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The influence of phenazinium upon glycolysis and the pentose pathway in human red cells*

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BIOLOGICAL interest in phenazines began with the discovery of the bacterial pigment pyocyanine[1–3] and with its suppressive effects on the growth of a variety of bacteria.[4–7] That some phenazines directly affect growth processes in metazoa,[8–11] including tumor growth[12, 14] has led recently to examination of a number of these substances as potential antitumor agents.[14, 15] One of these, 1:3-diamino-5-, methyl phenazinium chloride (phenazinium,† Fig. 1), has some activity against mouse leukemia 1210, and is at present undergoing clinical trial in human neoplasia. Its metabolic effects are being currently studied, and preliminary data upon glycolysis and the pentose pathway, with the human red cell as a model system, are here presented.

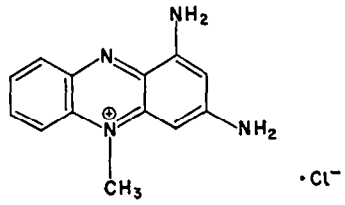

FIG. 1. Phenazinium.

EXPERIMENTAL

Studies were carried out in a standard Warburg apparatus at 37°, the period of incubation being 3 hr. The incubation medium was 0·15 M phosphate buffer, pH 7·7, 0·08 M in glucose. All determinations were performed in triplicate and the mean used as the final result. Oxygen consumption was determined manometrically, CO_2 production by the recovery of $^{14}CO_2$ obtained from substrate glucose labeled with ^{14}C in the 1-, 2-, 6-positions, and from universally labeled glucose, the counts being measured in a Packard Tri-carb scintillation counter by the method of Passmann *et al.*[16] Lactate production was studied by the methods of Barker and Summerson[17] and by a lactate dehydrogenase

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method.[18] Unless otherwise indicated, fresh red cells from normal human subjects were used in each case. Differentiation of these cells into "old" and "young" groups was achieved by the technique of Prankerd.[19] Varying concentrations of phenazinium and of other reagents were added to the flasks from the side arm as required.

RESULTS

Table 1 shows the general effect of phenazinium on red cell metabolism, contrasted with methylene blue. Phenazinium greatly augmented oxygen consumption, while lactate production did not keep pace with glucose utilization. The large amount of $^{14}CO_2$ produced was derived from glucose labeled in the 1-position. Phenazinium thus behaved much like methylene blue, acting as a powerful stimulus to the aerobic pentose pathway, and was at least as potent as methylene blue in this respect.

TABLE 1. EFFECTS OF PHENAZINIUM ON OVERALL RED CELL METABOLISM CONTRASTED WITH METHYLENE BLUE

Reagent and concentration (M)	O_2 Consumption (μl/3 hr)	Glucose consumption (μmoles/ml cells/hr)	Lactate production (μmoles/ml cells/hr)	CO_2 Production	
				counts/min added	counts/min recovered
Control	19·8	1·99	4·10	24,200	286
Methylene blue, 0·005	398·0	3·26	3·80	23,700	5723
Phenazinium, 0·0001	426·0	3·42	3·90	23,890	5988

Each Warburg flask contained 0·5 μc glucose 1-^{14}C.

In both older and younger cells, as prepared by differential centrifugation, phenazinium augmented the oxidative pathway, the young cells showing a greater response than the old. Normally, the older cells metabolized a larger percentage of glucose by the oxidative pathway than the younger cells, but phenazinium augmented this pathway in both cells. Methylene blue behaved similarly, though its effects, relative to concentration, were not so great. As an additional experiment, the effect of both agents on red cells stored for varying periods in acid–citrate–dextrose (ACD) at 4° was examined. Here, phenazinium again stimulated the pentose pathway, the effect being much less marked in cells stored for 21 days than in those stored for 7 days. Methylene blue, in higher concentration, produced a similar response. The increase in oxygen consumption produced by both these reagents was not inhibited by cyanide at a final concentration of 0·001 M and thus their mode of action, i.e. as hydrogen carriers, appeared to be identical.

The influence of phenazinium on red cell metabolism was further studied by measuring $^{14}CO_2$ production after varying the position of the ^{14}C label in the substrate glucose. Table 2 shows that, in the absence of phenazinium or methylene blue, very little $^{14}CO_2$ was recovered, wherever the label was located. In the presence of these compounds, a great increase in $^{14}CO_2$ production was noted, being maximal with glucose-1-^{14}C, but practically no activity was recovered from glucose-6-^{14}C. The proportion of activity recovered as $C^{14}O_2$ from the second glucose carbon approached about half the recovery from the first (aldehyde) carbon. This is in agreement with the findings of other workers[20] and is evidence of the recycling of some of the pentose phosphate to glucose 6-phosphate.

In concentrations greater than 10^{-3} M, phenazinium appears to be directly toxic, oxygen consumption being substantially reduced at these levels. Concentrations less than 5×10^{-5} M are without effect on carbohydrate metabolism.

It appears probable that the the profound effect of phenazinium on the pentose pathway occurs by virtue of its property as an excellent electron acceptor. It is likely that, in the presence of oxygen, phenazinium may effect a direct transfer of electrons from $NADPH_2$ to atmosphereric oxygen. Its main effects may thus occur on the initial dehydrogenations in the pentose cycle. A marked augmentation of these reactions might accelerate the entire pathway, as appears to be the case. Methylene blue

TABLE 2. $^{14}CO_2$ PRODUCTION FROM VARIOUSLY LABELED GLUCOSE-^{14}C BY HUMAN ERYTHROCYTES IN THE PRESENCE OF PHENAZINIUM (10^{-4} M) COMPARED WITH METHYLENE BLUE (5×10^{-3} M)

Reagent added	Glucose label	O_2 Consumption (μl/3 hr)	Glucose consumption (μmoles/ml cells/hr)	CO_2 Production		
				counts/min added	counts/mtn recovered	% recov.
Phenazinium	-1-^{14}C	422	3·44	25,450	5750	22·6
Phenazinium	-2-^{14}C*	413	3·42	10,650	1260	11·8
Phenazinium	-6-^{14}C	419	3·41	21,800	64	<1
Phenazinium	-U-^{14}C	418	3·41	26,600	2020	7·1
Methylene blue	-1-^{14}C	405	3·38	22,900	5600	24·5
Methylene blue	-2-^{14}C*	409	3·38	9800	1150	11·7
Methylene blue	-6-^{14}C	416	3·42	23,500	54	<1
Methylene blue	-U-^{14}C	419	3·41	26,990	2060	7·6
Control	-1-^{14}C	20·8	2·10	24,200	232	<1
Control	-2-^{14}C*	19·6	1·98	6950	63	<1
Control	-6-^{14}C	20·4	2·00	22,300	50	<1
Control	-U-^{14}C	19·5	1·99	22,500	64	<1

* 0·2 μc glucose-2-^{14}C/flask; all other flasks contain 0·5 μc/flask.

behaves in a similar manner, but higher concentrations are needed to achieve the degree of augmentation observed with phenazinium. The toxic action of phenazinium seen with concentrations greater than 10^{-3} M might be significant clinically if susceptible tumor cells can concentrate the drug.

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