same manner (Fig. 1, V). It may be noted that the ethanol precipitation was carried out according to BEAR's method[5] for the preparation of the "V" modification of starch with sharp X-ray interferences. We did not succeed, however, in obtaining such sharp interferences as shown by BEAR's diagrams.

A more detailed chemical study of both these starches is under way.

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PARTIAL PURIFICATION OF DNASE II FROM THYMUS*

by

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The abbreviations used in this paper follow the pattern suggested previously[1]. The first evidence that thymus contains a nucleodepolymerase (DNase II), which is different from the desoxyribonuclease of pancreas (DNase I), was obtained by MAVER AND GRECO[2]. Their findings were almost simultaneously confirmed by WEBB[3], and in this laboratory[4]. Furthermore, it was found[4] that the intracellular distribution of this enzyme does not coincide with the distribution of its substrate (DNA). The latter conclusion was confirmed by SCHNEIDER AND HOGEBOOM[5] on liver cells, by ALLFREY AND MIRSKY[6] on a number of tissues, and by WEBB[7] on thymus. WEBB[8] described a method for partial purification of the DNase II from thymus.

A method, devised in this laboratory, and leading to a considerable purification of the DNase II from thymus is described. The extraction procedures in both WEBB's and our methods are almost identical. Several other methods of extractions have been tried but none was found better[9]. Contrary to WEBB[8] we were unable to detect any increase of activity after 24 or 48 hours of autolysis either at pH 5, or in an unbuffered extract.

Step 1. Fresh calf thymus (5 lbs) is blended in portions in the Waring blendor and is extracted overnight with 11 liters of ice-cold 0.85% NaCl containing 0.02 *M* $CaCl_2$, after the pH of the mixture has been adjusted to 5.0. The mixture is strained through four layers of gauze, the cloudy liquid is mixed with 250 g of Celite No. 545 and is refiltered through a layer of Celite No. 512 (300 g) on 32 cm Whatman No. 1 paper**. The enzyme is precipitated from the clear filtrate between 30 and 90% saturation of $(NH_4)_2SO_4$.

Step 2. The precipitate from the Step 1 is dissolved in water, the pH is adjusted to 2.5 and the concentration of protein is adjusted to give an optical density reading, $E_{280}^{1\,cm}$, of 5.0 to 7.0. The proteins are fractioned at this pH at 40, 60, and 80% saturation. The third precipitate (between 60 and 80% saturation) contains most of the activity.

Step 3. This precipitate is dissolved in 0.1 *N* acetate buffer pH 5.5, protein concentration is adjusted to $E_{280}^{1\,cm} = 10$, and pH to 5.5. The precipitate obtained between 50 and 80% saturation of $(NH_4)_2SO_4$ contains most of the enzyme.

Step 4. The precipitate from the step 3, combined from three or four preparations, is dissolved in water and is dialyzed for 48 hours against 0.01 *M* sodium acetate pH 4.0. The protein concen-

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** On a large stainless steel Büchner funnel, Model 503, a gift from the American Biosynthetics Corporation, Milwaukee, Wisconsin.

tration is then adjusted to $E_{280}^{1\,cm}$ between 7.0 and 10.0, and pH to 4.0. The solution is cooled to 0° and is treated with cold alcohol to attain 20% concentration (v/v). The temperature is not allowed to rise over 5°, the mixture is centrifuged in the cold and the precipitate is rejected. The clear supernatant is treated with cold alcohol to attain 60% concentration (v/v). The mixture is allowed to stand for four hours at —17°, and is centrifuged at this temperature. The liquid is discarded.

Step 5. The precipitate is dissolved in water, pH is adjusted to 3.5, the protein concentration to $E_{280}^{1\,cm} = 12$, and the temperature to 0°. Cold alcohol is added to attain 33%, and the mixture is left overnight at 5°. After centrifugation at this temperature the supernatant is adjusted to 60% alcohol concentration, and is left at —17° for 24 hours. The mixture is centrifuged and the liquid is discarded.

Steps 4 and 5 should be repeated. The final precipitate is lyophilized in the same centrifuge tube and is kept in the refrigerator for several months without loss of activity. The extent of purification and yields are summarized in Table I.

From Step 2 on, the activity was always measured by the spectrophotometric method of KUNITZ[10] and expressed as ΔE_{260}/min. The potency of the preparation was expressed as activity/$E_{280}^{1\,cm}$ of the enzyme solution. Using the same units the potency of the crystalline pancreatic DNase I is 2.0, equal to the potency of our best preparations of DNase II. It must be remembered, however, that the measurements are made at pH 5, close to the optimum of DNase II and far from the optimum of DNase I.

TABLE I

EXTENT OF PURIFICATION OF DNASE II FROM THYMUS

	Potency $\Delta E_{260}/min/E_{280}^{1\,cm}$ *of enzyme*	*Yield Units activity per 5 lbs.*
Step 1	0.02	300
Step 2	0.12	100
Step 3	0.22	70
Step 4	0.4	40
Step 5	0.8	25
Best preparation after repetition of Steps 4 and 5	2.0	

Occasionally it was possible to measure the activity of the preparation at the Step 1 spectrophotometrically. In the majority of cases, however, it was necessary to use the viscosimetric method[11] and to recalculate the results. The degree of purification achieved between the thymus homogenate and Step 1 is roughly estimated as between 5 to 10 fold.

The purified DNase II has no detectable phosphatase activity, but contains detectable amounts of thymic ribonuclease (RNase II). The observation of WEBB[9] that purification of DNase II changes its behaviour toward Mg was confirmed, but details differed. Our purified DNase II was almost completely inhibited by 0.025 *M* $MgSO_4$, but was not affected by 0.005 *M*.

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