Artificial Diet for Cecropia and Other Saturniid Silkworms

Abstract. *A wheat-germ diet containing no leaf material has been perfected for the rearing of* Hyalophora cecropia, Hyalophora gloveri, *and* Samia cynthia.

Since silkworms are leaf-eaters, there has long been a search for an "artificial diet" which will permit their rearing throughout the year. The first successful dietary mixtures were prepared for the commercial silkworm (*Bombyx mori*) (*1*) and later for several species of "wild" saturniid silkworms (*2*). Until recently all these diets have required pulverata or extracts of leaves of the appropriate food plants (*3*).

Levengood (*4*) of the University of Michigan recently informed us of his successful rearing of a few cecropia silkworms on the "wheat germ diet" that Adkisson and Vanderzant formulated for the laboratory rearing of the "pink boll worm" (*Pectinophora gossypiella*) (*5*). The addition of kanamycin to this diet by Burton was crucial for the rearing of cecropia (*6*).

In rearing operations, I was able to confirm Levengood's findings by rearing 2 to 10 percent of cecropia larvae to pupation on his dietary regimen. I now report that the addition of 0.05 percent cholesterol or β-sitosterol (*7*) permits one to rear up to 80 percent of cecropia larvae from egg to pupation.

Table 1. Composition of the modified Adkisson-Vanderzant-Levengood diet (*4, 5*) for rearing cecropia larvae (*13*).

Constituent	Amount added
Agar	25 g
Water	840 ml
Group 1	
Salts, Wesson's	10 g
Sucrose	35 g
Wheat germ	30 g
Casein, vitamin-free	35 g
Alphacel (powdered cellulose)	5 g
Cholesterol or β-sitosterol*	0.5 g
Formaldehyde (37 percent)	1 ml
Inhibitor solution †	20 ml
10 percent KOH	10 ml
Group 2	
Aureomycin	2.5 g ‡
Kanamycin sulfate	0.14 g
Ascorbic acid	4 g
Choline chloride	1 g
Inositol §	0.15 g
Linseed oil ||	1 ml
Vitamin suspension ¶	2 ml

* Author's modification. † Consists of 40 g of sorbic acid and 30 g of methyl *p*-hydroxy-benzoate dissolved in 340 ml of 95 percent ethanol. ‡ This amount was used in all the diets reported here, but as little as 0.18 g has been sufficient in rearing other lepidopteran larvae on the wheat-germ diet (*14*). Present indications are that 0.5 g per kilogram of diet is satisfactory for rearing these saturniid larvae. § Not essential for *H. cecropia*, but greatly enhances survival of *S. cynthia* and *A. polyphemus*. || Eliminates adult wing abnormalities, which are most prevalent in *A. polyphemus*, if none is present as in the original formulations received from Dr. Levengood (*4*). ¶ The vitamin suspension consisted of 0.12 mg of biotin, 6 mg of calcium pantothenate, 1.5 mg of folic acid, 6 mg of niacinamide, 1.5 mg of pyridoxine hydrochloride, 3 mg of riboflavin, 1.5 mg of thiamine hydrochloride, and 0.012 mg of vitamin B_{12} per milliliter.

A slice of the diet is placed in a glass jar, and 10 to 20 freshly hatched cecropia larvae are added. To facilitate molting, several wooden applicator sticks are placed in the container, and the jar is capped with a sheet of perforated aluminum foil and placed at 25°C. When crowding develops, the caterpillars are transferred to larger containers; fresh slices of diet are added as necessary.

Under these conditions, 65 to 80 percent of cecropia larvae survive and spin cocoons after 41 to 44 days. The pupae are completely normal; the female pupae weigh 6 to 7.5 g, and the male pupae weigh 4 to 5.5 g. Irrespective of the photoperiod experienced during larval life (8, 12, or 17 hours), all cecropia entered diapause after pupation. The pupae produced normal moths after 15 weeks of preliminary exposure to 5°C or after injection of α-ecdysone. These moths mated, and the females produced fertile eggs.

Hyalophora gloveri, a difficult species to rear in the laboratory, thrived on the cecropia dietary regimen and spun cocoons after 26 to 30 days at 25°C. So did *Samia cynthia*, provided that inositol was present.

