much more favorable. For example, in the case of yeast alcohol dehydrogenase the reaction between the acetylpyridine analogue of DPN and ethylmercaptan is approximately a million times more favorable than when the reaction is carried out in the absence of enzyme. It is of interest to note that although DPN shows little reaction with yeast alcohol dehydrogenase and ethylmercaptan, crystalline horse liver alcohol dehydrogenase will readily form a complex with the thiol compound and DPN (see Table I).

The reaction between the enzyme, coenzyme and substrate analogue exhibits specificity. Only those thiol derivatives which show a sufficient structural resemblance to the normal substrate of the various dehydrogenases will react with the pyridine nucleotides. For example, beef heart lactic dehydrogenase will react with mercaptoacetic or mercaptosuccinic acids, but not with 2-mercaptoethanol. Similar specificity is shown by skeletal muscle lactic dehydrogenase. The alcohol dehydrogenases will only form complexes with the *n*-alkyl mercaptans. Malic dehydrogenase will form a complex with mercaptosuccinic acid but not with ethylmercaptan. This enzyme appears to give a far more favorable complex with pyridine-3-aldehyde analogue of DPN than with DPN. Crystalline beef liver glutamic dehydrogenase forms a complex with DPN and mercapto-acetic acid, but not with mercaptosuccinic acid. Crystalline rabbit muscle glycerophosphate dehydrogenase shows a complex with propane-1,2-dithiol but not with thioethanol. Again the complex with the pyridine-3-aldehyde analogue appears to be far more favorable than with DPN.

The chemical reaction between hydroxylamine and DPN has a maximum at 315 m$\mu$[5]; in the presence of the horse liver alcohol dehydrogenase the maximum is shifted to 300 m$\mu$[1]. In a similar manner, the absorption maxima of the enzymically-bound thiol complexes are shifted toward shorter wavelengths (Table I). In all cases, where an enzymic reaction has been observed, a non-enzymic complex has also been detected.

The results of this work strongly support the previously proposed hypothesis that an addition reaction of substrate to coenzyme may be the first step in the reaction catalysed by this class of dehydrogenase[5]. Details of the kinetic implications and properties of the thiol-pyridine nucleotide complexes will be published shortly.

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Received October 15th, 1956

## The enzymic oxidation of reduced vitamin $K_3$ (menadione)

Although enzymes catalysing the reduction of vitamin $K_3$ (menadione)[1] or of vitamin $K_1$[2] by reduced diphosphopyridine nucleotide (DPNH) are known, there are no reports in the literature that the resultant hydroquinones can be re-oxidized by mitochondrial preparations. Both hydroquinones are, in fact, rapidly auto-oxidizable by air at neutral pH, but at pH 6.24, in the presence of ethylenediaminetetraacetate (EDTA), the auto-oxidation of the hydroquinone of menadione ($K_3H_2$) is very slow. It has now been shown, by working at this pH, that sarcosomal (mitochondrial) fragments prepared from horse heart (Keilin and Hartree heart-muscle preparation) actively catalyse the oxidation of $K_3H_2$ in the absence of added cytochrome *c*.

The special interest of this reaction lies in the fact that, whereas a large number of substances (such as ascorbic acid, benzohydroquinone) can be oxidized by this preparation in the presence of added cytochrome *c*[3], only succinate, DPNH and *p*-phenylenediamine are oxidized in the absence of the added cytochrome. The oxidation of $K_3H_2$ resembles that of succinate and DPNH rather than that of *p*-phenylenediamine, since it is completely inhibited by antimycin A.

The $K_3H_2$ oxidase activity was measured spectrophotometrically by following the increase of the optical density at 262 m$\mu$, as shown in Fig. 1. After measuring the rate of the auto-oxidation, the enzymic reaction was started by adding a suitably diluted heart-muscle preparation to each cuvette. After the completion of the reaction, the spectrum was identical with that of menadione, with bands at 250, 262–263 and 340 m$\mu$. The course of the oxidation shows first order kinetics, and the activity of the enzyme was expressed in terms of the first order velocity constant, $k'$. Doubling the initial concentration of the $K_3H_2$ did not affect $k'$.

