were made at 5-min intervals for at least 1 hr after tipping. Aerobic manometric experiments in the presence of DTT could not be performed with sufficient accuracy, due to the large oxygen uptake of the blank.

Lactic acid determinations. When lactic acid was determined, the contents of the Warburg flasks were denatured by heating 5 min in hot water (95°). Initial and final lactic acid were determined with lactate dehydrogenase from yeast according to Wieland[7] and by the colorimetric method of Barker and Summerson.[8] Anaerobic CO_2 determinations are uncorrected for gas retention.[9]

Radioactive experiments. Incubation of the cells was carried out at 37° in 25-ml Erlenmeyer flasks equipped with a center well and a gas-tight serum stopper.[10] The flasks, containing Krebs–Ringer phosphate buffer,[6] were gassed for 1 min with either N_2–CO_2 (95:5) or O_2–CO_2 (95:5) and capped. Ascites cells (0·2 ml, 20–50 × 10^6 cells) were added through the stopper with a 1-ml tuberculin syringe fitted with a 20 gauge, 5 cm needle. The other components were added in the same manner. At the conclusion of the incubation period, 0·3 ml of a mixture of ethanolamine and 2-ethoxyethanol (1:1) was injected into the center well and 1·0 ml of 1 N HCl into the main compartment of the flask. Shaking was continued for 1 hr to insure complete CO_2 absorption. The center well contents were then transferred to scintillation vials containing 10 ml of a mixture of toluene and 2-ethoxyethanol (7:3) and 50 mg 2,5-diphenyloxazole (PPO). $^{14}CO_2$ was counted in a Packard Tri-Carb scintillation spectrometer.

Thin-layer chromatography (TLC). Ehrlich ascites cells (65 × 10^6 cells, 20 mg dry wt.) were incubated in 3 ml Krebs–Ringer bicarbonate buffer, pH 7·4, for 1 hr at 37° with 30 μmole of uniformly labeled ^{14}C-glucose (3 μc). This served as the control. Other flasks of identical composition were incubated with the addition of 2-PDS (3 μmole) or ATP (3·0 μmole) or of both. The cells were removed by centrifugation and the supernatant (2 μl) was spotted on TLC plates coated with cellulose. The plates were developed with acetone–acetonitrile–1 N HCl (64:26:10, v/v) as described previously.[11] After drying at 40°, the plates were sandwiched with no-screen Kodak X-ray plates; the exposure time was approximately 1 week.

RESULTS AND DISCUSSION

If the 2-PDS inhibition of glycolysis and respiration of Ehrlich ascites cells is due to extensive oxidation of cell components, it should be possible to put the machinery of the cell in motion again by an agent capable of reducing some of the disulfide bonds formed by action of 2-PDS. Accordingly, we have attempted to reverse this inhibition by treating inhibited cells with a thiol, dithiothreitol. In this compound, the cyclic disulfide form is thermodynamically favored[12] and thus DTT is capable of reacting with disulfides more completely than aliphatic monothiols. It was found that DTT has a stimulatory effect on the hexose monophosphate pathway. The effect of DTT and other compounds on this pathway in noninhibited cells was studied first.

Effect of 2-PDS and other compounds on the hexose monophosphate pathway in Ehrlich ascites cells. When washed Ehrlich ascites cells are incubated at 37° with 2-PDS (10^{-3}M) with 1-^{14}C-glucose (10^{-2}M) as the substrate, a rapid evolution of $^{14}CO_2$ takes place during the first 5 min, both in oxygen and in nitrogen (Fig. 2). After this time, virtually no further evolution of $^{14}CO_2$ occurs. The amount of CO_2 evolved in this "burst" is found to vary between 0·3 and 1·2 μmole/100 mg dry wt. No "burst" of radioactive CO_2 from 6-^{14}C-glucose is caused by 2-PDS under aerobic conditions. The CO_2 coming from the 1-position of glucose is presumably produced via the initial reactions of the HMP pathway, stimulated by 2-PDS, through the reactions outlined in Fig. 1.

