(*15*). The shifts in the ratios observed for this protein fraction are consistent with the idea that ribosomal structural proteins are synthesized in the nucleolus and form complexes with newly synthesized ribosomal RNA (*16*) in the nucleolar periphery (first alternative) or within the chromatin (second alternative).

As yet we cannot discriminate between these alternatives. The results we obtained using short term incubation periods, however, clearly show that the nucleolus plays an important role in the protein synthesis of pea and tobacco nuclei. In particular, the nucleolus synthesizes an appreciable portion of the nuclear basic proteins. Some of these proteins have been characterized as histones.

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Culture of Embryonic Cells of Drosophila melanogaster in vitro

Abstract. *Embryonic cells isolated from eggs of* Drosophila melanogaster *have been cultured continuously in a new medium. Generation time for cell division is 30 hours. Chromosome number remains constant for at least 10 days. Cells from embryos of the mutant* maroon-like *grow at the same rate as those from wild-type embryos, but cells from* rosy-2 *grow slower and at a lower optimum temperature.*

The potential value of the techniques of cell culture for studies in somatic cell genetics, in the problems of differentiation, and in virology is widely recognized. Full realization of these potentialities, however, has been limited by the genetic and cytological complexities of the organisms where true cell culture has been possible. *Drosophila melanogaster,* the classical object of genetic study, would seem to be ideally suited for this purpose, but successful cultivation of its cells in vitro has not previously been achieved. The successful culture of insect cells by any method has been achieved only rarely (*1*). In this paper we report the development of a new culture medium and techniques which have resulted in the successful cultivation of embryonic cells from various developmental stages of *D. melanogaster.*

Embryonic cells were obtained from eggs of a wild stock (Oregon R-EL2) and from two eye-color mutants, *rosy-2* (*ry*^{2}) and *maroon-like* (*ma-l*). Eggs of highly uniform developmental stages were collected by use of the ovitron described by Yoon and Fox (*2*). Eggs of lesser uniformity were collected after oviposition on filter papers moistened with a suspension of standard corn meal-agar-molasses and killed yeast. One thousand to three thousand eggs collected by these methods were immersed for 2 minutes in 3 percent sodium hypochlorite. They were then rinsed with twice-distilled water and sterilized for 15 minutes in 0.05 percent $HgCl_2$ in 70 percent ethyl alcohol. All subsequent steps were carried out in a sterile-transfer chamber. The sterilized eggs were rinsed three times with H-5 culture medium (Table 1), and were homogenized lightly and slowly with 1 ml of H-5 medium in a 2-ml glass homogenizer.

By this method the embryos were dissociated into single cells with minimum damage. The suspension was then passed through a sintered glass filter (maximum pore size, 170 to 220 μ) for removal of the broken vitelline membranes, and were mixed by means of a pipette with 15 ml of H-5 medium supplemented with 10 percent newborn calf serum.

For growth, 5-ml portions of the cell suspension were transferred to T-30 culture bottles and cultured without shaking in an incubator aerated with normal air. For the determination of cell multiplication, 1-ml portions of the cell suspension were distributed into 12 by 100 mm test tubes, and the tubes were incubated at an angle of 5 degrees to the horizontal. After 0, 2, 4, and 7 days of cultivation, the numbers of cells were determined with a hemocytometer of the Bürker type. The average of three culture tubes

Table 1. Composition of medium H-5.

Constituents	Amount (mg/1000 ml)
Salts	
$NaH_2PO_4 \cdot 2H_2O$	200
$NaHCO_3$	350
KCl	200
$CaCl_2 \cdot 2H_2O$	20
$MgCl_2 \cdot 6H_2O$	100
NaCl	7,000
Sugars	
Glucose	13,800
Sucrose	13,800
Antibiotics	
Penicillin G (sodium salt)	30
Streptomycin sulfate	100
Peptides	
Glutathione	5
Amino acids	
Lactalbumin hydrolysate	17,500
L-tryptophane	100
L-cysteine hydrochloride	25
Vitamins and nucleotides	
Yeast extract	1,500
Ascorbic acid	100
Niacinamide	0.1
Nicotinamide-adenine dinucleotide	5
Organic acids	
Sodium acetate	25
Malic acid	670
Succinic acid	60
pH Indicator	
Phenol red	10

*p*H adjusted to 6.5 with 1*N* KOH. Sterilization by passage through a Seitz filter.

