folia and in four isolated dystrophic fibers in the dorsal group of the occipital musculature.

The findings (*8*) were of interest for the following reasons: intraocular injection of embryos succeeded in egg-adaptation of a virus hitherto not cultivable by the egg-technique; the virus-induced lesions resembled the well-known pathologic expressions of E-hypovitaminoses in domestic birds (*9*); and the nonspecific lesions were similar to, and the specific lesions more extensive than, those observed by Love and Roca-Garcia (*10*) in egg-adapted poliomyelitis.

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References and Notes

1. E. E. Jones, *Science* **76**, 331 (1932).
2. P. K. Olitsky and H. Van Roekel, in *Diseases of Poultry*, H. E. Biester and L. H. Schwarte, Eds. (Iowa State College Press, Ames, 1952).
3. K. Schaaf ad W. F. Lamoreux, *Am. J. Vet. Research* **16**, 627 (1955).
4. F. M. Burnet, *Principles of Animal Virology* (Academic, New York, 1955).
5. F. Sumner, E. Jungherr, R. E. Luginbuhl, unpublished results (1955).
6. F. Feibel *et al.* *Am. J. Vet. Research* **13**, 260 (1952).
7. The strain was originally obtained from H. Van Roekel, University of Massachusetts.
8. Supported in part by a grant, B-171 (C4), from the National Institutes of Health, U.S. Public Health Service, and by the American Poultry and Hatchery Federation.
9. A. M. Pappenheimer, M. Goettsch, E. Jungherr, *Conn. Agr. Expt. Sta. Bull. 229* (1939).
10. R. Love and M. Roca-Garcia, *Am. J. Pathol.* **31**, 901 (1955).

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Crystallization of Cytidine Diphosphate Choline from Yeast

Evidence for the role of a new coenzyme, cytidine diphosphate choline (CDP-choline), in one of the biosynthetic pathways of lecithin synthesis was recently presented by Kennedy and Weiss (*1*), who had been working with mammalian liver preparations. This compound has now been isolated from extracts of baker's yeast, and the purified compound has been crystallized as the sodium salt from 80-percent ethanol at *p*H 8.5 (*2*). Purification was by adsorption and elution with acetone from charcoal, followed by chromatography on a column of Dowex-1, formate form (10-percent crosslinked; eluant, 0.01*M* formic acid). CDP-choline was crystallized, in the form of thin, diamond-shaped plates with parallel or nonparallel sides, from the combined fractions obtained between seven and nine resin-bed volumes of eluant. The crystals decomposed, starting at 250°C, without melting. The absorption spectrum (peak at 280 mμ, $\lambda 280/\lambda 260 = 2.08$, $\lambda 290/\lambda 280 = 0.74$, at *p*H 2) was identical with that of cytidine-5′-phosphate (C5P) (*3*) and the E_m 280 mμ at *p*H 2 = 13.0 .

The compound was tentatively identified as the monosodium salt of CDP-choline on the basis of analytic values (*4*) of cytosine, pentose, acid-labile phosphate, total phosphate, choline, and Na^+ of 1.00, 0.99, 0.00, 1.96, 0.92, 1.05, respectively. Choline was estimated spectrophotometrically according to the procedure of Appleton *et al.* (*5*) after acid hydrolysis (40 minutes at 100°C in 1*N* HCl) and treatment of the hydrolyzate with the acid phosphatase of semen (*6*).

The absence of additional carbon- or nitrogen-containing moieties was indicated by elementary analysis (*7*) of the crystals considered to be the monosodium salt of the compound. Calculated percentage composition values were C, 32.88; N, 10.96; P, 12.12; and H, 4.93. Values found were C, 32.45; N, 10.98; P, 12.20; and H, 4.96.

The content of C5P was studied after acid hydrolysis (1*N* HCl, 40 minutes at 100°C). Paper chromatography (solvent, 3 parts 1*M* ammonium acetate at *p*H 7.5 in 0.003*M* Versene and 7.5 parts 95-percent ethanol, *8*) of an aliquot of the hydrolyzed sample (1.75 μmole) revealed the disappearance of the ultraviolet-absorbing starting material (R_f = 0.23) and the appearance of a new ultraviolet-absorbing spot (R_f = 0.12). The new compound was identified as C5P (1.59 μmole) on the basis of its R_f and absorption spectrum and by its conversion with 5′-nucleotidase (*9*) to inorganic phosphate (1.53 μmole) and cytidine (1.47 μmole, R_f = 0.53 with the afore-described solvent system.

After removal of the C5P with Norit from an aliquot of the hydrolyzed sample (1.75 μmole), treatment with semen phosphatase released 1.76 μmole of inorganic phosphate and 1.54 μmole of choline. Choline could not be detected in the hydrolyzed sample before treatment with the phosphatase preparation or in the unhydrolyzed compound following treatment with the enzyme.

The presence of a pyrophosphate linkage was evidenced by cleavage of the compound with nucleotide pyrophosphatase (*10*). Acid phosphatase of semen and 5′-nucleotidase, on the other hand, did not attack the compound.

The crystalline compound was found by Kennedy and Weiss to undergo enzymatic pyrophosphorolysis (*1*) at an identical rate with that of synthetic CDP-choline and could not be distinguished from the synthetic P^{32}-labeled compound by anion-exchange chromatography. The possibility that the CDP-choline is contaminated with a small amount of cytidine diphosphate ethanolamine has not been eliminated.

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References and Notes

1. E. P. Kennedy and S. B. Weiss, *J. Am. Chem. Soc.* 77, 250 (1955).
2. This investigation was aided by a research grant from the National Institutes of Health, U.S. Public Health Service.
3. E. Volkin and W. E. Cohn, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1954).
4. Pentose was determined by an unpublished method of C. E. Carter; Na^+ was estimated with a flame spectrometer; the other determinations have been previously described [I. Lieberman, *J. Am. Chem. Soc.* 77, 3373 (1955)].
5. H. D. Appleton *et al.*, *Federation Proc.* 9, 146 (1950).
6. J. Wittenberg and A. Kornberg, *J. Biol. Chem.* 202, 431 (1953).
7. Microanalyses were performed by the Elek Micro Analytical Laboratories, 4763 W. Adams Blvd., Los Angeles 16, Calif.
8. E. Cabib and L. F. Leloir, *J. Biol. Chem.* 206, 779 (1954).
9. L. A. Heppel and R. J. Hilmoe, *ibid.* 188, 665 (1951).
10. A. Kornberg and W. E. Pricer, Jr., *ibid.* 182, 763 (1950).

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Nucleic Acid and Succinic Dehydrogenase of Rat Liver after X-irradiation

In the course of an investigation of the effect of x-irradiation on animal tissues, the succinic dehydrogenase levels, as well as the nucleic acid composition of livers from control and from irradiated rats, were ascertained and compared (*1*). Earlier work in this laboratory disclosed that, when unfiltered x-radiation of dosages up to 20,000 r is applied to the surviving liver of rats just after partial hepatectomy, the extent of liver regeneration is not altered over an 11-day observation period. Pathologic changes of varying intensity, however, were noted in those livers when they were subjected to an x-ray dosage of 20,000 r (*2*).

Adult male rats of the Sprague-Dawley strain were placed under pentobarbital anesthesia. The abdomen of each rat was incised and the entire liver was exteriorized. The liver was held in position by leaded rubber rings secured at the base, the remainder of the body then being completely shielded with lead. The liver was irradiated with x-ray for 10 minutes (total dosage, 6500 r). The x-ray source comprised a 140-kv therapy machine operating at 8 ma [half-value layer,