rum Allison). A fluorescent pattern similar to that of an *Anacystis* leach was observed in aqueous extracts of cells made at 100°C for 2 to 4 minutes and chromatographed in the same solvent. Widespread distribution and an essential metabolic role are further indicated for these compounds by the recent finding of an unconjugated pteridine as a growth factor for *Crithidia fasciculata* (*2*).

The parallel loss of photosynthetic activity and release of pteridines, together with the presence of relatively large concentrations of pteridine compounds in these photoautotrophs, suggest a role in the photosynthetic apparatus. The dramatic susceptibility to cold shock seen here in *Anacystis* is considered to be related to its thermotolerant nature. No precedent is known for such extreme cellular lability under what would seem to be a mild condition. The possible significance of pteridines in the metabolism of blue-green algae and other photosynthetic organisms is being investigated (*3*).

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References and Notes

1. H. S. Forrest and H. K. Mitchell, *J. Am. Chem. Soc.* 77, 4865 (1955).
2. H. A. Nathan, S. H. Hutner, H. L. Levin, *Nature* 178, 741 (1956).
3. This work was supported by the Robert A. Welch Foundation, Houston, Texas, and the Rockefeller Foundation.
4. W. A. Kratz and J. Myers, *Am. J. Botany* 42, 282 (1956).

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Distribution of Radioactivity in Cholesterol-C^{14} of Different Origins

The positions of the radioactive carbon atoms in the molecule of cholesterol-C^{14} made by biosynthesis from acetate-C^{14} have been established by the well-known classical experiments (compare review by Cornforth, *1*), which, however, were made with cholesterol that was obtained exclusively from the incubation of rat liver slices with acetate. In view of the varying patterns of distribution of radioactivity in cholesterol biosynthesis in different species and in different tissues (*2*), the question may be raised whether, in species other than the rat and under conditions different from those of the *in vitro* experiment with liver slices, the pathway of synthesis would be the same and thus give the same distribution of C^{14} in the radioactive cholesterol that is finally formed. The availability of cholesterol-C^{14} of different origins permitted an investigation of this question. The method employed was the comparison of the count of dehydroepiandrosterone-C^{14} prepared by chromic acid oxidation of cholesterol-C^{14} with the count of the starting material (*3*).

Table 1. Radioactivity in cholesterol-C^{14} from different sources. CH, cholesterol-C^{14}; DA, dehydroepiandrosterone-C^{14}; DAA, dehydroepiandrosterone-C^{14}-acetate.

Origin of cholesterol-C^{14}	Radioactivity (count/mmole min × 10^3)			CH/DA	CH/DAA
	CH	DA	DAA		
Intact rat	42.9	31.1	32.4	1.38	1.33
Rat liver slices	58.0	42.4		1.37	
Hen	138.0	108.4		1.27	
Pig liver perfusion	33.2	23.4	24.4	1.42	1.36

Samples of cholesterol-C^{14} had been obtained in experiments with acetate-1-C^{14} as described in earlier papers from this laboratory (*2*) and consisted of small amounts that had been purified through the dibromo compound and recrystallized from methanol for counting purposes. Before oxidation, each sample was diluted with enough nonradioactive cholesterol (purified through the dibromo compound) to give about 5 g and then again purified by bromination, and so forth, and finally recrystallized from methanol to make 3 to 3.5 g available for oxidation. The following samples were used: (i) from whole rats that had been previously injected intraperitoneally with acetate-1-C^{14}; (ii) from rat liver slices incubated as usual in Krebs-Ringer buffer (a total of 326.6 mg was diluted with 4.7 g of pure cholesterol); (iii) from intact hens (the acetate-1-C^{14} was injected intraperitoneally and the cholesterol-C^{14} isolated only from the liver and the gastrointestinal tract was used; a total of 2.1 g was mixed with 3.1 g of pure cholesterol); (iv) from perfusions of pig livers (a total of 790 mg was mixed with 4.21 g of pure cholesterol).

The oxidation of the purified samples was carried out, and dehydroepiandrosterone-C^{14}-acetate semicarbazone was isolated as described (*4*). The semicarbazone was split with pyruvic acid to obtain the steroid acetate by following the directions of Hershberg (*5*). The crude reaction product was purified by chromatography over silica and by repeated recrystallization from ether-pentane. Free dehydroepiandrosterone was obtained by hydrolysis of the acetate with alcoholic KOH and was again purified by chromatography on silica and by recrystallization from ether-pentane, methanol-water, and methanol.

Samples of the substances were combusted for the assay, and $C^{14}O_2$ was collected as $BaC^{14}O_3$ and counted with an end-window counter. All counts were corrected to infinite thickness, but since only relative values were required, no correction was applied for dilution and other factors.

According to the accepted mechanism of cholesterol biosynthesis from acetate-1-C^{14}, and in agreement with experimental findings (*1*), all radioactive carbon atoms of cholesterol and therefore also of the dehydroepiandrosterone obtained therefrom have the same count. Consequently, the quotient of the total specific activity of 1 mole cholesterol-C^{14} over the total specific activity of 1 mole dehydroepiandrosterone-C^{14} should be equal to the quotient of the number of radioactive carbon atoms of the two substances. Because the dehydroepiandrosterone represents the four-ring nucleus, this quotient mirrors the distribution of radioactivity in the molecule of cholesterol-C^{14}. When acetate-1-C^{14} is used as in the experiments of this report, the quotient should be 12/9 or 1.333. Table 1 gives the results obtained. The figures show that, for samples of cholesterol-C^{14} derived from different sources, the same quotient, nearly identical with the theoretical quotient, is found; this indicates that the pathway of biosynthesis is the same for cholesterol of various origins. The different patterns of distribution of this substance in biological experiments must therefore be caused by other factors.

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References and Notes

1. J. W. Cornforth, *Rev. Pure and Appl. Chem. (Australia)* 4, 275 (1954).
2. E. Schwenk, G. J. Alexander, C. A. Fish, *Arch. Biochem. and Biophys.* 58, 37 (1955).
3. This work has been supported by the Damon Runyon Fund, by the U.S. Public Health Service, by an institutional grant from the American Cancer Society, by the Schering Corporation, Bloomfield, N.J., and by the Jane Coffin Childs Memorial Fund.
4. E. Schwenk, N. T. Werthessen, A. F. Colton, *Arch. Biochem and Biophys.* 48, 321 (1954).
5. E. B. Hershberg, *J. Org. Chem.* 13, 542 (1948).

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