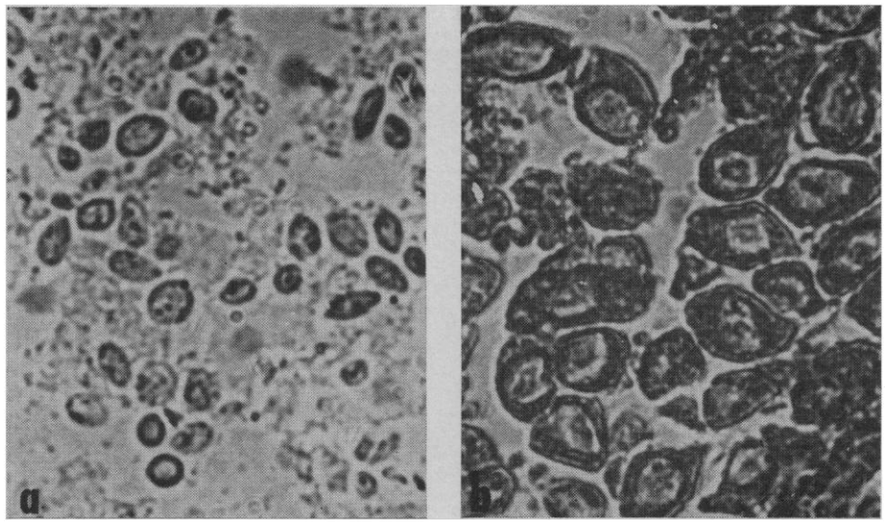

Fig. 1. Phase contrast photomicrographs of living cells isolated from eggs of *D. melanogaster* 8 hours after fertilization. (*a*) Small cells (5 to 10 μ in diameter). (*b*) Large cells (20 to 35 μ in diameter).

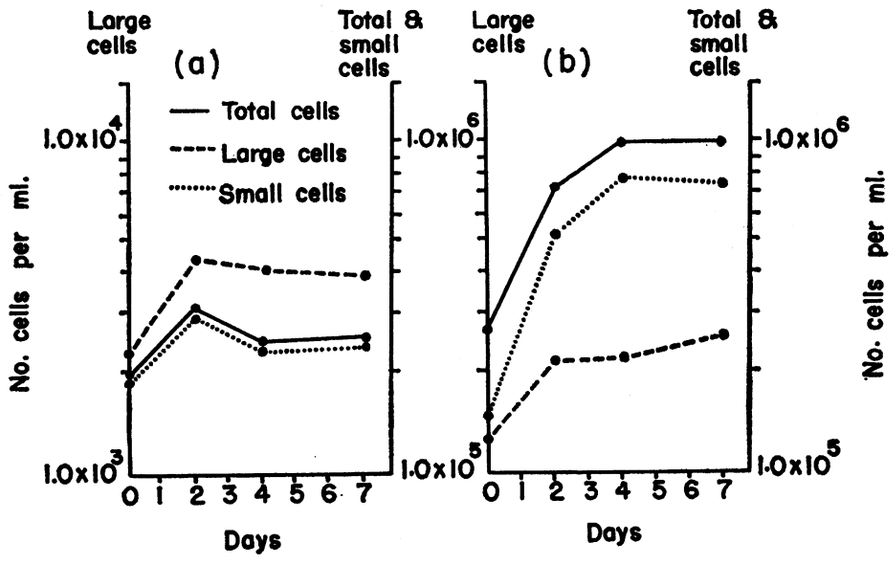

Fig. 2. Growth curves of embryonic cells in vitro. (*a*) Cells isolated from eggs 2 hours after fertilization. (*b*) Cells isolated from eggs 8 hours after fertilization. Medium H-5 supplemented with 10 percent calf serum. Temperature, 30°C.