The cecropia diet is not as satisfactory for rearing *Antheraea polyphemus*. Most newly hatched larvae failed to initiate feeding. For polyphemus, diets containing β-sitosterol are definitely superior to those containing cholesterol (*8*), and survival is further enhanced by the addition of 0.015 to 0.03 percent inositol (*9*) and of 0.001 to 0.1 percent *trans*-2-hexenal (*10, 11*). Under these conditions, about 90 percent will initiate feeding but only about 25 percent pupate. Cannibalism is prevalent, so rearing in isolation increases survival. The vast majority of individuals brought to pupation on diets without a source of fatty acids other than wheat germ showed foreshortened wings. This wing defect was partially rectified by the addition of 0.13 percent linolenic acid and completely by addition of 0.1 percent linseed oil (*12*).

The cecropia diet is even less satisfactory for the rearing of *Antheraea pernyi*; not a single individual has been reared to pupation. The addition of 0.001 percent *cis*-3-hexenol or *trans*-2-hexenal (Aldrich) (*11*) enhanced the feeding response, and a few fifth instar larvae have been obtained. All have died prior to spinning.

Despite these residual and unsolved difficulties, the artificial diet in Table 1 can confidently be recommended to students and fanciers of cecropia, gloveri, and cynthia silkworms.

Note added in proof: Bombyx mori larvae readily feed on the β-sitosterol diet and are presently in the third instar 2 weeks after hatching.

LYNN M. RIDDIFORD
Biological Laboratories,
Harvard University,
Cambridge, Massachusetts 02138

References and Notes

1. T. Fukuda, M. Suto, Y. Higuchi, *Nature* **187**, 669 (1960); T. Ito, *J. Sericult. Sci. Japan* **29**, 191 (1960).
2. For *Samia cynthia ricini*: T. Fukuda, Y. Higuchi, M. Matsuda, *Agr. Biol. Chem.* **25**, 417 (1961); T. Fukuda, *Bull. Sericult. Exp. Sta.* **19**, 220 (1964). For *Antheraea pernyi*: T. Fukuda and Y. Higuchi, *Agr. Biol. Chem.* **27**, 99 (1963). For *Hyalophora cecropia*: M. Garrison, F. Wittwer, R. Ziegler, D. Bucklin, *Amer. Zool.* **3**, 531 (1963); W. R. Harvey, personal communication.
3. *Bombyx mori* has been reared on an entirely artificial diet containing no leaf material with very limited success: T. Ito, *J. Sericult. Sci. Japan* **31**, 1 (1962). Much more successful is a totally artificial diet for *Samia cynthia ricini*: T. Fukuda, *J. Sericult. Sci. Japan* **36**, 309 (1967); T. Fukuda and Y. Higuchi, *Bull. Sericult. Exp. Sta.* **21**, 401 (1967).
4. W. C. Levengood, personal communication.
5. P. L. Adkisson, E. S. Vanderzant, D. L. Bull, W. E. Allison, *J. Econ. Entomol.* **53**, 759 (1960); E. S. Vanderzant, C. W. Richardson, S. W. Fort, Jr., *ibid.* **55**, 140 (1962). For the use of this diet in rearing other insects, see review by E. S. Vanderzant, *Ann. Entomol. Soc. Amer.* **60**, 1062 (1967).
6. Levengood's diet formulation was provided by R. L. Burton, *U.S. Agr. Res. Serv. ARS-33-117* (1967), 12 pp. The two antibiotics used in this diet also have been found to allow disease-free laboratory rearing of cecropia larvae on fresh cherry leaves; see L. M. Riddiford, *Science* **157**, 1451 (1967).
7. *Hyalophora cecropia* requires cholesterol and can convert the phytosterols to cholesterol. For a review of lipid metabolism in cecropia, see L. I. Gilbert, *Advan. Insect Physiol.* **4**, 69 (1967). Although the amount of cholesterol or β-sitosterol was arbitrarily selected from the range of concentrations found essential for *B. mori* [T. Ito, *Nature* **191**, 882 (1961); T. Ito and Y. Horie, *Ann. Zool. Jap.* **39**, 1 (1966); T. Ito, K. Kawashima, M. Nakahara, K. Nakanishi, A. Terahara, *J. Insect Physiol.* **10**, 225 (1964)], it was later noted that the same amount had previously been found essential for the boll weevil and salt-marsh caterpillar larvae (*5*).
8. β-Sitosterol enhances feeding in *B. mori* (*7*).
9. Inositol stimulates feeding in *B. mori* [S. Ishikawa, *J. Cell. Comp. Physiol.* **61**, 99 (1963); S. Ishikawa and T. Hirao, *Bull. Sericult. Exp. Sta.* **18**, 297 (1963); T. Ito, *J. Insect Physiol.* **5**, 95 (1960)] and in the tobacco hornworm