Since first order kinetics were obtained with $K_3H_2$, and zero order kinetics are found with comparable concentrations of DPNH[5], it is not possible to compare the specific activities of the

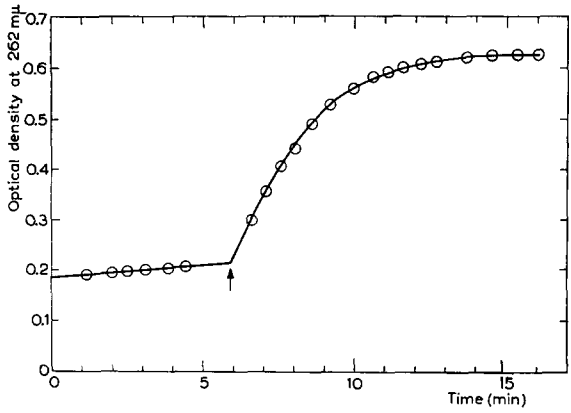


Fig. 1. Oxidation of $K_3H_2$ by heart-muscle preparation. The test cuvette contained $K_3H_2$ (prepared according to FIESER[4]), $3.8 \cdot 10^{-5} M$; phosphate buffer, pH 6.24, $0.04 M$; EDTA, $10^{-3} M$. The reference cuvette contained the buffer and EDTA. Heart-muscle preparation (final concentration, 26 μg/ml) was added to both cuvettes at the arrow. Temp., 22.2° C. The optical densities have been corrected for the dilution caused by the addition of the heart-muscle preparation.

two oxidase systems. The rate of oxidation of $K_3H_2$ is, however, a rapid reaction, the initial rate of oxidation in the experiment illustrated in Fig. 1 corresponding to a $Q_{O_2}$ (μl $O_2$/h/mg protein) of 365 at pH 6.24, and 22.2° C, compared with 284 for the DPNH oxidase system at pH 7.3 and 20° C. A good qualitative correlation was found between the $K_3H_2$ oxidase and the DPNH oxidase activities of different heart-muscle preparations.

The relative sensitivities of the different oxidase systems to antimycin are compared in Table I. The $K_3H_2$ oxidase system is the most sensitive; the DPNH oxidase system is considerably more sensitive than the succinic oxidase system, while as would be expected from the literature[7], the oxidation of *p*-phenylenediamine was completely unaffected. The $K_3H_2$ oxidase was inhibited 95% by $0.001 M$ cyanide and 99.5% by $0.01 M$ cyanide.

TABLE I

SENSITIVITY OF OXIDASE SYSTEMS TO ANTIMYCIN A

Concentrated heart-muscle preparation (35.5 mg protein/ml) was treated with different amounts of antimycin according to Method II of THORN[6], and after dilution, the rates of oxidation of succinate, DPNH and $K_3H_2$ were determined on the same suspension. The experiment with *p*-phenylenediamine was carried out with the same heart-muscle preparation treated separately with antimycin. The rates of oxidation of succinate, DPNH and *p*-phenylenediamine were measured as previously described[2,5].

| *Antimycin* (μg/g protein) | *Inhibition (%) of oxidation of* | | | |
|---|---|---|---|---|
| | *succinate* | *DPNH* | $K_3H_2$ | *p-phenylenediamine* |
| 0.07 | 22 | 24 | 46 | 0 |
| 0.105 | 41 | 80 | 85 | —1 |
| 0.14 | 99 | 97 | 91 | —1 |

Since the same particles catalyse the oxidation of DPNH, succinate and $K_3H_2$, it might be expected that, in the absence of oxygen, they would also catalyse reactions between the various substrates. A slow oxidation of DPNH by fumarate, under these conditions, has already been reported[5]. The oxidation of DPNH by $K_3$ (rate 15% that of the aerobic oxidation of DPNH) and a slow oxidation of succinate by $K_3$ have now been demonstrated. Thus the heart-muscle preparation is able to catalyse the following anaerobic oxido-reductions

$$\text{DPNH} + \text{fumarate} + \text{H}^+ \longrightarrow \text{DPN}^+ + \text{succinate} \quad (1)$$

$$\text{DPNH} + \text{K}_3 + \text{H}^+ \longrightarrow \text{DPN}^+ + \text{K}_3\text{H}_2 \quad (2)$$

$$\text{succinate} + \text{K}_3 \longrightarrow \text{fumarate} + \text{K}_3\text{H}_2 \quad (3)$$

Inhibition of reactions (1) and (3) by malonate shows that succinic dehydrogenase is involved in these reactions.