If, after 7 min of incubation, when the CO_2 evolution has subsided, a mixture of DTT, $NADP^+$ and ATP is added to the metabolically inert cells, the evolution of CO_2 from the C_1 of glucose resumes and continues at a rate about 50 per cent higher than that of untreated cells. This is true in oxygen (Fig. 3) and in nitrogen (Fig. 4). It is thus conceivable that this CO_2 production by Ehrlich ascites cells on treatment with 2-PDS may come to an end after 5 min because of exhaustion of the endogenous supply of ATP and $NADP^+$.

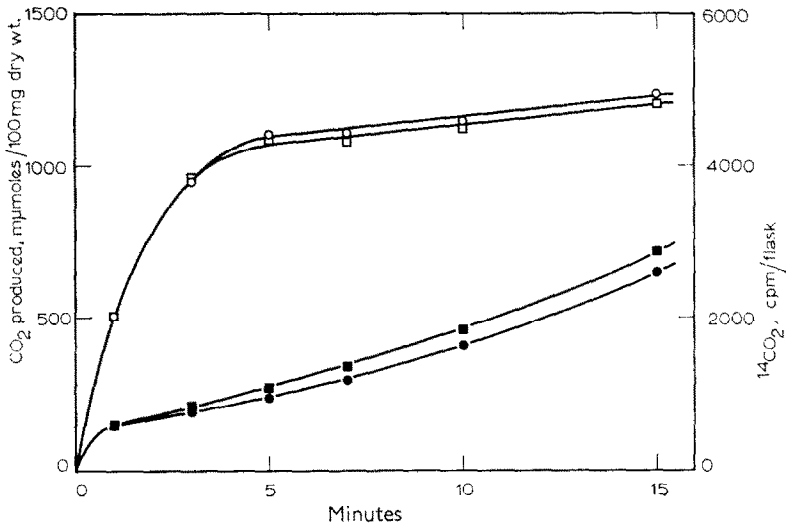


FIG. 2. Effect of 2-PDS on the production of CO_2 by Ehrlich ascites cells from 1-^{14}C-glucose. Each flask contained 30 μmole glucose (1 μc 1-^{14}C-glucose) and 32×10^6 washed Ehrlich ascites cells (15·0 mg dry weight) in a total volume of 3·0 ml Krebs–Ringer phosphate buffer, pH 7·2. Incubations were carried out at 37°. ● = CO_2 production by untreated Ehrlich ascites cells under N_2–CO_2 (95:5); ○ = CO_2 production in the presence of 2-PDS (3·0 μmole) under N_2–CO_2 (95:5); ■ = CO_2 production under O_2–CO_2 (95:5) by untreated cells; □ = CO_2 production in the presence of 2-PDS (3·0 μmole) under O_2–CO_2 (95:5).

When washed Ehrlich ascites cells are treated with the same compounds with 1-^{14}C-glucose as substrate, but in the absence of 2-PDS, the rate of $^{14}CO_2$ evolution is increased almost 3-fold, both in oxygen (Fig. 5) and in nitrogen (Fig. 6).

When washed Ehrlich ascites cells are treated with $NADP^+$ and ATP in the presence of glucose, no stimulation occurs; incubation of 2-PDS-inhibited cells with $NADP^+$ and ATP causes no restoration of $^{14}CO_2$ evolution from 1-^{14}C-glucose. The presence of DTT thus appears necessary for the stimulation and reversal of the HMP pathway. In preliminary experiments, it was found that DTT alone is capable of stimulating the oxidation of 1-^{14}C-glucose to $^{14}CO_2$ and of reversing its inhibition by 2-PDS to extents comparable to those given in the presence of added $NADP^+$ and ATP.

Effect of various compounds on the glycolysis and respiration of Ehrlich ascites cells. When ATP is added to glycolyzing cells, either alone or together with DTT or $NADP^+$ or both (Table 1), a moderate stimulation of anaerobic lactate production takes place.

