effect was overcome by cocaine 0.5 mg percent and by ephedrine 45 mg percent and was partially inhibited by ergotamine 0.5 mg percent. It was not inhibited, however, by neosynephrine or pitocin. Previous treatment with desiccated thyroid 1:100,000 for 1 wk did not inhibit the development of color. Injection of epinephrine 0.05 ml 1:1000 led to blanching within a few minutes.

The effect was caused by the dilation of surface and deep melanophores and lipophores, for not only did the black, brown, green, and blue shades become enhanced, but there might, as in *Betta splendens,* be the appearance of reds and golds that were not previously evident in this fish. On the other hand, fish that already had highly dilated lipophores, such as *Carassius auratus* (goldfish) and the red *Xiphophorous helleri* ("helleri"), did not exhibit enhancement of their coloration. The mechanism of dilatation and aggregation of chromophores in fish is still imperfectly understood (*3*). The speed with which melanophores responded to excitation in *Ae. portalegrensis* suggested that excitement led to epinephrine secretion in the vicinity of the melanophores. Since reserpine inhibited this effect and since only a large dose of ephedrine or injection of epinephrine caused blanching, the effect of reserpine was probably directed to the chromophores themselves rather than through the mediation of the nervous system or by way of hormones such as intermedin or thyroxin.

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Intermediates in the Biosynthesis of Porphyrins from Porphobilinogen

It has been demonstrated that porphobilinogen (PBG), an α-methylamino monopyrrole, can be used as a substrate for the enzymatic synthesis of a number of porphyrins, including protoporphyrin IX, by cell-free preparations of *Chlorella* cells (*1*) and of chicken red cells (*2*). Investigations of early steps in the utilization of PBG in the biosynthesis of porphyrins indicate that a colorless precursor of uroporphyrin, rather than uroporphyrin itself, is the direct intermediate in the biosynthetic chain of the porphyrins (*3*).

Aqueous extracts of spinach-leaf acetone powder catalyze the disappearance of PBG and the appearance of uroporphyrin I [characterized by the method of Falk and Benson (*4*)]. These reactions occur aerobically or anaerobically. However, the porphyrin does not appear when the reaction is carried on anaerobically in the presence of 0.1M cysteine.

The enzyme porphobilinogen deaminase has been purified approximately 45-fold and has been separated from the other enzymes in extracts of spinach-leaf acetone powder that act in the synthesis of porphyrins from PBG. During the course of the reaction catalyzed by this enzyme, there is a mole-for-mole correspondence between the liberation of ammonia and the disappearance of PBG (as measured by the formation of the *p*-dimethylaminobenzaldehyde complex). This reaction proceeds aerobically, anaerobically, or anaerobically in the presence of 0.1M cysteine. The product of this reaction, in addition to ammonia, is colorless and does not react with *p*-dimethylaminobenzaldehyde. Under aerobic conditions, a small amount of porphyrin may form during the incubation period; for example, in one experiment of 4-hr duration, in which the initial concentration of PBG was 0.96 μM/ml, 0.94 μM of ammonia per milliliter was liberated and 0.03 μM of porphyrin per milliliter accumulated. When this product is allowed to stand at room temperature in air, additional uroporphyrin I accumulates. The optimum *p*H for the action of PBG deaminase is about 8.2. The enzyme does not catalyze the deamination of β-phenylethylamine, glutamine, or cysteine.

When small amounts of 30 to 40 percent or 40 to 50 percent of ammonium sulfate fractions of spinach-leaf acetone powder extracts are added to a solution containing the colorless product described in the preceding paragraph (but not containing detectable PBG), uroporphyrin I is formed rapidly via an intermediate that is characterized by a strong absorption maximum at about 500 mμ. On the other hand, when a solution containing the colorless product is incubated with a preparation of frozen and thawed *Chlorella* cells (*1*), usually relatively little uroporphyrin is recovered; but porphyrins with from three to seven carboxyl groups per molecule have been identified on paper chromatograms of the porphyrin products. Coproporphyrin has usually appeared as the major product. From these experiments, it appears that the colorless product, or something derived from it, may serve as a substrate for enzymes that mediate the decarboxylation of porphyrin precursors.

Chlorella preparations appear to be incapable of utilizing uroporphyrin I or III as substrates for the synthesis of porphyrins with fewer than eight carboxyl groups per molecule. This observation indicates that the colorless enzymatic product described here is not converted, for example, to coproporphyrin via uroporphyrin.

Uroporphyrins and very small amounts of coproporphyrins have been recovered after incubation of uroporphyrinogen I or III (prepared by the reduction of the corresponding porphyrins with palladium and hydrogen in alkaline solution) with *Chlorella* preparations. The relatively low yields of copropor-

phyrin suggest that the porphyrinogens supplied in these experiments serve as less satisfactory substrates than the colorless enzymatic product for the production of porphyrins with fewer than eight carboxyl groups per molecule. It thus appears doubtful that the enzymatic product is identical with the bulk of the porphyrinogen produced by the palladium-hydrogen reduction of uroporphyrins. Inquiry into the nature of the enzymatic product is being continued (*5*).