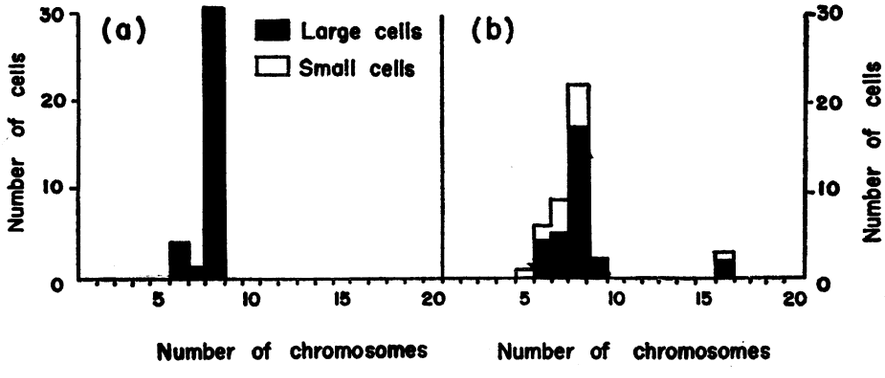

Fig. 3. Distribution of chromosome numbers. (*a*) Cells freshly isolated from eggs 8 hours after fertilization. (*b*) Cells after 10 days of growth in vitro.

was taken as the cell number. Chromosome counts of cultured cells were made by the squash method in water, with 1 percent aceto-orcein.

There were two distinct types of cells, small and large, in the initial cell suspensions. The small cells (5 to 10 μ in diameter) were round, with a clear outline and clear cytoplasm (Fig. 1*a*). The large cells (20 to 35 μ in diameter) were rather flat and the cytoplasm was finely granulated (Fig. 1*b*). The proportion of small cells decreased as development proceeded. In eggs of 2, 4, and 8 hours after fertilization they constituted 95.4 percent, 72.0 percent, and 48.8 percent, respectively, of the total cell number.

Cells isolated from eggs 2 hours after fertilization were incapable of multiplication in vitro (Fig. 2*a*). Since blastoderm formation is not yet complete at this stage, it is probable that most of the "small cells" isolated at this time were in fact nuclei with some cytoplasm but no cell membrane. The large cells may have been yolk cells. On the other hand, when cells isolated from eggs 8 hours after fertilization were cultured, the large cells divided only once while the small cells entered logarithmic multiplication (Fig. 2*b*). At the optimum temperature for growth, 30°C, the mean generation time of the small cells during the logarithmic phase of growth was 30 hours. The logarithmic growth phase lasted for 2 to 3 days, and the maximum cell number was reached in 5 days, at which time the cell number had increased about four times. Growth also occurred in unsupplemented H-5 medium, but at a slower rate.

Chromosomes could be seen readily in dividing large cells, and with some difficulty in dividing small cells. As shown in Fig. 3, the chromosome number probably remained constant at the diploid number (that is, eight) through 10 days of growth. Due to the difficulty of counting chromosomes in small cells, counts of less than eight or of nine were probably artifactual, while cells with 16 chromosomes were those in mitotic anaphase.

Cultures have been maintained through repeated transfers. At the time of writing this report, one culture has been maintained for 110 days in T-30 flasks, having been transferred every 10 days. At each transfer, the cells from a single bottle are divided into two parts and resuspended in fresh medium.

The mutants ry^2 and *ma-l* effect the synthesis of the enzyme xanthine dehy-

drogenase and the eye color of the adult. In vivo, ry^2 is lethal in larvae reared at high temperatures (30°C), but, if reared at lower temperatures, larvae with ry^2 survive. Connected with this is our finding that cells isolated from *ma-l* embryos exhibited the same growth rate and optimum temperature as those from embryos of wild-type *D. melanogaster*, while cells from ry^2 exhibited a lower optimum temperature (25°C) and grew more slowly, with a mean generation time of 42 hours in the log phase.