Studies on the effect of DTT + $NADP^+$ + ATP on the metabolism of the C_6 of added glucose by intact Ehrlich ascites cells are reported in Fig. 7. It is seen that

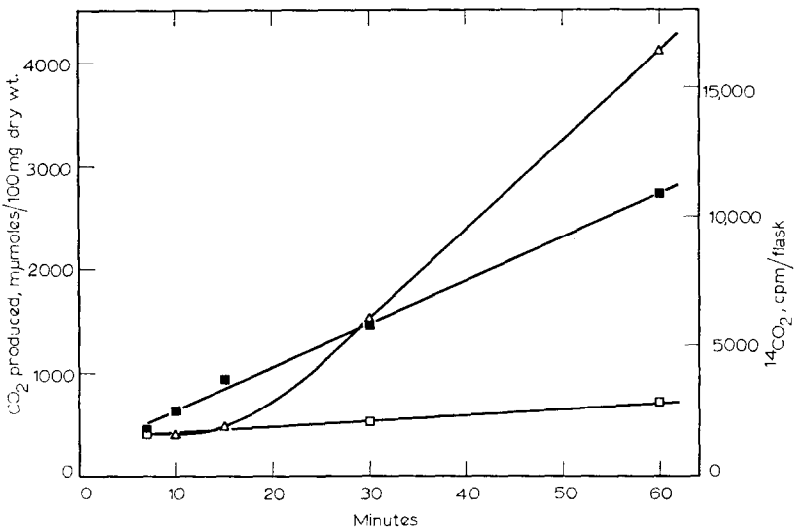


FIG. 3. Effect of DTT + $NADP^+$ + ATP on aerobic CO_2 production by 2-PDS-inhibited Ehrlich ascites cells from 1-^{14}C-glucose. Each flask contained 30 μmole glucose (1 μc 1-^{14}C-glucose) and 24 × 10^6 washed Ehrlich ascites cells (15·0 mg dry wt.) in a total volume of 3·0 ml Krebs–Ringer phosphate buffer, pH 7·2. The gas phase was O_2–CO_2 (95:5). Incubations were carried out at 37°. ■ = CO_2 production by untreated Ehrlich ascites cells; □ = CO_2 production in the presence of 2-PDS (3·0 μmole); △ = CO_2 production by 2-PDS-inhibited cells in the presence of 30 μmole DTT, 3 μmole $NADP^+$ and 3 μmole ATP. Cells were incubated with 2-PDS (3·0 μmole) for 7 min; at this time the other compounds were added.

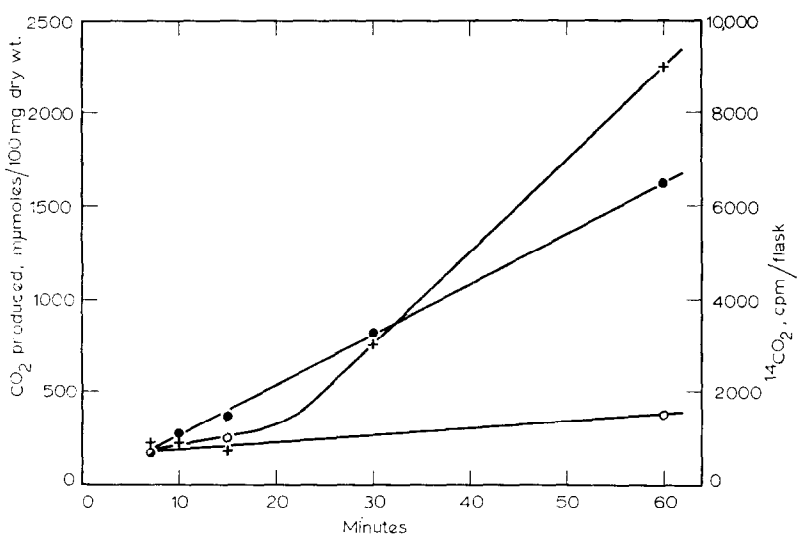


FIG. 4. Effect of DTT + $NADP^+$ + ATP on anaerobic CO_2 production by 2-PDS-inhibited Ehrlich ascites cells from 1-^{14}C-glucose. Each flask contained 30μmole glucose (1μc 1-^{14}C-glucose) and 44 × 10^6 washed Ehrlich ascites cells (14·5 mg dry wt.) in a total volume of 3·0 ml Krebs–Ringer bicarbonate buffer, pH 7·4. The gas phase was N_2–CO_2 (95:5). Incubations were carried out at 37°. ● = CO_2 production by untreated Ehrlich ascites cells; ○ = CO_2 production in the presence of 3·0 μmole 2-PDS; + = CO_2 production by 2-PDS-inhibited cells in the presence of 30 μmole DTT, 3 μmole $NADP^+$ and 3 μmole ATP. Cells were incubated with 2-PDS (3·0 μmoles) for 7 min; at this time the other compounds were added.