(*Manduca sexta*) [R. T. Yamamoto and G. S. Fraenkel, *Ann. Entomol. Soc. Amer.* **53**, 499 (1960); L. M. Schoonhoven and V. G. Dethier, *Arch. Neer. Zool.* **16**, 497 (1966)], both of which have been found to have specific gustatory receptors for this compound.
10. *Trans*-2-hexenal was added since it is an attractant for *B. mori* larvae (*11*) and is also the substance in oak leaves which initiates the release of sex pheromone by female polyphemus [L. M. Riddiford, *Science* **158**, 139 (1967)] and stimulates oviposition in this species (J. W. Truman, unpublished observations in this laboratory).
11. T. Watanabe, *Nature* **182**, 325 (1958); T. Ito and Y. Horie, *J. Insect Physiol.* **8**, 569 (1962).
12. Such wing defects are presumably a result of fatty acid deficiency; see G. Fraenkel and M. Blewett, *J. Exp. Biol.* **22**, 172 (1946).
13. Nutritional Biochemical Corporation now markets a mixture of the first five ingredients under group 1 as the "Vanderzant Adkisson Wheat Germ Diet" and a mixture of all the compounds in group 2 except the aureomycin, kanamycin, and linseed oil as "Vanderzant's Vitamin Mixture for Insect Diets."
14. C. M. Ignoffo, *Ann. Entomol. Soc. Amer.* **56**, 178 (1963); T. M. O'Dell and W. D. Rollinson, *J. Econ. Entomol.* **59**, 741 (1966).
15. The aureomycin was provided by the Lederle Laboratories; the kanamycin by the Bristol Laboratories. The work was supported by research funds of Harvard University and NSF grant GB 6730. I thank Mrs. Su-Mei Wang for technical assistance and Prof. C. M. Williams for reading the manuscript.

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Lectins in Extracts of Certain Polygonaceae Seed Precipitate Animal and Human Serums

Abstract. *Seeds of four species of Polygonaceae were tested for lectins that precipitate human and animal serums.* Rumex crispus, Polygonum convolvulus, *and* Polygonum pennsylvanicum *developed specific precipitate bands on double diffusion on agar gel plates. These bands were enhanced and increased in number when extracts were tested against serums from patients with certain diseases. When tested against lyophilized serum, no precipitate bands developed. The active substance cannot be dialyzed through cellulose membrane against running tap water for 16 hours, and it is heat stable. Extracts from* Fagopyrum esculentum *developed no precipitate bands.*

Agglutinating lectins from extracts of various plant seeds are specific for blood group A and its subgroups (*1*). Specific lectins against M, N, and H groups have also been discovered (*2*). These lectins have aided in elucidating the ABO and MNSs blood groups, and they are of practical value in legal medicine and anthropology. If a satisfactory lectin against group B (*1–3*) were discovered, the use of lectins in blood grouping for transfusion would become routine, since the present methods are more expensive.

The precipitating lectins in plant extracts have not been as extensively studied as the agglutinating lectins (*4*). One probable reason is that agglutination of red cells is very sensitive and the tests are easy to perform; another is that agglutinating lectins are used in blood grouping for transfusion. The precipitating tests present more difficulties. Some preciptating lectins are specific for blood-group substances in various body fluids (*5*). Because some leguminous plant lectins give specific precipitates with serum proteins (*6*), there is the possibility that they may be used in the diagnosis of certain diseases.

Rumex crispus (Curly dock), *Polygonum convolvulus* (Wild buckwheat), *Polygonum pennsylvanicum* (Smartweed), and *Fagopyrum esculentum* (Domestic buckwheat) (*7*) were examined. The active precipitating substances could be extracted with water or ethanol. For extraction in ethanol, 6 g of seed was ground in a hand coffee mill, then pulverized to an average fineness of 500 μm in a water-cooled micropulverizer. Five grams of the pulverized seed were then transferred to a flask with 100 ml of absolute ethanol and extracted overnight (16 hours) in a refrigerator at 7°C.