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Mycotoxins: Aflatoxin Isolated from Penicillium puberulum

Abstract. Penicillium puberulum *Bainer was found growing on a sample of moldy peanuts. It also grows on shredded wheat, potatoes, and laboratory culture media such as wort, potato dextrose, and Sabouraud agars, and synthesizes aflatoxin on these substrates. Thin-layer chromatograms of the chloroform-soluble toxin produced by the mold when grown on shredded wheat show fluorescent bands with* R_F *values identical with those of the fractions* B_1, B_2, G_1, *and* G_2 *of the toxin produced by* Aspergillus flavus. *This extract produces typical bile duct proliferation type of liver damage in 2- to 3-day-old Peking white ducklings.*

Initially aflatoxin was isolated by extraction from toxic peanut meal contaminated with *Aspergillus flavus* Link ex Fries, as described by Austwick, and identified by the strain number V-3734/10 (*1*), in our nomenclature, M-3. For some time, the toxin was considered to be a unique product of these particular strains of *A. flavus*. Now there is evidence (*2*) that certain other molds produce aflatoxin, such as *A. parasiticus* (Austwick, strain number IMI 15, 957 ii). The *A. parasiticus* is a subculture of the original strain isolated and described by Speare (*2*). We now report that *Penicillium puberulum* Bainer (Hodges, strain number M-56) produces aflatoxins. This strain of *Penicillium* was isolated from a sample of rejected moldy peanuts.

Mold mycelia were found on the inner surface of the cotyledons of many nuts. Samples of the different mycelia were transferred with a sterile needle to wort agar medium in a petri dish, and after 2 to 3 days of incubation at room temperature, mold colonies were transferred to Czapek agar slants. One of the mold cultures was initially recognized as a *Penicillium* species (*3*) and was later identified as *P. puberulum* Bainer (*4*).

This strain of *P. puberulum* has been cultured on sterilized potato plugs, moist shredded wheat, Sabouraud's agar slants, and potato dextrose agar slants. Chloroform extracts of all these cultures exhibited a strong fluorescence when excited by 365 mμ of light. A substrate of shredded wheat was chosen for toxin production after a preliminary evaluation of the relative intensity of fluorescence of the extracts from the several cultures.

The chloroform-soluble extract (M-56) was a light tan amorphous powder, (*5*). Analytical tests were performed on this preparation with ascending thin-layer chromatography on silica gel G-HR (distributed by Brinkmann Instrument Co.). The chromatograms were developed with a mixture of chloroform and methanol (95 : 5) in sealed, lined tanks. The chromatographed M-56 extract exhibited four characteristic blue and green fluorescent spots with R_F's identical to known B_1, B_2, G_1, and G_2 aflatoxins. Thin-layer quantitative analysis of the extract, with pure aflatoxins B_1 and G_1 as standards, indicated that the extract is composed of 15 percent each of aflatoxins B_1 and G_1 and 1 percent each of aflatoxins B_2 and G_2 (by weight).

A propylene glycol solution of the M-56 extract, when tested by our qualitative screening test in 2- to 3-day-old Peking white ducklings, produced the typical bile duct proliferation type of liver damage that is characteristic of the aflatoxin effect. The oral acute toxicity of the extract in similar ducklings was measured, ten birds being used for each dose, and may be expressed in milligrams per kilogram with 95 percent confidence limits (*6*) as follows: The 50-percent lethal dose, $LD_{50} = 2.30$ (2.12 to 2.48); slope = 1.14 (0.05 to 1.39).

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Reduction of Cardiac Stores of Norepinephrine in Experimental Heart Failure

Abstract. *Constriction of the ascending aorta in the guinea pig resulted in ventricular hypertrophy and congestive heart failure. In animals with heart failure which were killed 5 to 40 days after constriction, the norepinephrine stores in both ventricles were strikingly reduced; the extent of reduction was related to the severity of the constriction.*

Marked reductions of the norepinephrine concentration in the atrial appendage have recently been found in some patients with chronic congestive heart failure (*1*). This finding has led to the suggestion that there may be depletion of the norepinephrine stores during heart failure which could interfere with sympathetic nervous transmission. In view of the important positive inotropic effects on the heart of activity of the sympathetic nervous system (*2*), such interference with adrenergic function could possibly further impair cardiac performance in heart failure. To study this problem in a controlled experimental situation, we have produced heart failure in guinea pigs by graded aortic constriction and have examined its effects on the stores of norepinephrine in each ventricle and in the kidney.