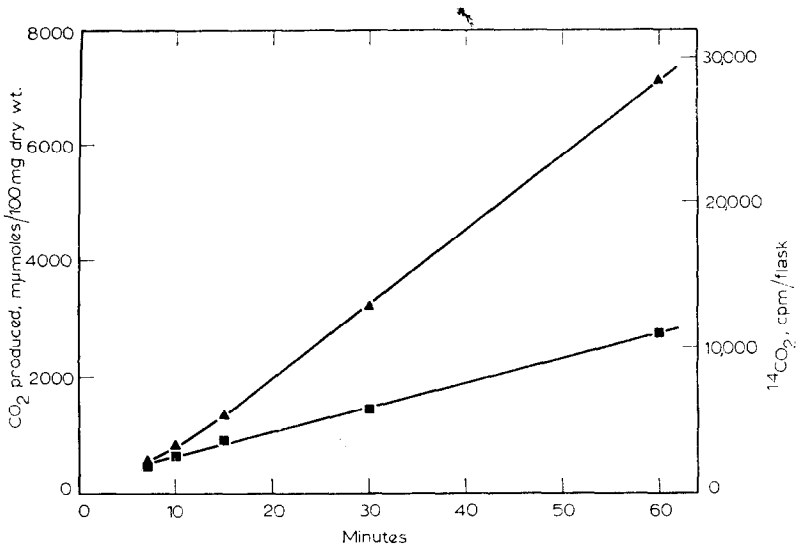


FIG. 5. Effect of DTT + $NADP^+$ + ATP on aerobic CO_2 production by Ehrlich ascites cells from 1-^{14}C-glucose. Each flask contained 30 μmole glucose (1 μc 1-^{14}C-glucose) and 24 × 10^6 washed Ehrlich ascites cells (15·0 mg dry wt.) in a total volume of 3·0 ml Krebs–Ringer phosphate buffer, pH 7·2. The gas phase was O_2–CO_2 (95:5). Incubations were carried out at 37°. ■ = CO_2 production by untreated cells; ▲ = CO_2 production in the presence of 30 μmole DTT, 3 μmole $NADP^+$ and 3 μmole ATP.

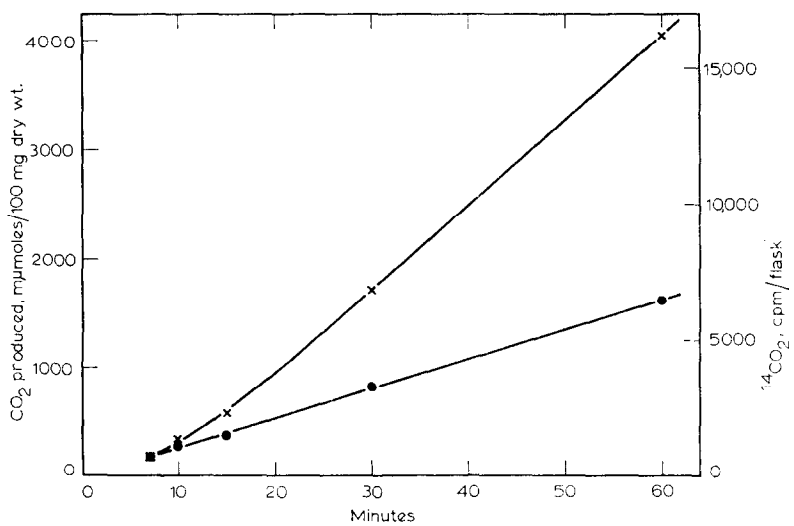


FIG. 6. Effect of DTT + $NADP^+$ + ATP on anaerobic CO_2 production by Ehrlich ascites cells from 1-^{14}C-glucose. Each flask contained 30 μmole glucose (1 μc 1-^{14}C-glucose) and 44 × 10^6 washed Ehrlich ascites cells (14·5 mg dry wt.) in a total volume of 3·0 ml Krebs–Ringer bicarbonate buffer, pH 7·4. The gas phase was N_2–CO_2 (95:5). Incubations were carried out at 37°. ● = CO_2 production by untreated cells; × = CO_2 production in the presence of 30 μmole DTT, 3 μmole $NADP^+$ and 3 μmole ATP.

