Stimulation of Erythrocyte Metabolism by Menadione.* (25961)

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The human erythrocyte in the presence of an autoxidizable dye such as methylene blue can metabolize glucose *via* the hexose monophosphate shunt. In fact, as much as 85% of oxygen consumed by the erythrocyte can be accounted for by this mechanism(1). Since the demonstration(2,3) that respiration of the erythrocyte can be stimulated by methylene blue, investigators have sought a physiological electron carrier to substitute for the dye. Most of these attempts have met with little success, although it has been reported that a toxoflavin from *Pseudomonas cocoven* stimulates the enzyme system(4). Substances reported to be without effect are riboflavin, flavin adenine dinucleotide, reduced glutathione, ascorbic acid and ergothionine(3). The present study indicates that menadione will substitute for the artificial carrier in the erythrocyte system and stimulate oxygen consumption by the cells. It is further demonstrated that such stimulation is dicumarol sensitive and can be appreciably decreased when dicumarol is present in the reaction flasks.

Methods. Blood collected in acid citrate-dextrose was centrifuged to remove the buffy coat and to collect the cells. These were subsequently washed with additional acid citrate-dextrose followed by 3 washings with Krebs-Ringer phosphate buffer pH 7.4. The cells were finally resuspended in the buffer, volume of which was adjusted to give a final hematocrit of 50. Adenosine (10 μmoles/ml) was added to all preparations(3). Oxygen uptake was determined by the conventional Warburg technic. Coenzyme Q_{10}† and α-tocopherylquinone were added to the reaction in bovine plasma albumin, ethanol, buffer suspension, 5 mg coenzyme Q_{10} or α-tocopherylquinone, 2 mg albumin, 0.1 ml ethanol per ml suspension.

Results. Table I summarizes the results obtained when the erythrocytes were incubated with menadione. Upon incubation, oxygen consumption of the cells compared favorably with that obtained with methylene blue or other autoxidizable dyes. When concentration of menadione was varied, oxygen consumption also varied. For example, menadione (.15 μmole) caused an oxygen consumption of 50.9 μl/hr/hematocrit of 50; increasing the concentration to 0.31 μmole resulted in an oxygen consumption of 76.7 μl/hr/hematocrit of 50.

Since it has been demonstrated that dicumarol is an effective inhibitor of Vit. K(5), it was felt to be of interest to assess its role in the menadione stimulated erythrocyte system. Addition of dicumarol to the system resulted in inhibition of oxygen consumption produced by menadione (Table II). On the other hand, dicumarol had no effect when methylene blue was used to stimulate the system.

The fact that menadione, a napthoquinone, was capable of maintaining oxygen consumption by the erythrocyte prompted evaluation of like compounds in this study. Coenzyme Q_{10} and α-tocopherylquinone were without effect.

Other compounds capable of acting in redox systems were also tried. The following were without effect: ergothionine, dehydro-ascorbic

TABLE I. Effect of Menadione on Qo_2 of Human Erythrocytes.

Additions (μmole)		Qo_2, μl/hr/hematocrit of 50 Mean	Range
Methylene blue	(.31)	79.5	71.1-88.4
Menadione	(.31)	76.7	72.6-82.5
"	(.15)	50.9	48.7-55.0
"	(.05)	14.1	12.8-15.2
"	(.03)	6.0	4.5-10.6

Each Warburg flask contained: 1 ml erythrocytes, 30 mmoles glucose, 1 ml buffer pH 7.4, 0.2 ml 6 N NaOH. Menadione or methylene blue added as indicated. Total vol 3.2 ml. Oxygen uptake values corrected for endogenous respiration.

Menadione was dissolved in 7% alcohol prior to use. Alcohol controls exerted a negligible effect on the system.

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† Kindly supplied by Merck, Sharp & Dohme.

TABLE II. Effect of Dicumarol on Erythrocyte System.

Dicumarol, μmoles	% inhibition	
	Menadione (.31 μmole)	Methylene blue*
.31	25.9	0
1.5	44.0	0
3.1	50.1	0

System same as indicated under Table I. Dicumarol added at the expense of buffer.

* A slight potentiation of action of methylene blue was noted with dicumarol which appeared to be independent of dicumarol concentration.

acid, hypoxanthine, and oxidized glutathione.

Discussion. The results presented here would suggest that in addition to autoxidizable dyes, menadione is capable of supporting oxygen consumption in the erythrocyte system. It is tentatively assumed that oxygen uptake in the presence of menadione, like that in the presence of methylene blue, is a measure of the glucose oxidative pathway in this tissue since, in the human erythrocyte, oxygen utilization occurs almost entirely through the hexose monophosphate shunt. Similar results have been reported(6) on a rat liver fraction which reoxidizes TPNH (reduced triphosphopyridine nucleotide), where it was noted that the enzyme preparation reacted with electron acceptors, notably Vit. K and coenzyme Q_{10}, but the diaphorase was particularly active in presence of Vit. K_3 (menadione).

The sensitivity of the menadione stimulated system to dicumarol and the lack of effect with dicumarol in presence of methylene blue implies that the effects noted with dicumarol are specific for menadione and do not necessarily imply interference with the action of the erythrocyte system.

Menadione has been previously demonstrated to be effective in increasing the activity of the phosphogluconate pathway in ascites tumor cells(7). Subsequent studies(8) also noted the dicumarol inhibitory effect on C-1 oxidation *via* the pentose pathway. However, the present experiments are the first demonstration of such an effect in the human erythrocyte.

Of added importance to this study is the nature of the defect in congenital methemoglobinemia (Type I). Since this deficiency can be corrected by methylene blue, it would be of interest to know if menadione would substitute for methylene blue in the treatment. This would appear to be a reasonable possibility because both compounds are most likely serving in the electron transport system between reduced pyridine nucleotides and methemoglobin.

It is not immediately apparent why coenzyme Q_{10} and α-tocopherylquinone did not support oxidation by the pentose pathway. The high concentration of alcohol required to solubilize these compounds may have inactivated the system; also, the side chain on these compounds may have prevented access to sites within the cell necessary for activity. Of interest also is the fact that addition of CoQ_{10} to ascites cells failed to stimulate decarboxylation *via* the phosphogluconate oxidative pathway(8). Studies are now in progress on isolation of the enzyme system and the role of coenzyme Q_{10} and like substances will be evaluated on the isolated system.

Summary. In the human erythrocyte, menadione is capable of supporting oxygen consumption. In this respect, it can replace autoxidizable dyes such as methylene blue that have been previously used. Oxygen consumption, as a measure of the pentose pathway, is inhibited by dicumarol.

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