The flask was occasionally shaken, when convenient. The ethanol was filtered off, and the residue was washed four times, each time with 50 ml of absolute ethanol. The fourth washing was free of color. The ethanol filtrate was evaporated to dryness at 37°C. At this stage, depending upon the intended tests, the dry residue was suspended either in 50 ml of distilled water or in 0.85 percent unbuffered saline and the *p*H was adjusted to 7.0 with 0.4*N* NaOH. For routine gel-diffusion tests, the dry residue was suspended in saline containing 0.01 percent Merthiolate as a preservative. The suspension was centrifuged, filtered, distributed approximately equally into six test tubes, and stored at −15°C. As needed the extract was thawed at 37°C in a water bath, adjusted to *p*H 7.0 with 0.1*N* NaOH, and centrifuged at 2000 rev/min for 10 minutes. The supernatant was used for gel-diffusion tests or was filtered and used in ring-precipitin tests. The extract was stored at 7°C.

Serums were obtained from the hospital blood bank and from patients admitted to the hospital. Animal serums, obtained from local sources, were first used fresh, and then after 0.01 percent Merthiolate was added as a preservative. All serums were stored at 7°C. Initially, the ring-precipitin test was done with these serums; if significant precipitate developed, then the Ouchterlony gel-diffusion procedure was employed (*8*). We prepared our own plates using Noble agar in phosphate buffer at *p*H 7.4. Merthiolate (0.01 percent) was added as a preservative. The test plates were allowed to develop at room temperature. Ethanol extracts in saline were used in red-cell agglutination and complement fixation tests.

Extracts of the four species were tested against serum from a patient afflicted with lupus erythematosus (Fig. 1). Both extract of *R. crispus* dialyzed through a cellulose membrane against running tap water for 16 hours and undialyzed extract developed three precipitate bands. The extracts from *P.*

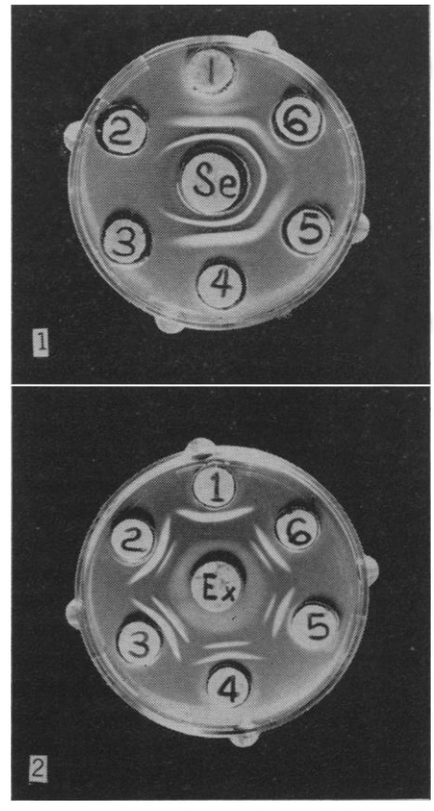

Fig. 1. Comparison of precipitates in Noble agar gel diffusion of four species of Polygonacae against lupus erythematosus serum. Well 1, dialyzed *R. crispus* extract; wells 2 and 3, *F. esculentum*, silver hull and black varieties, respectively; well 4, undialyzed *R. crispus*; well 5. *P. convolvulus;* and well 6, *P. pennsylvanicum; Se,* lupus erythematosus serum. Note the identity of precipitate bands and that *F. esculentum* varieties develop no precipitates. Photographed at 24 hours. Fig. 2. Precipitate bands formed by *R. crispus* extract against different animal serums including human donor serum in Noble agar gel with phosphate buffer at *p*H 7.4. Well 1, human donor serum; well 2, Rhesus monkey serum; well 3, turkey serum; well 4, horse serum; well 5, cow serum; and well 6, hog serum; *Ex, R. crispus* extract. Note only one precipitate band with donor serum. Photographed at 48 hours.