

meal (expeller process) was substituted for a considerable part of the meat element in meat scraps, or when a fine grade of low-temperature sardine meal was substituted for dry milks.

Further, it was brought out in chicks on 2 different rations that there was no difference in mortality from infective inoculations of 100,000 and 200,000 oöcysts (Experiment 3).

10447

Tests for the Eye-Color Hormones of *Drosophila* in Other Insects.

L. W. LAW.* (Introduced by C. C. Little.)

From the School of Biological Sciences, Stanford University.

Diffusible substances hormone-like in nature are concerned in the eye-color development of *Drosophila*¹ and certain other insects (*Ephestia*,² *Bombyx*,³ and *Habrobracon*⁴). It has been shown that the substances in *Ephestia*, *Habrobracon* and *Drosophila* are not species-specific.⁴ Many of the chemical properties of these substances have been determined.^{5, 6} In *Drosophila* they are active when injected (extract form) into,⁷ or fed to,⁸ appropriate test larvae. The *v*⁺ substance brings about a modification of the eye color of vermilion brown (*v bw*) test larvae from a pale pink towards brown, and the *cn*⁺ substance induces a similar modification of cinnabar brown (*cn bw*) eye color from colorless towards brown.

A question of interest in regard to the nature of these hormone-like substances is: Are they normal constituents of tissue; are they restricted to certain insect groups, or is their occurrence general throughout the class Insecta? It is the purpose of this paper to pre-

* Parker Fellow from Harvard University, 1937-38.

¹ For review of literature see Ephrussi, B., *Am. Naturalist*, 1938, **72**, 5.

² Kühn, A., *Z. indukt. Abst. u. Verer.*, 1937, **73**, 419.

³ Kikkowa, H., *Zoöl. Mag. (Japan)*, 1937, **49**, 348.

⁴ Beadle, G. W., Anderson, R. L., and Maxwell, Jane, *Proc. Nat. Acad. Sci.*, 1938, **24**, 80.

⁵ Khouvine, Y., and Ephrussi, B., *Compt. Rend. Soc. Biol.*, 1937, **124**, 885.

⁶ Tatum, E. L., and Beadle, G. W., *J. Gen. Physiol.*, 1938, **22**, 239.

⁷ Beadle, G. W., Clancy, C. W., and Ephrussi, B., *Proc. Roy. Soc. B*, 1937, **122**, 98.

⁸ Beadle, G. W., and Law, L. W., *Proc. Soc. Exp. Biol. and Med.*, 1938, **37**, 621.

sent data on feeding tests made on tissues of representatives of most of the insect orders.

A few tests have been made for these substances in other organisms. Khouvine and Ephrussi were unable to demonstrate any hormonal activity in nitrogenous extracts of sheep brain. The author was unable to detect any activity in embryonic tissues of mice by both the injection and feeding technics.

The insects tested were collected in larval and pupal stages and identified in nearly all cases as to family. Where possible several growth stages have been included in a test to be sure of detecting any hormonal activity. The tissues were placed in boiling H₂O for 30 to 40 seconds to inactivate the enzyme, dried in the oven for ½ hour at 100°C, and crushed in a few drops of Ringer's. This material was then placed in vials on a 1% agar, 0.25% yeast medium.

Eggs from which the *v bw* and *cn bw* test larvae were obtained were collected over a 2-hour period at 25° and the larvae were grown on a standard cornmeal-molasses-agar medium seeded with yeast. The test larvae were removed from the food at approximately 75-84 hours after egg laying and transferred to the vials. In this manner there can result no change in the eye color due to "starvation effect".⁹ From 10 to 15 larvae were used in each test. The resulting responses in the adult *Drosophila* eyes have been graded according to a standard color scale.⁶

Feeding tests were first made using representatives of the 3 orders known to possess the *v** and *cn** hormones. In the Diptera *Chaoboris* and *Culex* (Culcidae) and a Chironomid midge (Chironomidae) gave strong positive tests for both substances. In addition to *Bombyx* and *Ephestia* 2 other Lepidoptera have been tested, *Vanessa* and *Pseudohazis*. Both gave relatively strong tests. Four additional families were tested in the Hymenoptera, Formicoidae (ant), *Bombidae* (bumble-bee), *Tenthridinidae* (saw fly) and *Apidae* (honey-bee and wasp). There was found to be no influence on the eye color of test larvae by tissues of the ant, bumble-bee or honey-bee. However, tissues of the wasp (also *Apidae*) produced a decided color change of 0.8 units in *v bw* and 0.5 units in *cn bw* animals, and tissues of the saw fly produced a comparable modification. All tests giving negative results were repeated 2 or 3 times.

In addition to these 3 orders, representatives of 10 other insect orders have been tested (Table I). Modifications were obtained in all orders except Isoptera, where only one family was available for study at the time. The greatest modification in eye color was pro-

⁹ Beadle, G. W., Tatum, E. L., and Clancy, C. W., *Biol. Bull.*, 1938, **75**, 447.

