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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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

Table 1. Effect of long-term pectin consumption on changes in body weight, food intake, cholesterol content of plasma and liver, and severity of atherosclerosis in adult cockerels fed on a diet containing nonnutritive fiber (cellulose) or pectin.

Measurements	Diet supplement	
	Cellulose	Pectin
Body weight (g) at start	2327 ± 49*	2240 ± 58
After 18 months	2511 ± 50	2317 ± 52
Food consumption (g/day)	118 ± 5	124 ± 4
After 18 months		
Plasma cholesterol (mg/100 ml)	96 ± 4	111 ± 6
Liver cholesterol (mg/g wet wt)	5.19 ± 1.3	5.87 ± .20
Abdominal aorta:		
Macroscopic score	2.04 ± .12	1.39 ± .08
Severe scores†	58	20
Weight-area ratio	53.3 ± 1.4	50.7 ± 1.5
Histological grading:		
Intima†	32	21
Localized fibrous plaques†	68	45
Media†	26	8

* Mean ± standard error for 24 and 20 birds surviving on the nonnutritive fiber and pectin supplemented diets, respectively. † Frequency of severe scores (%); severe scores were those with grade 2.0 or above; scoring was based on a 1 to 3 scale with 1 indicating essentially no lesions and 3 very severe plaques.

pectin (first group) or 5 percent nonnutritive fiber (second group). The animals, kept in individual cages, had continuous access to food and water and were maintained on these diets for 18 months. Body weights and food consumption were recorded at monthly intervals. Toward the end of the experimental period, blood was taken from a wing vein of each bird for determination of the cholesterol content of the plasma (*2*).

At the end of the 18-month period the surviving birds were killed; livers were removed for cholesterol determination, and aortas removed from the heart to the iliac bifurcation. After careful cleaning, each aorta was visually scored on a scale from 1 to 3 with 0.5-unit intervals in which a value of 1 indicated an essentially clean blood vessel, and a value of 3 represented an aorta practically occluded by plaques. After the macroscopic scoring, the aortas were weighed and the area was determined by planimetry to obtain the weight-area ratio (*3*). They were then examined histologically by techniques described previously (*4*), on the basis of (i) diffuse intimal proliferation, (ii) localized fibrous plaque formation, and (iii) medial lesions. Within each of these characterizations, lesions were graded as to severity on a 1 to 3 scale.

The pectin-fed birds had significantly ($p < .001$) fewer atherosclerotic plaques in the abdominal aorta than the control birds, measured both macroscopically and histologically (Table 1). Only the weight-area ratio did not show a significant difference, suggesting that this measurement is more a reflection of the diffuse intimal proliferation than of the localized plaque of which the macroscopic score appears to be more representative. In addition to the aortic measurements given in Table 1, three aortas from control birds only showed calcium deposition in localized fibrous plaques. No hypocholesterolemic action could be ascribed to the pectin feeding, in contrast to reports for the rat and for man (*5*). Indeed, the pectin-fed animals had significantly higher concentrations of cholesterol in the plasma and liver.

The birds fed with pectin consumed slightly more food throughout the experiment than did the control birds fed with cellulose (Table 1). The control birds, however, gained almost three times as much weight as did the pectin-fed birds, indicating that pectin reduced the utilization of nutrients.

Table 2 shows the results of a separate study in which analyses were made of the excreta of 10-week-old chickens fed pectin or cellulose in diets which contained 10 percent fat and 0.5 percent cholesterol. There was no difference in the moisture content of the excreta, although the excreta from the birds fed pectin gave the appearance of being diarrhetic. However, the pectin-fed birds lost three times as much lipid and almost twice as much cholesterol per gram of excreta as did the control birds.

Table 2. Moisture, total lipid, and cholesterol content of excreta from chickens fed pectin or nonnutritive fiber (cellulose).

Excreta measurement	Diet supplement	
	Cellulose	Pectin
Moisture (%)	73.4	73.5
Extractable lipid (mg/g dry excreta)	48.7	145.2
Cholesterol (mg/g dry excreta)	8.7	15.6

These results confirm our previous suggestion (*1*) that pectin may be efficacious in retarding spontaneous avian atherogenesis. The excreta analyses, in conjunction with the food intake and patterns of body weight, suggest two possible modes of action for pectin: it might accelerate food passage or interfere with the digestive processes so that less nutrients become available for absorption; or it might specifically interfere with the absorption or reabsorption of atherogenic substances (cholesterol). With regard to the first possibility we have recently shown that a restricted pattern of food intake was beneficial in retarding avian atherosclerosis (*6*). The higher plasma and liver cholesterol values of the pectin-fed birds make the second possibility appear unlikely.

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Formation and Degradation of Cyclic Dextrins by Intracellular Enzymes of Bacillus macerans

Abstract. *The enzymes of* Bacillus macerans *that participate in the formation and degradation of Schardinger dextrins were shown to be intracellular.* Bacillus macerans *amylase converts starch into cyclic dextrins. A newly discovered enzyme, cyclodextrinase, catalyzes the degradation of cyclic dextrins.*

Bacillus macerans amylase, also known as cyclodextrin transglycosylase, is an enzyme that converts starch into Schardinger dextrins, a homologous series of cyclic oligosaccharides contain-