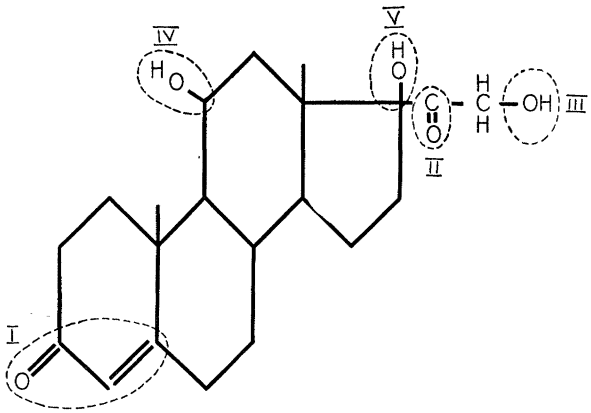

FIG. 1. I. Is essential for all known physiological activity. II. Is essential for all known physiological activity. III. Enhances sodium retention; necessary for carbohydrate activity. IV. (Either a hydroxyl or a carbonyl group.) In the presence of III, decreases sodium retention and increases carbohydrate activity. V. In the presence of III, and ? IV, increases carbohydrate activity and induces sodium excretion.

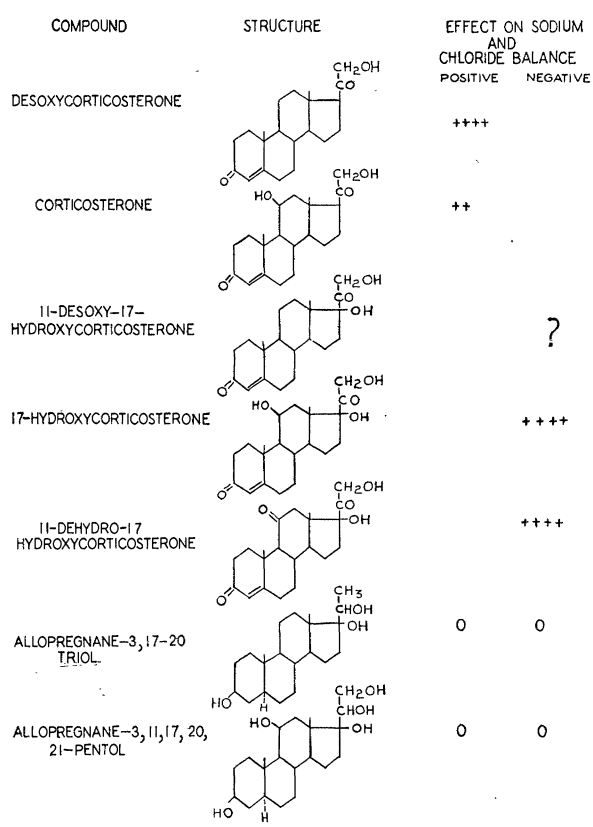

FIG. 2

These studies help to clarify a number of controversial experimental data in regard to the effect of various cortical extracts and their derivatives on electrolyte metabolism. It is also apparent from this study why desoxycorticosterone acetate therapy, (sodium-retaining factor) in Addison's disease produces edema so readily in contrast to treatment with adequate doses of potent adrenal cortical extract which contains a mixture of "sodium-retaining" and "sodium-excreting" factors.

We are indebted to Dr. E. C. Kendall, of the Mayo Clinic, Rochester, Minnesota, and Professor T. Reichstein, of Basel, Switzerland, for the crystalline compounds used in this study.

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THE ENZYMATIC LINK BETWEEN DIHYDRO-DIPHOSPHOPYRIDINE NUCLEOTIDE AND CYTOCHROME C

ALTHOUGH it has been generally held that reactions involving diphosphopyridine nucleotide (DPN) are linked to oxygen through cytochrome C, no isolated enzyme system has as yet been shown to catalyze the reduction of cytochrome C by reduced DPN (DPN · H_2). Corran, Green and Straub[1] have suggested that heart flavoprotein performs this function, but no evidence has been presented on which such a suggestion can be based. Lockhart and Potter[2] demonstrated the existence in crude heart muscle extract of such an enzyme system, but the active agent was apparently not capable of being extracted in a soluble form and therefore could not be subjected to fractionation and purification. In this note we are reporting the extraction from baker's yeast of a soluble enzyme which is very active in catalyzing the reduction of cytochrome C by DPN · H_2.

A spectrophotometric test similar to that used by Haas, Horecker and Hogness[3] in the isolation of cytochrome reductase was used. The DPN was reduced by a system consisting of hexose disphosphate, arsenate and an acetone dried enzyme powder containing zymohexase, isomerase and phosphoglyceraldehyde oxidase prepared according to the method of Warburg and Christian.[4] The DPN is incubated with this mixture for one-half hour at 25° and then heated for five minutes to 85° to destroy all the enzymes present. The DPN · H_2 is unaffected by this heating process and is stable for several days. Upon mixing an excess of DPN · H_2 and cytochrome C in an absorption cell,

[4] John D. Archbold Fellow-in-Medicine.

[1] H. S. Corran, D. E. Green and F. B. Straub, *Biochem. Jour.*, 33: 793, 1939.

