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

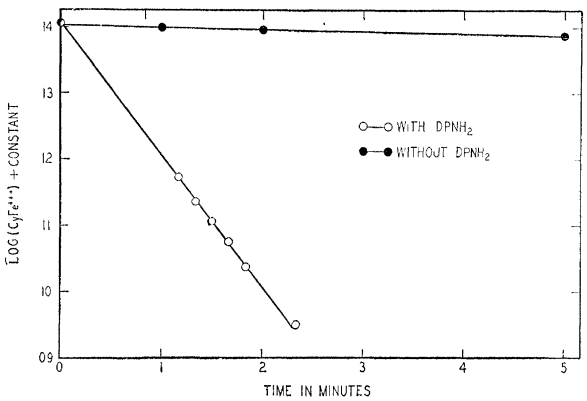

FIG. 1. Enzymatic reduction of Cytochrome C, with and without DPN · H_2.

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.

We are indebted to the Works Progress Administration, to Mr. Fred Johnson for valuable technical assistance, and particularly to the Rockefeller Foundation for a grant in aid which made this work possible.

A. M. ALTSCHUL
H. PERSKY
T. R. HOGNESS

THE UNIVERSITY OF CHICAGO

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.

ment could not be grown in this medium (BV), probably because younger embryos are less capable of synthesizing their own growth factors than older ones. Coconut milk[4] proved to be an excellent source of these additional growth factors necessary for very young embryos. For example, results such as the following were obtained: Pro-embryos, 0.14 mm in diameter (0.00144 mm³) were isolated from ovules of $2n$ plants 14 days after pollination and transferred to media B, BV and BV to which was added non-autoclaved coconut milk, and BV to which was added autoclaved coconut milk. After 4 days in the medium containing non-autoclaved coconut milk 4 of 7 embryos were on the average 1.9 mm long and 0.6 mm in diameter. These embryos grew below the surface of the medium. Two other embryos which were placed at the surface of the medium did not grow and 1 culture was infected. Thus, the 4 embryos that had grown had within 4 days increased their volume over 300 times. After 10 days in culture the two largest of the embryos measured 10×1.3 mm and hence had increased in volume 8,000 times. No growth occurred in the other media.

The following is another example: 7 embryos from ovules of $4n$ plants 11 days after pollination were removed. The embryos were in a slightly more advanced stage of development than the $2n$ embryos mentioned above. They measured 0.3 mm in diameter (0.014 mm³) and showed small cotyledon primordia. After 3 days below the surface of the medium (BV) to which non-autoclaved coconut milk was added all embryos cultured had grown on the average 2.0 mm in length and 0.9 mm in width. This corresponds to a volume increase of 90 times. After 10 days in the above medium the two largest embryos measured 8×1.5 mm, corresponding to a 1,000-time increase in volume. The embryos in the two experiments cited showed a good development of cotyledons and hypocotyl. The primary leaves also developed to a length almost equal to the cotyledons. Roots did not develop, but could be made to develop by transferring the embryos to medium (B) or (BV) without the additional coconut milk. A heat-stable root inhibitor which may be auxin is probably present.

In the case of these $4n$ embryos, growth also occurred in half of the cultures kept on medium (BV) to which autoclaved coconut milk was added. However, no differentiation occurred. After 10 days they had developed into lens-shaped bodies about 2 mm in diameter.

The success of coconut milk in furnishing some accessory substances which stimulate the growth of isolated embryos *in vitro* suggests its applicability to other species and prompts this preliminary report. Ultimately it is hoped to secure information regarding the nature of the substances in coconut milk which give it its peculiar properties.

J. VAN OVERBEEK
MARIE E. CONKLIN
A. F. BLAKESLEE
CALIFORNIA INSTITUTE OF TECHNOLOGY AND
CARNEGIE INSTITUTION OF WASHINGTON,
DEPARTMENT OF GENETICS,
COLD SPRING HARBOR, N. Y.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MINCER ADAPTABLE TO SMALL QUANTITIES OF TISSUE[1]

IN preparing skeletal muscle for oxygen uptake determinations we were confronted with the problem of obtaining a relatively uniform mince of small specimens obtained at biopsy. It became necessary to design and construct the apparatus described here, since we could find no adequate micromincer on the market.

The essential elements of the mincer (Fig. 1) are three telescoping parts: (*1*) The easily removable tubular steel jacket (*A*) which is held in a fixed position during mincing by a pin which fits into a slot in flange *D*. The mince emerges from holes drilled in the side of this tube, and the particle size can be regulated by varying the diameter and number of the holes. (*2*) The steel knife unit (*B*), which is solid at the shank end and slotted, as shown, to engage with pin *E*, and tubular at the opposite end to accommodate the plunger, which forces the contained tissue through

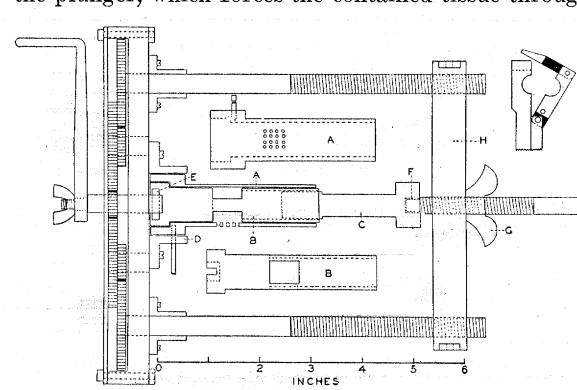

FIG. 1

[4] Coconuts were obtained from the local markets. The activity of the milk from different nuts varied considerably.

[1] Supported by the Wisconsin Alumni Research Foundation.