TABLE 1. EFFECT OF VARIOUS COMPOUNDS ON ANAEROBIC CO_2 AND LACTATE PRODUCTION BY EHRLICH ASCITES CELLS

Additions*	No.	CO_2 production ± S.E. (range)† (μmole/hr/100 mg dry wt.)		No.	Lactate production ± S.E. (range)† (μmole/hr/100 mg dry wt.)	
Glucose	6	126 ± 5	(109–142)	4	141 ± 9	(125–166)
DTT, glucose		123 ± 4	(112–138)		149 ± 6	(139–163)
Glucose	8	125 ± 3	(115–142)	5	142 ± 7	(125–166)
DTT, $NADP^+$, ATP, glucose		162 ± 3	(151–180)		160 ± 3	(161–171)
Glucose	5	131 ± 4	(123–142)	3	145 ± 12	(125–166)
$NADP^+$, ATP, glucose		176 ± 5	(164–186)		176 ± 10	(165–196)
Glucose	2	116	(112–120)	2	134	(131–137)
$NADP^+$		6			0	
Glucose	3	133 ± 3	(125–142)	3	151 ± 7	(142–166)
ATP, glucose		169 ± 6	(159–180)		179 ± 4	(172–185)
Glucose	2	128	(125–132)	2	144	(142–146)
ATP		30			3	

* Final concentrations: 10^{-2}M for DTT and glucose; 10^{-3}M for $NADP^+$ and ATP.
† At 37°, in N_2–CO_2 (95:5); S.E. = standard error; No. = number of determinations.

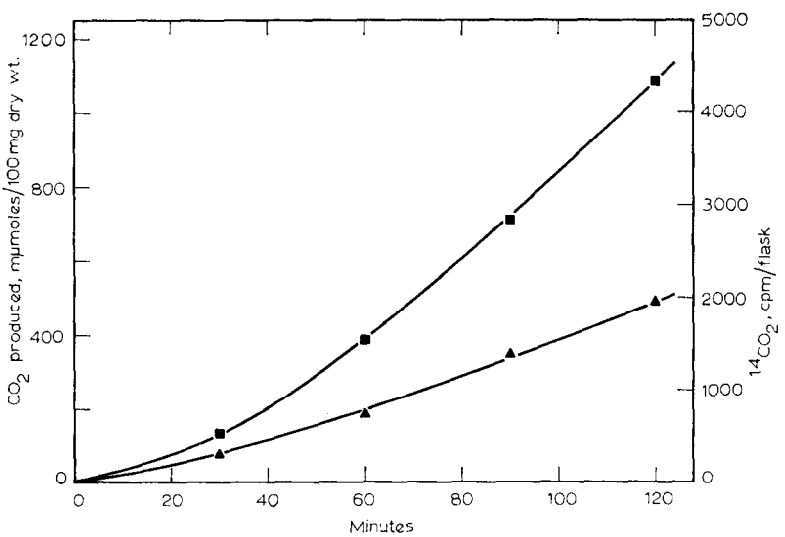


FIG. 7. Effect of DTT + $NADP^+$ + ATP on aerobic CO_2 production by Ehrlich ascites cells from 6-^{14}C-glucose. Each flask contained 30 μmole glucose (1 μc 6-^{14}C-glucose) and 22 × 10^6 washed Ehrlich ascites cells (13·7 mg dry wt.) in a total volume of 3·0 ml Krebs–Ringer phosphate buffer, pH 7·2. The gas phase was O_2–CO_2 (95:5). Incubations were carried out at 37°. ■ = CO_2 production by untreated cells; ▲ = CO_2 production in the presence of 30 μmole DTT, 3 μmole $NADP^+$ and 3 μmole ATP.

