# EFFECT OF INHIBITORS ON THE METABOLISM OF SPECIFICALLY LABELLED GLUCOSE BY BRAIN

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## SUMMARY

Arsenite and menadione alter the normal metabolic pathways of guinea-pig brain cortex in a manner which may be interpreted as permitting a greater participation of the direct oxidative pathway, in contrast to the Embden-Meyerhof pathway. The means by which these two compounds accomplish this change appear to be fundamentally different. Chlorpromazine, two dodecylpyridinium compounds, iodoacetate and probably arsenate seem to be general metabolic inhibitors which have little selective influence on these two routes. Similarly, in uninhibited brain metabolism, potassium chloride stimulation of respiration or its omission have no selective influence.

## INTRODUCTION

Mammalian brain is generally considered to utilize the Embden-Meyerhof glycolysis route almost exclusively[1,2]. For example, brain slices consistently give a value of approximately 1 for the ratio $^{14}CO_2$ from [6-$^{14}$C]G/$^{14}CO_2$ from [1-$^{14}$C]G. It appeared interesting, therefore, to test whether some metabolic inhibitors might be capable of dissociating this ratio by altering the metabolic pathways.

Although the use of inhibitors may give a distorted picture of normal metabolism, the interesting question underlying these studies is less one of what biochemical pathways are normally used than one of how existing pathways may be modified and, qualitatively, in view of the limitations of the present method[3], to what extent alternate pathways may be utilized.

## MATERIALS AND METHODS

### *Metabolic experiments*

Warburg vessels were used which were equipped with a center-well and two side-arms. When assembled, vessels contained the following: in one side-arm, 50 $\mu$moles glucose (radioactive plus non-radioactive) in 0.2 ml 1.5 *M* KCl[4] (in 0.2 ml distilled water for the experiment in which KCl stimulation was omitted); in the other side-arm 0.15 ml 5 *N* sulfuric acid; in the center-well 0.15 ml 20 % KOH and a filter paper wick; and in the main compartment approx. 50 mg of guinea-pig brain cortex slices and sufficient phosphate saline buffer[4] to give a final volume of 3.0 ml. When other compounds were added, they were dissolved in the buffer and the pH was checked to maintain 7.4. At the end of a 3-h incubation period, 30°, $O_2$ atmosphere, the reaction

was stopped by the addition of acid and the contents of the center-well were assayed as previously described[5].

*Inhibitors*

The following quaternary nitrogen derivatives, the abbreviations of which are given, were used: pyridine-2-aldoxime methiodide, PAM[6]; pyridine methiodide, PMI[7]; pyridine-2-aldoxime dodeciodide, PAD[8]; pyridine dodeciodide, PDI[7]. The methylated members of this series are lipid-insoluble; the dodecyl analogues, lipid-soluble. All of the pyridine derivatives were synthesized by Dr. S. GINSBURG of this Department. Chlorpromazine hydrochloride was a gift from Smith, Kline, and French Laboratories, Philadelphia, and Synkavite was kindly given by Hoffmann-La Roche Inc., Nutley, New Jersey.

The methylphosphonate diester of menadione, 2-methyl-1,4-naphthohydroquinone di(methylphosphonic acid) ester, was synthesized by the following steps. Methylphosphonyl dichloride (5 g, 0.04 mole), obtained through the courtesy of Chemical Warfare Laboratories, Army Chemical Center, Maryland, was reacted with 1.74 g (0.01 mole) of 2-methyl-1,4-dihydroxynaphthalene in an excess of anhydrous pyridine. About 15 min after mixing, a mildly exothermic reaction commenced and a white precipitate formed over a period of several hours. The reaction mixture was filtered, care being taken to avoid contact with atmospheric moisture, and the filtrate was concentrated to a pale yellow syrup at about 80° and 20 mm pressure. Ice was added to the syrup and sufficient ether to give, finally, about 1:25 ether : water. This mixture was shaken violently and, after the ice had melted, filtered. The white precipitate was washed with ether, dissolved in hot ethyl alcohol, and recrystallized on addition of ether and cooling. The product melted at 204–206° and had an equivalent weight by titration of 174.2. Another recrystallization from alcohol–ether and a recrystallization from water gave a sharp-melting white crystalline product; m.p., 204–205°, equivalent weight, 167.0. Calc. for $C_{11}H_8O_2[CH_3P(O)OH]_2$, 165.1, u.v. absorption spectrum virtually identical with that of the diphosphate ester, Synkavite.

