tain the enzyme which brings about the phosphorylation of glycerol; hence, clinical insulin had no effect in experiments with rat brain homogenates.

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References and Notes

1. G. Bhattacharya, *Science* **123**, 505 (1956).
2. H. M. Kalckar, *Enzymologia* **2**, 47 (1937).
3. Since this paper was completed, my attention was drawn to abstracts of two papers by A. Ruffo *et al.* [*Chem. Abstr.* **48**, 8274d (1954); *ibid.* **49**, 1212d (1955)] in which the presence of a substance in commercial insulin preparations stimulating oxidative phosphorylation in rat liver homogenates was reported. Furthermore, the active material was also shown to be glycerol.
4. I wish to express my gratitude to F. G. Young for his kind interest in this work and for granting the use of laboratory facilities. My best thanks are due to C. G. Pope of the Wellcome Physiological Research Laboratories for helping me to obtain samples of the Wellcome insulin. Samples of the Lilly insulin were kindly donated by A. J. Kenny of this department. My thanks are also due to the Imperial Chemical Industries Fellowship Fund, University of Cambridge, for the grant of a fellowship.

22 January 1957

Influence of Prenatal Maternal Anxiety on Emotionality in Young Rats

The purpose of the observations reported in this article (*1*) was to test the hypothesis that emotional trauma undergone by female rats during pregnancy can affect the emotional characteristics of the offspring. By now, a good deal of evidence favoring this possibility has accumulated from diverse sources, including teratology (*2*), pediatrics (*3*), experimental psychology (*4*), and population biology (*5*). While none of the studies done has directly confirmed this hypothesis, many of them indicate that such hormones as cortisone, adrenalin, and adrenocorticotropic hormone, injected into the mother during pregnancy, have drastic effects on the fetus via the maternal-fetal blood exchange. Since strong emotion may release such substances into the mother's blood stream, there are grounds for supposing that it may have an important influence on fetal behavioral development. This experiment was the first in a projected series designed to examine this question in detail.

The rationale of the procedure was to create a situation which would predictably arouse strong anxiety in female rats, and to provide them with a standard means of reducing this anxiety; then to expose them to the anxiety-arousing situation during pregnancy, but block the accustomed means of escaping it. The assumption was that strong, free-floating anxiety would be generated in the pregnant females, and that any endocrine changes resulting would be transmitted through the maternal-fetal blood exchange to the fetus. The experiment was done by training five randomly chosen female hooded rats in a double compartment shuttlebox, first to expect strong shock at the sound of a buzzer, and then to avoid the shock by opening a door between the compartments and running through to the safe side. When the rats had learned this, the five experimentals, together with five control females, were mated to five randomly chosen males in a large cage. As soon as the experimentals were found to be pregnant (by vaginal smears), they were exposed to the buzzer three times every day in the shock side of the shuttlebox, but with the shock turned off and the door to the safe side locked. This procedure was terminated by the birth of a litter. The controls were placed in breeding cages during the same time.

Possible postnatal influences were controlled by cross-fostering in such a way as to yield a design with six cells, each containing ten offspring with two main variables—namely, prenatal and postnatal treatment. The data obtained from tests given to the young were examined by means of analysis of variance. In all tests of significance, three error estimates were used: the within-cell variance, the within-plus-interaction variances, and the within-plus-interaction plus between-postnatal-treatment variances. Thus, as shown in Table 1, all tests of significance reported involve three F values.

The emotional characteristics of the 30 control and 30 experimental offspring were compared by two tests given at 30 to 40 and 130 to 140 days of age. In test A, measures of amount and latency of activity in an open field were taken in three daily sessions of 10 minutes each. In test B, emotionality was measured by latency of leaving the home cage, and latency of reaching food at the end of an alley way leading out from the cage after 24 hours' food deprivation. In the second test, the maximum time allowed an animal to reach food was 30 minutes. In the measures used, low activity and high latency were taken as indices of high emotionality.

The results are summarized in Table 1. On test A, striking differences between experimentals and controls were obtained in amount of activity, both at 30 to 40 days and at 130 to 140 days. On the first testing, a significant interaction was obtained which probably represents genetic variation. On the second measure, experimental animals showed a much higher latency of activity than controls at both ages of testing. In neither of these activity measures were there any significant differences due to postnatal treatment or interaction besides the one mentioned.

In test B, experimental animals were slower to leave the home cage than controls at the first age of testing. There was no significant difference between groups in this measure, however, at 130 to 140 days of age. Similarly, experimentals showed a much higher latency than controls in getting to food at the end of the alley way at the first age of testing. The difference was less at the later age of testing. At both ages, significant interaction variances were found. As before, both may well be due to genetic variation. On neither of the measures used in test B were any significant differences found between methods of postnatal treatment.

