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 F. Sumner, E. Jungherr, R. E. Luginbuhl, unpublished results (1955).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,

3.92 mm Al; distance, 12.5 cm; 650 r/min (air)].

The controls were manipulated in a similar manner, except that no radiation was applied. The animals were maintained in individual cages, and ground Dixie cubes and water were administered *ad libitum*. At periods of 1, 2, 5, and 11 days, the respective groups of control and irradiated rats were sacrificed by decapitation, and the livers were extirpated. Small sections were removed for histological examination, and the remainder was reserved for succinic dehydrogenase or nucleic acid analyses. In one series, a 0.50-percent cysteine diet, prepared by incorporation of the hydrochloride (Merck) into the powdered control ration, was fed to rats 2 weeks prior to surgery and over a 48-hour period preceding necropsy.

For the determination of succinic dehydrogenase, each of the liver samples was homogenized with 0.067*M* phosphate buffer at pH 7.4 (*3*). The enzyme content was ascertained manometrically in a Warburg assembly at 37.5°C (*4*), according to the procedure of Schneider and Potter (*5*). The method used for the determination of nucleic acids was based on the phosphorus content (*6*). The respective dry-liver powders, prepared by the scheme of Schmidt and Thannhauser (*7*), served as the starting material for the analysis of desoxyribose nucleic acid (DNA) and total nucleic acid (*7, 8*). Ribose nucleic acid (RNA) was obtained by subtraction. The DNA and RNA values, in terms of milligrams of phosphorus per 100 g of fresh tissue, with Fisher *t* values, are given in Table 1. Succinic dehydrogenase contents, expressed as the conventional Q_{O_2}, together with body-weight data are entered in Table 2. In no case were the livers pooled. Only values for the individual samples were averaged. Analysis of variance applied to the succinic dehydrogenase results showed significance only in the comparison of controls and irradiated animals of group C (48 hours after treatment, significant at the 1-percent level).

Table 1. Nucleic acid content of liver from control and x-irradiated animals (sacrificed on the second and fifth days after treatment).*

Treatment	No. of animals†	DNAP‡	RNAP‡
Group A			
Controls§	12	17.68±0.69	66.88±5.11
Irradiated (2 days)	10	21.38±4.35	64.57±5.83
Irradiated (5 days)	10	17.00±0.60	75.62±3.53

* The standard error (S.E.) follows each ± sign.
† The respective body weights (g ± S.E.) of the control rats and those sacrificed 2 days and 5 days after irradiation were 270.5 ± 5.5, 262.3 ± 6.4, and 263.4 ± 4.0 at surgery and 260.6 ± 5.4, 253.2 ± 7.3, and 250.8 ± 5.0 at necropsy.
‡ Milligrams of P per 100 g fresh liver. Fisher *t* values for the comparison of controls with the 2-day and 5-day treatment series were 1.89 and 0.73 (DNAP) and 0.29 and 1.36 (RNAP), in the order given ($P > 0.05$).
§ Average value for controls, groups of six that were sacrificed on the second and fifth days after sham surgery.

Table 2. Succinic dehydrogenase levels of control and x-irradiated rat liver.*

Treatment	Post-treatment (days)	No. of animals†	Succinic dehydrogenase activity, Q_{O_2}±S.E.‡
Group B			
Controls	1	10	94.92±3.93
Irradiated	1	10	94.27±4.54
Group C			
Controls	2	13	91.60±2.44
Irradiated	2	13	83.50±2.58
Group D			
Controls	5	13	93.33±3.85
Irradiated	5	12	93.64±3.78
Group E			
Controls	11	14	95.29±2.79
Irradiated	11	13	91.70±2.53
Group K			
Controls (0.50-percent cysteine diet)	2	14	89.22±4.78
Irradiated (0.50-percent cysteine diet)	2	15	86.41±2.22

* Except for group K, all rats received the control ration.
† Initial body weights (g ± S.E.) for rats of groups B, C, D, E, and K were 260.3 ± 5.6, 233.4 ± 2.2, 251.2 ± 3.1, 243.9 ± 3.2, and 286.1 ± 3.1, respectively. The corresponding values at necropsy were 270.7 ± 4.1, 239.5 ± 1.7, 238.5 ± 2.2, 258.6 ± 4.4, and 279.1 ± 2.9.
‡ A statistically significant difference was noted solely for group C (analysis of variance).

It will be noted that hepatic DNA and RNA values of rats 2 or 5 days after exposure to x-ray at a dosage of 6500 r did not differ significantly from the respective control values (Table 1). Although the differences between liver succinic dehydrogenase contents of irradiated animals that were sacrificed 1, 5, or 11 days after treatment and those contents for the corresponding controls were not statistically significant, a definite, though minimal, depression in enzyme activity was evident with the 48-hour series (group C, Table 2). Under the same conditions of irradiation and time, but with recourse to the 0.50-percent cysteine diet (group K), no decrease was observed. This might be indicative of a protective action exerted by cysteine on the sulfhydryl-dependent enzyme. As is shown in the tables, the average body-weight changes were quite small for all groups.

The aforementioned results are in marked contrast to those of Di Bella, who reported succinic dehydrogenase decreases of 30 percent or greater for liver removed 24 or 48 hours after x-irradiation at dosages of 600 r and 800 r (*9*). The enzyme activity remained unchanged 24 hours after treatment at 500 r, with only a slight decrease after 48 hours at a level of 500 r. Cysteine or glutathione failed to alleviate the enzyme inhibition (48 hours, 800 r). However, Di Bella's conditions comprised total body exposure of immature rats to filtered x-ray (maximal dosage, 800 r) rather than exposure of the liver alone. Although his results lack statistical rigor, such trends might derive indirectly from damage to more sensitive tissues.

The absence of any remarkable alterations in the succinic dehydrogenase and nucleic acid levels of irradiated intact liver, which are rather sensitive criteria for the evaluation of radiation involvement, indicates a relative radio-resistance for this organ under these conditions. This is further reflected in the gross and microscopic examination of the liver sections, none of which exhibited any noteworthy changes either in the cells or tissue architecture.

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References and Notes

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2. L. L. Gershbein, *Am. J. Physiol.*, in press.
3. V. R. Potter and C. A. Elvehjem, *J. Biol. Chem.* 114, 495 (1936).
4. Cytochrome *c* for this analysis was purchased from the Nutritional Biochemicals Corporation.
5. W. C. Schneider and V. R. Potter, *ibid.* 149, 217 (1943).
6. J. E. Logan, W. A. Marinel, R. J. Rossiter, *Biochem. J. (London)* 51, 470 (1952).
7. G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.* 161, 83 (1945).
8. W. C. Schneider, *ibid.* 161, 293 (1945).
9. S. Di Bella, *Boll. soc. ital. biol. sper.* 29, 1147 (1953).

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What is most needed for learning is an humble mind.—CONFUCIUS.