## RESULTS

The results are summarized in Table I, which shows oxygen uptake by slices of guinea-pig brain cortex, the production of $^{14}CO_2$ from [1-$^{14}$C]G and from [6-$^{14}$C]G, and the ratios $^{14}CO_2$ from [6-$^{14}$C]G/$^{14}CO_2$ from [1-$^{14}$C]G. It is known that oxygen uptake and glucose metabolism in brain slices are stimulated by an increase in the potassium ion concentration from about 0.005 *M* to about 0.1 *M*[4,9]. The difference in results between low and high potassium ion concentrations is shown in the first two lines of Table I. All of the other compounds added, shown in the next three groups of results in Table I, were tested under KCl stimulation, *i.e.*, in the higher potassium ion concentration. The first five compounds produced little or no deviation from the normal values. Chlorpromazine and iodoacetate, at the concentrations used, caused marked inhibition of both radiochemical yields and oxygen consumption. Both compounds, however, had little effect on the ratios of the radiochemical yields. Both PAD and PDI also caused marked metabolic inhibition but, again, did not greatly alter the ratio of the radiochemical yields from the two labelled substrates.

TABLE I

METABOLISM OF RADIOACTIVE GLUCOSE BY SLICES OF GUINEA-PIG BRAIN CORTEX

K ion concentration sufficient for stimulation of respiration in all experiments except the second, as indicated. First experiment average of 5 (n = 4 for calculation of standard deviation); all others single experiments. Other conditions given in text.

| *Compound added* | *Molarity,* $- \times 10^{-4}$ | *Radiochemical yield of* $^{14}CO_2$, *% per g fresh tissue, from* [1-$^{14}$C]G | [6-$^{14}$C]G | $O_2$ *uptake, µl per g fresh tissue* | *Ratio,* $\frac{[6\text{-}^{14}C]G}{[1\text{-}^{14}C]G}$ |
|---|---|---|---|---|---|
| None | | 20.3 ± 4.8 | 18.4 ± 5.4 | 3208 ± 599 | 0.90 ± 0.11 |
| None, no $K^+$ stim. | | 7.56 | 7.88 | 1948 | 1.04 |
| Benzene | 300 | 19.1 | 23.0 | 3405 | 1.20 |
| PAM | 5 | 20.2 | 18.5 | | 0.92 |
| PMI | 5 | 19.7 | 15.1 | 3175 | 0.77 |
| Dodecyl iodide* | | 17.1 | | 3125 | |
| Methylphosphonate diester of menadione | 10 | 25.6 | 17.9 | 3005 | 0.70 |
| Chlorpromazine | 1 | 4.11 | 3.56 | 1205 | 0.87 |
| Chlorpromazine | 5 | 2.54 | 1.95 | | 0.77 |
| Iodoacetate | 4 | 7.64 | 6.80 | 1798 | 0.89 |
| PAD | 5 | 7.81 | 5.37 | | 0.69 |
| PDI | 5 | 6.93 | 5.88 | 1425 | 0.85 |
| Arsenate | 5 | 15.2 | 11.0 | 2780 | 0.72 |
| Arsenite | 2 | 10.9 | 0.47 | 400 | 0.04 |
| Arsenite | 8 | 2.87 | 0.095 | 828 | 0.03 |
| Synkavite | 5 | 46.4 | 14.8 | 3650 | 0.32 |
| Synkavite | 10 | 63 | 16.0 | 3840 | 0.25 |

* 1 mg per Warburg vessel; approx. $10 \cdot 10^{-4}$ *M*, had it been soluble.

Arsenate ion, at the concentration shown, appears to have a small, but similar, effect on glucose metabolism.