It is clear from this analysis that the experimental and control animals differ strikingly on the measures of emotionality used, and that these differences persist to a great extent into adulthood. While there is no question about the reliability of these differences, there is some ambiguity regarding their cause. Thus, we do not know exactly how the stress used had effects. It is possible that

Table 1. Comparison of experimental and control animals on two tests of emotionality.

Item	Test A		Test B	
	Amount of activity (distance)	Latency of activity (seconds)	Latency to leave cage (minutes)	Latency to food (minutes)
	Tests given at age 30 to 40 days			
Experimentals	86.0	146.3	14.9	23.7
Controls	134.5	56.8	5.2	11.8
F values	(15.79, 14.21, 13.57)	(8.51, 7.91, 8.07)	(16.13, 16.46, 15.62)	(31.73, 25.66 25.87)
p	$< .001$	$< .01$	$< .001$	$< .001$
	Tests given at age 130 to 140 days			
Experimentals	114.5	71.5	4.8	11.6
Controls	162.3	26.8	2.1	6.2
F values	(9.77, 9.12 8.76)	(4.95, 4.79, 4.57)	(2.39)	(4.48)
p	$< .01$	$< .05$	$> .05$	$< .05$

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

1. This research was done at Queens University, Kingston, Ontario, and supported by grants from the Queens Science Research Council and the National Science Foundation. Grateful acknowledgment is made to C. H. Hockman for his invaluable aid in helping to build the apparatus and to test the animals.
2. F. C. Fraser and T. D. Fainstat, *Am. J. Diseases Children* 82, 593 (1951).
3. L. W. Sontag, *Am. J. Obstet. Gynecol.* 42, 996 (1941).
4. W. D. Thompson and L. W. Sontag, *J. Comp. and Physiol. Psychol.* 49, 454 (1956).
5. D. Chitty, "Adverse effects of population density upon the viability of later generations," in *The Numbers of Man and Animals*, (Oliver and Boyd, London, 1955).

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.

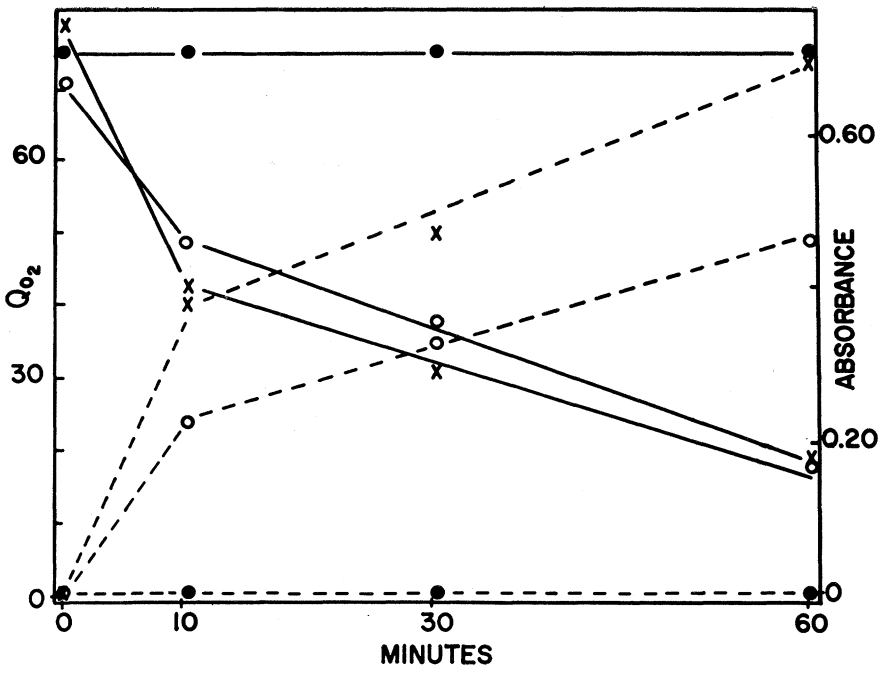
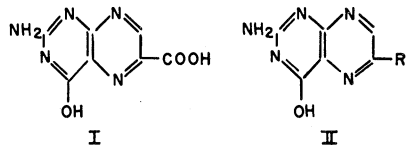


Fig. 1. Loss of photosynthetic activity and release of pteridines at 4°C. Cells of *A. nidulans* were grown in a continuous-culture chamber in medium C (*4*), washed and suspended in distilled water, and incubated at 4° or 25°C. At indicated times, aliquots were removed and centrifuged. Supernatants 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-*