Culture of Insect Salivary Glands in a Chemically Defined Medium

Abstract. *A method for culturing the salivary glands of* Sciara coprophila *in a chemically defined medium has been developed. During a 24-hour period, the polytene chromosomes from six larval stages undergo normal developmental changes, including puff formation, puff condensation, and DNA synthesis.*

Wyatt, Loughheed, and Wyatt (*1*) analyzed the chemical constitution of hemolymph of *Bombyx mori, Galleria mellonella,* and *Diprion hercyniae* and found similar distributions of solutes. Wyatt (*2*) formulated a medium, based on this analysis, to culture ovarian tissue from *Bombyx mori* larvae. Grace (*3*) modified the medium by adding cholesterol, the B vitamins, and plasma in order to subculture ovarian sheath cells from diapausing pupae of *Callosamia promethea.* Wyatt and Kalf (*4*) found that α-trehalose was the principal sugar in the hemolymph of ten different species of insects. The medium used for the salivary gland culture presented here is based on the Grace modification of the Wyatt medium, but it is altered by the addition of a large amount of trehalose and the exclusion of plasma. The medium is unlike previous media (*5*) which had been used for insect tissue culture in that it is chemically defined. Its composition (mg/200 ml) is as follows:

Inorganic salts: NaH_2PO_4, 220; $MgCl_2 \cdot 6\ H_2O$, 608; $MgSO_4 \cdot 7\ H_2O$, 740; KCl, 596; $CaCl_2$, 162.

Sugars: glucose, 140; fructose, 80; sucrose, 80; trehalose, 1000.

Organic acids: malic, 134; α-ketoglutaric, 74; succinic, 12; fumaric, 11.

Amino acids: L-arginine-HCl, 140; DL-lysine-HCl, 250; L-histidine, 500; L-aspartic acid, 70; L-asparagine, 70; L-glutamic acid, 120; L-glutamine, 120; glycine, 130; DL-serine, 220; L-alanine, 45; L-proline, 70; L-tyrosine, 10; DL-threonine, 70; DL-methionine, 20; L-phenylalanine, 30; DL-valine, 40; DL-isoleucine, 20; DL-leucine, 30; L-tryptophane, 20; L-cystine, 5; cysteine HCl, 16.

Vitamin B complex: thiamine hydrochloride, 0.004; riboflavin, 0.004; nicotinic acid, 0.004; pantothenic acid, 0.004; biotin, 0.004; folic acid, 0.004; inositol, 0.004; choline, 0.004.

Other: cholesterol, 6; penicillin, 12; phenol red, 20.

Subsequent to the experiments with this medium, a similar chemically defined medium has been reported to facilitate differentiation of larval eye-antennal discs of *Drosophila* (*6*).

The medium was prepared by dissolving the various solutes in water that had been distilled three times. Inorganic salts, excepting $CaCl_2$, were dissolved in 60 ml; $CaCl_2$ in 14.4 ml; sugars in 20 ml; organic acids in 10 ml; amino acids in 80 ml; vitamins, cholesterol, penicillin, trehalose, and phenol red in 10 ml. The solutions were mixed, with $CaCl_2$ being added last. The *p*H was adjusted to 6.35 by the addition of KOH, and H_2O was added to bring the total volume to 200 ml. The solution was filtered through a Millipore filter and stored in tightly capped bottles at 4°C.

The method is one of organ culture in that whole salivary glands rather than individual cells are cultured. In order to have an indication of the exact stage of chromosome development at the time of culture, one of the two glands, which are synchronous in chromosome development, can be squashed and stained after the dissection. The other gland is cultured for a 24-hour period and then is compared to the uncultured gland. Because a hormonal source has been shown to be important for chromosome development (*7*), in early experiments the brain and overlying ring gland were dissected and placed adjacent to the salivary gland. If DNA synthesis was to be compared in the two glands, one gland was given a pulse labeling of tritiated thymidine (2 c/mmole from the New England Nuclear Corporation) at a final concentration of 5 μc/ml for 30 minutes after the dissection. The other gland was cultured for the 24-hour period and then given the pulse labeling.

The ring gland and brain were unessential for puff formation in the chromosomes of the anterior lobe of the salivary gland. However, no puffs developed in the posterior lobe chromosomes without the hormonal source. Puff condensation, which is a change of the fully extended chromatin to a more densely staining, more compact state and which normally occurs around the time of pupation, occurred at a slightly earlier time in culture. Synthesis of DNA was similar before and after culture both in patterns of DNA uptake and percentage of nuclei labeled (*8*).

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References and Notes

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9. Supported by USPHS postdoctoral fellowship GPD-16,503 under the sponsorship of Prof. J. H. Taylor and by NSF grant GB42, which provides maintenance and support of Dr. Helen Crouse's Sciara laboratory, Department of Botany, Columbia University.

12 October 1964

Avian Atherosclerosis: Retardation by Pectin

Abstract. *A highly significant retardation of spontaneous atherosclerosis was observed in 2-year-old cockerels fed on a standard diet supplemented with 5 percent pectin for 18 months. The pectin-fed birds excreted three times as much lipid extract and almost twice as much cholesterol as did the control cockerels fed the standard diet supplemented with 5 percent nonnutritive fiber.*

For a prolonged period animal species (fowl) highly susceptible to spontaneous atherosclerosis was fed with a mixed human-type diet relatively rich in pectin-containing foodstuffs, and a significant retardation in the development of the disease was noted (*1*). Because of the complexity of the diet used previously and the uncertainty as to the nature of the effective substance responsible for the retardation of atherogenesis, we conducted an experiment in which pectin (National Formulary grade) was used as the only variable in the diet.

Two groups of 2-year-old cockerels were used, with 30 birds in each group. Both groups were fed on a corn-soybean ration containing 15 percent protein, to which was added 5 percent