A very different picture was obtained with the third group of inhibitors tested. A concentration of arsenite which caused about half-inhibition of the radiochemical yield from [1-$^{14}$C]G caused an 88 % inhibition of oxygen uptake and virtually abolished the radiochemical yield of $^{14}CO_2$ from [6-$^{14}$C]G. This, in turn, reduced the ratio $^{14}CO_2$ from [6-$^{14}$C]G/$^{14}CO_2$ from [1-$^{14}$C]G from 0.90 to 0.04. A higher concentration of arsenite had a consistently similar effect except for a slightly higher oxygen uptake. Synkavite also markedly reduced the ratio of the radiochemical yields, but in a strikingly different way from that observed with arsenite. Here, there was no inhibition of oxidation or of $^{14}CO_2$ production from [6-$^{14}$C]G, but instead a several-fold stimulation of the radiochemical yield of $^{14}CO_2$ from [1-$^{14}$C]G. The oxygen uptake, although not statistically significantly higher than the average controls, was, in both cases, increased by about 20 % over the individuals controls.

## DISCUSSION

It appears from these results that brain tissue, which generally metabolizes glucose essentially by the Embden-Meyerhof pathway[1,2], can be made to utilize what must be a pre-existing alternate route. Arsenite has accomplished this by the inhibition of a later common reaction, probably the one involving fumarase or the enzyme or

enzymes which catalyze the oxidative decarboxylation of pyruvate to give acetyl coenzyme-A[10,11].

The means by which Synkavite has accomplished this is probably related to the uncoupling and accompanying stimulatory action[12,13] of its hydrolysis product, 2-methyl-1,4-naphthohydroquinone or the corresponding quinone, menadione. This conclusion is supported by the lack of effect of enzymically unhydrolyzable methylphosphonate diester of menadione, the latter it was found, being unhydrolyzed after 5 h incubations in liver brei or in intestinal brei, treatments which completely hydrolyzed Synkavite. Such resistance to enzyme action has been noted previously for a methylphosphonate ester[14]. It is safe to assume that the total dissociation and hydrolysis of the Synkavite, which would have increased the sodium ion content of the buffering medium by only about 3 % and the phosphate content by only about 20 %, respectively, would not account for the effects observed. Whether the results obtained here with Synkavite could be realized at lower concentrations or with other uncoupling agents or even with vitamin $K_1$ would require further investigation. However, it is noteworthy that other investigators have concluded, on the basis of results obtained with inhibitors and specifically labelled glucose, that the hexose monophosphate oxidative pathway may be involved in the intermediary metabolism of erythrocytes[15] and retinal tissue[16].

Chlorpromazine, in contrast to arsenite, appears to exercise its inhibitory action on an early common step, as has been partly indicated in studies of the effects of chlorpromazine on purified hexokinase[17].

Two predominantly lipid-soluble compounds, benzene and dodecyl iodide, and two predominantly water-soluble compounds, PAM and PMI, are without effect, so far as tested, but the lipid- and water-soluble compounds PAD and PDI are inhibitory. This effect may be accounted for by two factors: first, the greater ability of PAD and PDI to penetrate tissue[8]; and second, the ability of these compounds to react unspecifically with proteins due to Van der Waal's and Coulombic forces when applied in high concentration (unpublished results of S. EHRENPREIS AND HANNA GREENBERG of this Laboratory; see also NACHMANSOHN[18]). At lower concentrations ($10^{-6}$ *M* and lower) such lipid-soluble quaternary nitrogen ions as, *e.g.*, PAD and PDI show specific interactions with the acetylcholine system. These and related specific, directed attractions are responsible for the powerful reactivating action of PAM on inhibited acetylcholinesterase.

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# A MAGNETO-KINETIC STUDY OF THE REACTION BETWEEN FERRIMYOGLOBIN AND METHYL HYDROGEN PEROXIDE

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## SUMMARY

1. The reaction between ferrimyoglobin and methyl hydrogen peroxide has been studied with a new instrument for measuring rapid changes in the magnetic susceptibility of dilute aqueous solutions of proteins.

2. A comparison of the magnetically and spectrophotometrically obtained rate data shows differences, increasing toward lower temperatures, which can be explained in part by the production of free radicals.

3. The molar susceptibility at 20° C of the myoglobin compound formed is $3300 \cdot 10^{-6}$ emu with a standard error of $500 \cdot 10^{-6}$ emu.

## INTRODUCTION

The reactions of the hemoproteins with peroxides are of interest in themselves and pertinent to the elucidation of the mechanisms of peroxidase and catalase action. They have been studied spectrophotometrically and magnetometrically for many years. The recent development of rapid spectrophotometric techniques[1] has permitted

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