[2] E. E. Lockhart and V. R. Potter, *Jour. Biol. Chem.*, 137: 1, 1941.

[3] E. Haas, B. L. Horecker and T. R. Hogness, *Jour. Biol. Chem.*, 136: 747, 1940.

[4] O. Warburg and W. Christian, *Biochem. Zeits.*, 303: 40, 1939.

no change in the extinction at 550 mμ (α band of reduced cytochrome C) is observed. However, when 35 γ of a partially purified enzyme preparation is added, the cytochrome C is rapidly reduced, the color of the solution changes from brown to pink, and the extinction at 550 mμ is increased. The rate of the reaction is apparently first order with respect to cytochrome C concentration, proportional to the enzyme concentration, and independent of small variations in the concentration of DPN · H_2.

In Fig. 1 is shown the effort of DPN · H_2 on the rate

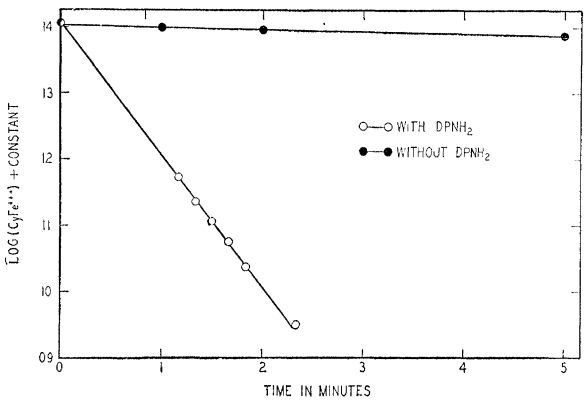

FIG. 1. Enzymatic reduction of Cytochrome C, with and without DPN · H_2.

of reduction of cytochrome C by a dialyzed and somewhat purified enzyme preparation. The slopes of the straight lines shown in the drawing are directly proportional to the rate of the reduction. When DPN · H_2 is added to the test solution, 5×10^{-7} moles of cytochrome C are reduced per minute per cc of enzyme solution used. In the absence of DPN · H_2, the rate of reduction is reduced to 1 per cent. of the above rate.

The activity of this enzyme can also be observed by measuring the change in absorption at 340 mμ (position of absorption band of DPN · H_2). When DPN · H_2 is oxidized, the light absorption at this wavelength is decreased. The results of a series of experiments are shown in Table 1.

TABLE I

Experiment	cc DPN·H_2 (10^{-6} moles/cc)	cc DPN (2.5×10^{-6} moles/cc)	cc Cytochrome C (1.5×10^{-6} moles/cc)	cc Enzyme (20 mg./cc)	Δ*
1	0.20	...	0.05	0.05	0.212
2	0.20	...	...	0.05	0.025
3	...	0.05	0.05	0.05	0
4	0.20	...	0.05	...	0

* Δ is the decrease in log $\frac{I_0}{I}$ at 340 mμ, upon addition of enzyme. A 0.5 cm absorption cell was used. $\frac{M}{40}$ phosphate buffer, pH = 7, was used to bring volume to 1.25 cc.

It is to be noted that in the absence of cytochrome C, very little DPN · H_2 is oxidized, even though there is a considerable excess of O_2 dissolved in the test solution. This fact would seem to indicate that this enzyme solution is far less reactive toward O_2 as the oxidizing agent than cytochrome C.

This enzyme can be precipitated by ammonium sulfate, alcohol and acetone, may be dialyzed without great loss in activity and is destroyed by heating. Further work toward purification of this enzyme is in progress.

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FACTORS IN COCONUT MILK ESSENTIAL FOR GROWTH AND DEVELOPMENT OF VERY YOUNG DATURA EMBRYOS

IN the course of our investigations on artificial parthenogenesis it became necessary to grow embryos *in vitro* in their early stages of development. In addition, a method by which this could be accomplished might insure the success of many wide crosses hitherto impossible. Although embryos isolated from mature or nearly mature seeds have often been grown *in vitro*, no success with very young embryos has been reported in the literature.

The embryos were removed from ovules of *Datura stramonium* and transferred to a basic medium (B) containing 1 per cent. agar, 1 per cent. dextrose and a mixture of mineral salts according to Tukey.[1] Additional substances were added to this basic medium, as will be mentioned below. The entire procedure was carried out under aseptic conditions. In the basic medium alone, embryos approximately 2 mm long when isolated (the embryos in mature seeds are approximately 6 mm long) showed root and hypocotyl growth but no growth of the cotyledons. No viable seedlings resulted. When, however, a mixture of physiologically active substances[2] was added to the basic medium (BV) cotyledons developed also and viable seedlings resulted when they were kept in dim light.

Pro-embryos[3] and slightly older stages of develop-

[1] *Bot. Gaz.*, 99: 630, 1938.

[2] Concentrations in mg per liter. Glycine (3), Thiamin (0.15), ascorbic acid (20), nicotinic acid (1), vitamin B_6 (0.2), adenine (0.2), succinic acid (25), pantothenic acid (0.5). This mixture was made up arbitrarily and because it proved effective was not further investigated as to essentiality of all components or optimum concentrations.

[3] Terminology follows Souèges, according to whom an embryo is called a pro-embryo as long as it remains radially symmetrical, hence, before the cotyledon primordia develop.