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References and Notes

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5. The porphobilinogen used in these experiments was recovered from the urine of a patient suffering with acute porphyria. The urine was obtained through the cooperation of George V. LeRoy of the University of Chicago and E. O. Willoboughy, M. Arkin, J. Lindberg, and J. Chapell of the Veteran's Administration Hospital, Hines, Ill. The technical assistance of Sandra Jacobsohn in performing the experiments reported here is gratefully acknowledged.

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On the Supposed Contamination of Thymus Nuclear Fractions by Whole Cells

In a recent communication Brown (*1*) has claimed that thymus nuclear fractions prepared in sucrose-$CaCl_2$ solutions (*2*) are made up in large part of intact thymocytes. It should be emphasized that this claim is not based on the visible demonstration of the intact cells but rests rather on some differences in osmotic behavior shown by individual components of a nuclear suspension. Because these differences in osmotic behavior did not fit certain hypothetical expectations, they were taken to indicate a distinction between isolated cell nuclei and intact cells, and the conclusion was drawn that small thymocytes comprise a considerable proportion of the isolated "nuclear fraction." It was admitted, however, that such thymocytes could not be seen under ordinary or phase-contrast microscopy.

The difficulties in establishing the purity of nuclear preparations by light microscopy alone have concerned workers in this laboratory for some time. It is the purpose of this communication to demonstrate that thymus nuclei prepared in 0.25*M* sucrose-0.0018*M* $CaCl_2$ solutions are contaminated by thymocytes to only a slight extent. This demonstration rests on the observation and examination of the isolated nuclei under the electron microscope.

The appearance of thymus nuclei, isolated as previously described (*2*), is shown by the electron micrographs in Fig. 1 (*3*). Only one thymocyte (indicated by the arrow in the bottom photograph) can be detected in these two fields. The proportion of such intact cells in microscope fields selected at random is very low; of the order of 29 small thymocytes per 1000 nuclei. A careful scrutiny of electron micrographs shows that whole cell contamination is not a problem in such nuclear preparations. (It is realized that details of structure that are strikingly obvious in the original electron micrograph may not be as evident when the plates are reduced to the small size of Fig. 1.)

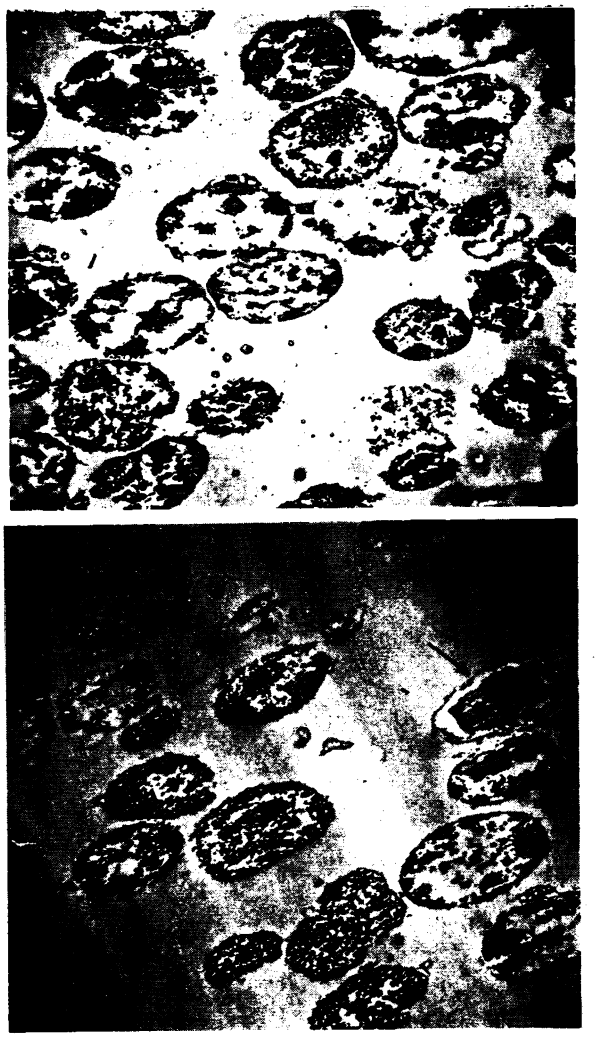

Fig. 1. Electron micrographs of thin sections of calf thymus nuclei following isolation in 0.25*M* sucrose-0.0018*M* $CaCl_2$ solution. A thymocyte is indicated by the arrow in the bottom photograph. (× 10,000)

What is evident is that thymus nuclei prepared in sucrose-$CaCl_2$ solutions are not entirely free of small amounts of adhering cytoplasm. This type of contamination is more difficult to evaluate. Its extent