

Conclusion. Rapidly repeated pregnancy does not affect the adenomatous component of our transplantable rat adenofibroma 5-B 1 beyond that expected to occur in mammary tissue in single pregnancy. Retention of secretory material was observed but no tendency toward malignant hyperplasia was detected.

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**Influence on Eye Color of Feeding Diffusible Substances to
Drosophila melanogaster.***

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Diffusible substances of hormone-like nature concerned in eye color development are known in *Drosophila* and in certain other insects. First evidenced in *Drosophila* by observations of interactions between tissues of different constitutions in genetic mosaics,¹ such substances have been studied extensively by means of transplantation and injection experiments.²⁻⁵ The substances designated v^+ substance and cn^+ substance have been obtained in solution and certain of their chemical properties determined.⁶⁻⁸ One test for these substances has been injection of solutions into the blood of test larvae. Presence of active v^+ substance results in a modification of the eye color of vermilion brown ($v\ bw$) animals from a very pale pink toward brown.⁷ A similar modification is produced by cn^+ substance on the eye color of cinnabar brown ($cn\ bw$) test larvae. Experiments have shown that the substances mentioned are taken up effectively when fed to test larvae. It is the purpose of the present paper to describe such experiments.

Preliminary trials showed that if wild type pupae are immersed

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⁴ Beadle, G. W., Clancy, C. W., and Ephrussi, B., *Proc. Roy. Soc., B*, 1937, **122**, 98.

⁵ Harnly, M. H., and Ephrussi, B., *Genetics*, 1937, **22**, 393.

⁶ Khouvine, Y., Ephrussi, B., and Harnly, M. H., *C. R. Acad. Sci., Paris*, 1936, **203**, 1542.

⁷ Thimann, K. V., and Beadle, G. W., *Proc. Nat. Acad. Sci.*, 1937, **23**, 143.

⁸ Khouvine, Y., and Ephrussi, B., *C. R. Soc. Biol.*, 1937, **124**, 885.

in boiling water for a short time (30-40 seconds), crushed, and fed on a thin layer of plain agar to vermilion brown or cinnabar brown test larvae taken from regular cultures at about 24 hours before puparium formation, the eye color of both types of test animals is modified toward brown. Unheated pupae, otherwise similarly fed, have no effect. This loss of activity in unheated crushed pupae has been noted previously in injection tests and has been attributed to enzymic oxidation of the substances concerned.⁷

Control experiments show that pupae of genetic types deficient in one or both substances produce no modification in eye color of appropriate test animals. Thus, vermilion pupae fed to vermilion brown test larvae produce no eye color response; similarly, cinnabar pupae give negative tests for *cn*⁺ substance.

In an attempt to standardize and improve the feeding test method, test larvae of known ages were fed at various stages of development. Individual tests were made in the following way: One cc. of 2% agar was pipetted into 25x90 mm. shell vials. Fifty wild type pupae, taken at 48 hours after puparium formation, were heated in boiling water for 30 to 40 seconds, crushed and placed on the agar. Twelve test larvae were placed in the vial. Eggs from which test larvae were obtained were collected over a 2½ hour period and the test larvae were cultured, up to the beginning of the feeding test, on standard cornmeal-molasses-agar medium seeded with yeast. All experiments were carried out at 25°C. The results of a series of such tests are summarized in Table I. It is seen that, under the conditions of the tests, the maximum effects were observed when test larvae were taken for feeding at from 60 to 84 hours after egg-laying. Weaker responses of larvae put in the test vials at an earlier stage may be accounted for by assuming a gradual loss of activity

TABLE I.
Effect of Feeding Crushed Wild Type Pupae on the Eye Color of vermilion brown (*v bw*) and cinnabar brown (*cn bw*).

Age at feeding (hours after egg laying)	<i>v bw</i> test animals		<i>cn bw</i> test animals	
	No.	color value* (mean and range)	No.	color value* (mean and range)
36±1½	12	1.0 (0.7-1.5)	10	0.2 (0.0-0.2)
48±1½	9	0.5 (0.0-0.7)	9	0.1 (0.0-0.2)
60±1½	12	2.1 (1.5-4.0)	12	2.2 (1.5-2.3)
72±1½	10	1.7 (1.5-2.3)	12	1.9 (1.0-2.3)
84±1½	10	1.8 (1.0-2.3)	12	1.7 (1.0-2.3)
96±1½	12	0.1 (0.0-0.3)	12	0.9 (0.0-1.5)
108±1½†	9	0.0	12	0.0

*In the numerical scale of color values, 0 represents no effect, 5 the maximum possible effect.⁷

†Puparium formation took place within a few hours after this time.

of the eye color substances in the crushed pupae. The decline in effect in older test larvae is explained by the fact that they take in less food before puparium formation than do younger larvae.

Tests have shown that somewhat stronger effects are obtained on feeding crushed pupae if the material to be fed is not allowed to come in contact with the agar. The active substances are known to diffuse into agar where presumably they are less available to the larvae.

Active water solutions of the two substances under consideration, prepared and made available by Dr. Edward L. Tatum, have given positive reactions when mixed with yeast and fed as described above.

Summary. The hormone-like v^+ and cn^+ substances concerned in the development of eye colors in *Drosophila* produce their characteristic reactions when administered with the food of test larvae. In this way they are comparable with certain hormones of the vertebrates, for example, thyroxin.

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Electrophoresis of Prolan.*

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No gonadotropic substance has ever been isolated in pure form. Nevertheless, an empirical idea of the chemical nature of these substances has been deduced from their solubility, their failure to diffuse through membranes, and their inactivation by certain reagents. The gist of these experiments¹⁻⁴ is that the gonadotropic hormones are protein-like in character and perhaps are muco-proteins similar to certain enzymes.

The object of the present work is to bring a new technique, namely that of electrophoresis, to bear upon the problem of the characterization of gonadotropic substances. The behavior of an amphoteric substance such as a protein in an electrophoretic cell

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