after a 2-hr incubation period the production of $^{14}CO_2$ from 6-^{14}C-glucose is inhibited about 55 per cent. Since the same additions do not inhibit lactate production from glucose, but rather stimulate it (Table 1), it would appear that this inhibition is due to a blockage of the Krebs cycle and not of the glycolytic pathway. Similar experiments have shown that the addition of ATP alone and $NADP^+$ alone has no significant effect on the aerobic production of $^{14}CO_2$ from 6-^{14}C-glucose. DTT alone, however, elicits an inhibitory effect somewhat smaller than that found when $NADP^+$ and ATP are also added. It was further established that the oxidized forms of DTT (10^{-3} M) and 2-TP (2×10^{-3}M) do not appreciably affect the rate of $^{14}CO_2$ evolution from 6-^{14}C-glucose under aerobic conditions. 2-PDS (10^{-3}M) inhibits aerobic CO_2 evolution from the C_6 of glucose (see Fig. 8 and below).

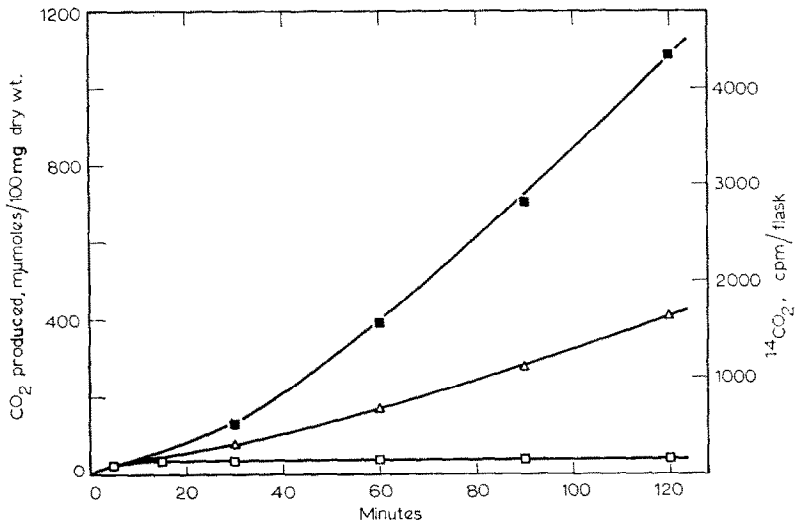


FIG. 8. Effect of DTT, $NADP^+$ and ATP on aerobic CO_2 production by 2-PDS-inhibited Ehrlich scites cells from 6-^{14}C-glucose. Each flask contained 30 μmole glucose (1 μc 6-^{14}C-glucose) and 22×10^6 washed Ehrlich ascites cells (13·7 mg dry wt.) in a total volume of 3·0 ml Krebs–Ringer phosphate buffer, pH 7·2. The gas phase was O_2–CO_2 (95:5). Incubations were carried out at 37°. ■ = CO_2 production by untreated Ehrlich ascites cells; □ = CO_2 production in the presence of 3·0 μmole 2-PDS; △ = CO_2 production by 2-PDS-inhibited cells in the presence of 30 μmole DTT, 3 μmole $NADP^+$ and 3 μmole ATP. Cells were incubated with 3·0 μmole 2-PDS; other compounds were added after 7 min.

Reversal of 2-PDS inhibition of glycolysis and respiration. Treatment of the inhibited Ehrlich ascites cells with DTT (10^{-2}M) (Table 2) brings about an 18 per cent reversal of glycolysis. If, in addition to DTT, $NADP^+$ (10^{-3} M) and ATP (10^{-3} M) are added, the reversal of lactate formation is increased to 30 per cent that of untreated cells. The presence of DTT is essential for this reversal, as is apparent from the data in Table 2.