The physiological significance of the reactions studied is not yet clear. We have not succeeded in preparing a solution of the hydroquinone of the naturally occurring vitamin $K_1$ in order to test whether it is oxidized in the same way as $K_3H_2$. The sarcosomal fragments of the heart-muscle preparation do not themselves contain any vitamin K[8], but it is possible that the vitamin is lost during the preparation of the fragments from the intact sarcosome. MARTIUS[9] has reported the

presence of vitamin $K_1$ in heart sarcosomes after the injection of radioactive vitamin $K_3$. The observations of MARTIUS AND NITZ-LITZOW[10] suggesting a role of vitamin $K_1$ in oxidative phosphorylation must also be kept in mind in this connection. The work is continuing, and will later be published in greater detail.

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Received October 27th, 1956

## An extract promoting the synthesis of deoxyribonucleic acid in U.V.-irradiated *Salmonella typhimurium*

A very striking effect of radiation on some micro-organisms is the alteration of deoxyribonucleic acid (DNA) synthesis[1,2,3]. KANAZIR AND ERRERA[2] have established that DNA synthesis is temporarily inhibited immediately after irradiation in *E. coli*, while the growth rate and the synthesis of ribonucleic acid (RNA) and protein are not fundamentally altered. This inhibition of DNA synthesis is followed by an accumulation of DNA precursors and adenosinetriphosphate (ATP), suggesting that a relationship exists between the synthesis of DNA and the metabolism of ATP[4]. This is also true in irradiated *Salmonella*[5].

The aim of this work was to look for factors able to promote DNA synthesis during the period of its immediate inhibition, subsequent to U.V. irradiation, and to elucidate the reasons for the continual occurrence of death as a result of irradiation, in spite of a resumed synthesis of DNA.

Experiments were carried out on *Salmonella typhimurium*, strain LT2, nonlysogenic, grown on aerated synthetic media, whose composition has already been described[5]. The bacteria were irradiated in logarithmic phase, with a Mineralight low-pressure mercury vapour lamp, from a distance of 30 cm, using a dose that gave about 50% survivors. All incubations were done in the dark. The determinations of the acid-soluble fraction, DNA, RNA and protein were carried out as already described[5].

An active principle was extracted from a normal strain of *Salmonella* grown on an aerated broth medium, by grinding fresh cells with 0.15 *M* KCl at 0°. The supernatant was recentrifuged at 0° C and 16,000 r.p.m., and dialysed against phosphate buffer pH 7.0. The crude extract was fractionated with solid ammonium sulphate. The precipitates obtained at different degrees of saturation were dissolved in potassium phosphate buffer at pH 7.4 and dialyzed against the same buffer.

In all tests, irradiated bacteria and controls were grown on synthetic aerated media in the presence of this extract or its ammonium sulphate fractions. An identical amount of protein derived from this extract, 30 $\gamma$/ml of culture, was used per assay. The activity of the extract and its fractions was destroyed either by heating or by trypsin digestion, while DNase, and RNase did not cause any inactivation. The extract was added either immediately after irradiation, or after the irradiated cultures had been allowed to grow for 120 minutes. In both cases, the cultures were grown for 20 min after the addition of the extract.

If, after irradiation, the bacteria are grown aerobically in the presence of the crude extract and then plated, the number of survivors increases: it is two or three times higher than in irradiated untreated cultures (Table I). The number of viable cells in the treated cultures is approximately as high as in normal cultures grown for the same length of time (Table I). This table also shows that the synthesis of DNA does not occur in irradiated untreated cells over a period of 20 min growth, while in the presence of our factor this synthesis follows a pattern similar to that of the normal cells and is accompanied by a more extensive utilization of acid-