TABLE I.
Effect of Feeding Crushed Tissues of Various Insects on the Eye-color of Vermilion Brown (*v bw*) and Cinnabar Brown (*cn bw*) Larvæ.

Insects used	<i>v bw</i> test animals		<i>cn bw</i> test animals	
	No.	Color value ⁶ (mean and range)	No.	Color value (mean and range)
Trichoptera*				
Leptoceridæ				
caddis fly	10	0.85 (0.5-1.0)	10	0.56 (0.5-0.7)
Plecoptera*				
stone fly	11	0.34 (0.2-0.5)	6	0.20 (0.1-0.4)
Orthoptera*				
Acridiidæ				
grasshopper	10	2.7 (2.0-3.0)	8	0.95 (0.8-1.0)
Gryllidæ				
Jerusalem cricket	14	0.0	11	0.0
Hemiptera*				
Aphididæ				
plant lice	8	1.6 (1.0-3.0)	8	0.8 (0.7-1.0)
Corixidæ				
water boatman	10	2.3 (2.0-3.0)	12	2.3 (1.5-3.0)
Dytiscidæ				
giant water beetle	10	0.5 (0.0-0.7)	11	slight
nymphal Hem.	11	1.3 (1.0-2.0)	9	1.2 (1.0-1.5)
Odonata				
Lestidæ				
dragon fly	11	0.7 (0.5-1.0)	16	0.62 (0.5-1.0)
Isoptera				
Termitidæ				
termites	14	0.0	10	0.0
Ephemeroptera				
may fly	12	1.3 (0.5-2.0)	9	1.3 (1.0-1.5)
Thysanura*				
Machilidæ				
fish moth	7	1.6 (1.0-2.0)	9	1.4 (1.0-2.0)
Neuroptera				
Raphidioidæ				
Raphidia	9	2.2 (2.0-3.0)		
Myrmeleonidæ				
ant lion	10	0.96 (0.5-1.5)	6	2.0
Coleoptera				
Bruchidæ				
bean weevil	15	0.0	12	0.0
Coccinelidæ*				
lady beetle				
<i>C. bipunctata</i>	12	2.2 (2.0-2.5)	12	1.1 (0.5-1.5)
Dermestidæ*				
<i>Dermestis lardarius</i>	13	0.61 (0.5-1.0)	12	slight
Chrysomelidæ				
tortoise beetle	13	1.9 (1.5-2.5)	12	1.2 (0.5-1.5)
Controls				
1% agar; 0.25% yeast	19	0.0	13	0.0

*Cross-comparable.

duced by tissue of the grasshopper (Acridiidae) and by *Coccinella bipunctata*. However, in both these orders there were found insects of other families which produced no modification whatsoever. No

explanation can be given for these results at the present time.

In every case where there was obtained a test for *v*⁺ substance there was also obtained a test for *cn*⁺ substance, giving further indication of their close chemical relationship. Likewise, in all tests which were cross comparable, there always resulted a greater modification of *v bw* eyes than of *cn bw* eyes.

Acknowledgment is made to Mr. Leigh Chadwick of Harvard University for supplying some of the insects used in this test.

Summary. The diffusible substances concerned in eye color development in *Drosophila* are widely distributed throughout the insect group. They are probably normal constituents of insect tissues only, since tests in other animals have been entirely negative.

Tissues producing a modification of *v bw* eyes also produce a modification of *cn bw* eyes. In all cross comparable cases there has resulted a greater modification of *v bw* eyes than of *cn bw* eyes.

10448 P

Depression of Polycythemia by Choline Hydrochloride.

JOHN EMERSON DAVIS. (Introduced by C. S. Leonard.)

From the Departments of Pharmacology and Biochemistry, University of Vermont, Burlington, Vermont.

The author¹ presented work which showed that the feeding of raw beef or hog liver to polycythemic (experimental) dogs caused a prompt reduction in their erythrocyte numbers, by depressing hemopoiesis.

Recently, Jacobs² has reported that liver extract, prepared for oral use, contains at least 1% of choline.

This report led us to the investigation of the effect of orally administered choline hydrochloride upon polycythemic dogs. Polycythemia was produced experimentally in 2 dogs by placing them for 6 hours daily in a low pressure chamber in which the environmental air pressure was reduced to about 430 mm of mercury by a motor driven vacuum pump. Within 10 days, this procedure had induced consistent increases (27 and 30%) in the erythrocyte numbers of the animals when in an approximately basal state. The hemoglobin percentage (Sahli) was increased correspondingly and reticulocytosis occurred.

¹ Davis, J. E., *Am. J. Physiol.*, 1938, **122**, 397.

² Jacobs, H. R., *J. Lab. and Clin. Med.*, 1938, **24**, 128.