When 2-PDS-inhibited cells are incubated with ATP-containing mixtures, the CO_2 production exceeds the lactate production by about 20 μmole/hr/100 mg dry wt. Even in the absence of lactate production, CO_2 production is about 20 μmole. When uniformly labeled ^{14}C — glucose is used as substrate, the addition of ATP (Table 3) causes no significant change in the amount of $^{14}CO_2$ evolved anaerobically. The amount of $^{14}CO_2$ produced from uniformly labeled ^{14}C-glucose under anaerobic conditions

is only 1 per cent of that obtained manometrically (Tables 1 and 2). These experiments prove that the extra amount of CO_2 produced by the cells in the presence of ATP is not due to the oxidation of the carbons of added glucose, but must come from the medium.

It is known[13] that the surface of Ehrlich ascites cells contains an ATPase acting on extracellular ATP. It is reasonable to assume that the ATP added in our experiments

TABLE 2. REVERSAL OF ANAEROBIC GLYCOLYSIS OF 2-PDS-INHIBITED EHRLICH ASCITES CELLS

Additions*	No.	CO_2 production ± S.E. (range)† by 2-PDS-inhibited‡ cells (μmole/hr/100 mg dry wt.)		No.	Lactate production ± S.E. (range)† by 2-PDS-inhibited cells‡ (μmole/hr/100 mg dry wt.)	
Glucose	17	4 ± 0·4	(1–9)	11	0·6 ± 0·3	(0–3)
DTT, glucose	6	20 ± 0·6	(9–41)	4	25 ± 9	(9–42)
DTT, $NADP^+$, ATP, glucose	11	62 ± 6	(32–96)	6	43 ± 10	(18–76)
$NADP^+$, ATP, glucose	7	27 ± 1	(24–32)	4	1	
$NADP^+$	2	3		2	0	
ATP, glucose	3	24 ± 1	(21–26)	3	0	
ATP	2	24	(22–26)	2	4	

* Final concentrations: 10^{-2}M for DTT and glucose; 10^{-3}M for $NADP^+$ and ATP.

† At 37°, in N_2–CO_2 (95:5); S.E. = standard error; No. = number of determinations.

‡ Actively glycolyzing cells in the presence of 10^{-2}M glucose, were treated with 10^{-3}M 2-PDS, 20–30 min prior to addition of the other compounds. Before inhibition, the cells produced 125 ± 2 μmole CO_2/hr/100 mg dry wt. (average of 18 determinations), and 142 ± 4 μmole lactate hr/100 mg dry wt. (average of 12 determinations). Manometric CO_2 determinations are not corrected for CO_2 retention.[9]

TABLE 3. EFFECT OF VARIOUS COMPOUNDS ON ANAEROBIC GLUCOSE OXIDATION BY EHRLICH ASCITES CELLS

Condition of cells	Additions*	$^{14}CO_2$ production (μmoles/hr/100 mg dry wt.)
Untreated	Glucose	1·42
	$NADP^+$, ATP, glucose	1·46
	DTT, $NADP^+$, ATP, glucose	1·60
2-PDS-inhibited	Glucose	0·05
	$NADP^+$, ATP, glucose	0·03
	DTT, $NADP^+$, ATP, glucose	0·48

* Concentrations: Glucose and DTT, 10^{-2}M; 2-PDS, $NADP^+$ and ATP, 10^{-3}M. The substrate was uniformly labeled ^{14}C-glucose. The experiments were carried out as described for the manometric determinations, using 3 μc of uniformly labeled ^{14}C-glucose per flask. The gas phase was N_2–CO_2 (95:5). The figures given are the average of 2–4 closely agreeing determinations.

is hydrolyzed to ADP + inorganic phosphate, with attending liberation of CO_2 from the bicarbonate of the medium. The data reported in Table 1 and 2 for the addition of ATP show that ATPase activity is found both in 2-PDS-inhibited cells and in intact cells. Thus we can conclude that 2-PDS does not affect the activity of ATPase.*

* In order to rule out the possibility that the acid responsible for CO_2 evolution may be some phosphorylated glycolytic intermediate, we examined the reaction mixture with the use of thin-layer chromatography combined with autoradiography. Both intact and inhibited cells were incubated with uniformly labeled ^{14}C-glucose and ATP as described under Methods. The cells were removed by centrifugation and the supernatant fractions chromatographed on thin-layer plates. The following results were obtained: (1) ATP accelerated the disappearance of glucose in untreated Ehrlich ascites cells; (2) no radioactive metabolite other than glucose could be detected with 2-PDS-inhibited cells; (3) in no case did we find spots corresponding to phosphorylated intermediates.

