the buzzer was strong enough to act on the fetuses directly rather than indirectly by causing release of hormones in the mother. Only a more careful repetition of the experiment will throw light on this problem.

A more serious objection than this is that, besides the main factor of prenatal stress, genetic variation could also have been responsible for the offspring differences if there had been inadvertent selection of nonemotional mothers for the control group and emotional mothers for the experimental group. However, several points argue against this possibility. Choice of female animals for the two groups was carried out randomly, and at least some of the genetic variance was included in the error estimates used to test the main effects. Further, an examination of scores within and between individual litters indicates that interlitter variances tend to be smaller than intralitter differences. This means that, in the population used, genetic variation was relatively slight compared with environmental variation. Consequently, it is improbable that even if accidental selection had occurred it could have resulted in an experimental group genetically very different from the control group.

Accordingly, we may state that there are some grounds for supposing that prenatal maternal anxiety does actually increase the emotionality of offspring. This conclusion is offered tentatively until further experimentation has been completed.

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References and Notes

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25 January 1957

Occurrence of Pteridines in a Blue-Green Alga

In the course of photochemical studies on the blue-green algae a loss of photosynthetic activity in *Anacystis nidulans* was observed when an aerated suspension of cells in water was allowed to stand for a short period at 4°C in darkness. Paper chromatographic examination (butanol, acetic acid, and water, 4:1:5 by volume) of the supernatant (leach) after removal of the cells revealed the presence of a number of fluorescent materials. In addition several areas could be seen which reacted with ninhydrin. Of these, the only area in quantity appeared to be glutamic acid. No ultraviolet quenching, no organic phosphate compounds, and no phycocyanin or chlorophyll could be detected.

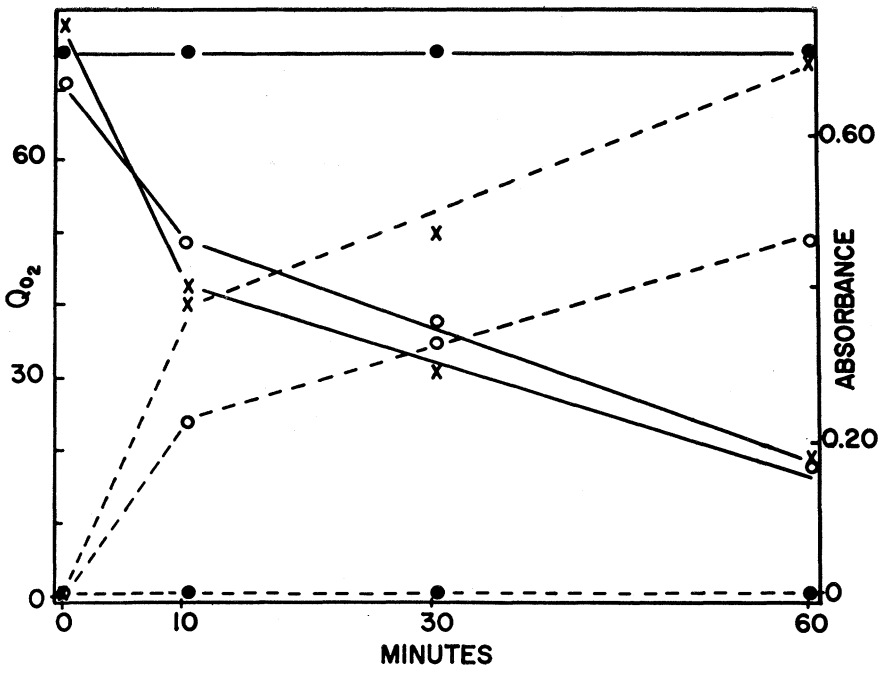
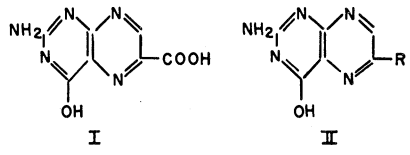


Fig. 1. Loss of photosynthetic activity and release of pteridines at 4°C. Cells of *A. nidulans* were grown in a continuous-culture chamber in medium C (*4*), washed and suspended in distilled water, and incubated at 4° or 25°C. At indicated times, aliquots were removed and centrifuged. Supernatants were examined in a 1-cm cell with a Beckman spectrophotometer. The packed cells were diluted in Warburg buffer No. 9 and their rate of photosynthesis was measured in saturating light from red neon. Solid lines, Q_{O_2} of photosynthesis (μl/mg hr); dotted lines, increase in absorbance at 270 mμ; ○ and ×, cells incubated at 4°C, duplicate experiments; ●, control cells at 25°C.

Aeration of the crude leach intensified its light yellow color. Its absorption spectrum revealed a major peak at 270 mμ and a smaller one at 410 mμ. The principal fluorescent material in the leach has been isolated from whole cells as a crystalline yellow compound in a yield equivalent to 0.05 to 0.1 percent dry weight of cells. Its ultraviolet absorption spectrum showed peaks at 285 and 400 mμ in 0.1*N* hydrochloric acid and at 268 and 430 mμ in 0.1*N* sodium hydroxide. On treatment with potassium permanganate in 0.1*N* sodium hydroxide (*1*) the yellow compound yielded 2-amino-4-hydroxy-6-carboxypteridine (I), and this same compound was obtained in the same manner from what appeared to be the principal blue fluorescent material. Another blue fluorescent compound has been identified spectrophotometrically and chromatographically as 2-amino-4-hydroxypteridine. Thus all fluorescent materials appear to be closely related and to have the general structure II. Work is continuing on the elucidation of the structural formulas of these compounds.



A parallel relationship between loss of photosynthetic activity of cells subjected to 4°C and increase in absorbance at 270 mμ of the resulting aerated leach was demonstrated by the experiment illustrated in Fig. 1. The absorbance at 270 mμ was taken as a measure of total pteridine released (see ultraviolet absorption data given in a preceding paragraph). The value for total pteridine released from the cells during cold treatment is approximately the same as that recovered in crystalline form. Separate experiments showed that quinone Hill activity after 60 minutes' incubation of cells at 4°C was affected in a manner similar to photosynthesis.

The general occurrence of pteridines in relatively large concentrations in blue-green algae was indicated by paper chromatographic examination of three other species (*Anabaena variabilis; Nostoc muscorum* Gerloff; and *Nostoc musco-*

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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4 February 1957

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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22 January 1957