Aerobic incubation of 2-PDS-inhibited Ehrlich ascites cells with 6-^{14}C-glucose shows that these cells do not oxidize the C_6 of glucose to CO_2. Treatment of these metabolically inert cells with DTT + $NADP^+$ + ATP, however, restores the oxidation of C_6 to about 40 per cent that of untreated cells (Fig. 8). It is interesting to note that the rate of CO_2 evolution from the C_6 of glucose obtained by treating 2-PDS-inhibited cells with DTT + $NADP^+$ + ATP (about 400 μmole/120 min/100 mg dry weight, or about 40 per cent the activity of intact cells) is approximately the same as that obtained by treating noninhibited cells with the same mixture (about 500 μmole/120 min/100 mg dry wt., or about 45 per cent the activity of intact cells). It would appear that the amount of reversal obtained with this mixture corresponds approximately to the maximum amount of CO_2 from the C_6 of glucose that the cells are capable of producing in the presence of these compounds, even without prior inhibition with 2-PDS. Under anaerobic conditions, no CO_2 production from the C_6 of glucose takes place in the presence or absence of these additions.

Comment. From the data reported here and from data previously presented,[3, 4] it would appear that 2-PDS inhibits the metabolism of Ehrlich ascites cells by oxidizing cellular thiols as well as some nonsulfhydryl compounds. The reversal obtained by treatment with DTT can be interpreted as being due to reduction of enough cellular disulfides to allow the energy metabolism to be partially restored. The fact that the reversal of respiration and of glycolysis is not complete indicates that 2-PDS may have some irreversible effects on cell components, such as denaturation of specific enzymes. The results obtained show that 2-PDS does not appreciably inhibit ATPase. DTT (reduced form) has a moderate inhibitory action of its own, most likely at the level of the Krebs cycle.

These studies are being continued, to include the effect of various disulfides and mixtures on the metabolism of tumors and of normal tissues.

Acknowledgements—The authors wish to thank Dr. S. Abraham for helpful discussions and Miss H. T. Ruan for technical assistance.

REFERENCES

1. D. R. GRASSETTI, M. E. BROKKE and J. F. MURRAY, JR., *J. med. Chem.* **8**, 753 (1965).
2. D. R. GRASSETTI and J. F. MURRAY, JR., *Archs Biochem. Biophys.* **119**, 41 (1967).
3. D. R. GRASSETTI and J. F. MURRAY, JR., *Biochem. Pharmac.* **16**, 2387 (1967).
4. D. R. GRASSETTI and J. F. MURRAY, JR., *Analyt. Biochem.* **21**, 427 (1967).
5. W. MARCKWALD, W. KLEMM and H. TRABERT, *Ber. dt. Chem. Ges.* **33**, 1556 (1900).
6. W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER, *Manometric Techniques*, p. 149. Burgess, Minneapolis (1957).
7. O. WIELAND, in *Methods of Enzymatic Analysis* (Ed. H. U. BERGMEYER), p. 271. Academic Press, New York (1963).
8. S. B. BARKER and W. H. SUMMERSON, *J. biol. Chem.* **138**, 535 (1941).
9. W. W. UMBREIT, R. H. BURRIS and J. F. STAUFFER, *Manometric Techniques*, p. 99. Burgess, Minneapolis (1957).
10. H. BARUSCH and I. L. CHAIKOFF, *Proc. Soc. exp. Biol. Med.* **86**, 97 (1954).
11. D. R. GRASSETTI, J. F. MURRAY, JR. and J. L. WELLINGS, *J. Chromat.* **18**, 612 (1965).
12. W. W. CLELAND, *Biochemistry, N.Y.* **3**, 480 (1964).
13. G. ACS, W. OSTROWSKI and F. B. STRAUB, *Acta physiol. hung.* **6**, 261 (1954).