in concentration in L-RNA and commercially obtained ribonucleotides. This contaminant appears to be removed by repeated ether washes of the RNA sample.

Ether is used in the modified Kirby extraction method to remove contaminating phenol. We routinely check the purity of the RNA samples by subjecting the extracted RNA to tests for DNA (diphenylamine), for protein (biuret), and for protein and phenol (Folin). The Folin test alone shows consistent and significant differences among the preparations (Table 2). There is always a higher concentration of Folin-positive material in the unwashed samples of B-RNA and H-RNA than there is in L-RNA. After five ether washes, the concentration of Folin-positive material decreases in the H-RNA and B-RNA and approaches that routinely found in L-RNA that has not been washed with ether. These differences are consistent and are found among all of the tissue extractions.

The experimental results reported above have led us to reevaluate the role of RNA in the induction of specific morphological changes in competent ectoderm. Since the ectoderm responds to extracts from both heart and brain and since this response is also found in ectoderm exposed to boiled B-RNA, it appears that the specificity of the induction is open to question and that the role of RNA in the inductive process should be further tested experimentally. Our results further suggest that a Folin-positive material in the RNA preparation may be responsible for the nonspecific induction which we have observed.

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Adaptation of an Insect Cell Line (Grace's Antheraea Cells) to Medium Free of Insect Hemolymph

Abstract. *Cultures of the insect cell line derived by Grace from* Antheraea eucalypti *Scott* [Austrocaligula eucalypti *(Scott)*] *were successfully adapted to medium supplemented with fetal bovine serum, whole-egg ultrafiltrate, and bovine plasma albumin instead of insect hemolymph. Cells, now in their 37th passage, have a population doubling time of 2.5 days; those of unadapted cultures, 4.2 days.*

The cell culture derived from ovarian tissues of *Antheraea eucalypti* Scott [*Austrocaligula eucalypti* (Scott)] (Lepidoptera: Saturniidae) by Grace (*1*) constitutes the first true line of arthropod cells and has considerable potential value for studies of insect and insect-borne viruses as well as for fundamental aspects of insect physiology. Although quickly disseminated to laboratories throughout the world, practical use of these cells has been limited because the medium must be supplemented with significant volumes of lepidopteran hemolymph, which is expensive and often difficult to obtain. The cell line was developed in a medium containing homologous hemolymph; later that of the related species *Antheraea pernyi* Guerin, *Austrocaligula helena* (White), and *Samia cynthia* (Drury) was found suitable (*2*). In the absence of hemolymph, cells of this line fail to grow, quickly undergo degenerative changes, and die within 3 or 4 weeks (*3*). We report here the rapid growth of a subline of these cells in medium free of hemolymph and supplemented only by readily available bovine and avian factors.

The adaptation experiments were done at the Rocky Mountain Laboratory. The original cell cultures and *A. pernyi* hemolymph were obtained from T. Grace, CSIRO, Canberra, Australia. Cells were cultivated at 28°C in 30-ml, disposable, tissue-culture flasks (Falcon Plastics) containing 4 ml of culture medium. Minimal culture medium used was Grace's insect tissue culture medium (Grand Island Biological Co.), which contains the antibiotics penicillin and streptomycin sulfate. Substances to be tested, over 60 kinds or combinations thereof, were empirically selected and added aseptically to make up the complete medium. Their effects on growth of cells were judged by inspection in comparison with control cultures grown continuously in the minimal medium enriched with 10 percent *A. pernyi* hemolymph. [Later it was shown that no more than 3 percent hemolymph was required by *Antheraea* cells (*3*) and the concentration of hemolymph in our controls was adjusted accordingly.]

Many of the additives did not permit survival or growth of the insect cells; those that did included fetal bovine serum, rabbit serum, and hemolymph of lobsters (*Homarus americanus* Milne-Edwards).

The most successful medium was one in which 10 percent heat-inactivated fetal bovine serum (Grand Island Biological Co.), 10 percent whole chicken-egg ultrafiltrate (Microbiological Associates, Inc.), and 1 percent bovine plasma albumin (fraction V; Armour Co.) were added to the minimal medium. When first transferred to this, cells survived, but increased only slowly until, after 49 days, their number was sufficient to warrant subcultivation. Growth rate increased with 12 successive passages until the cultures required subcultivation at weekly intervals. This subline has been established for 17 months and subcultivated 44 times.

At the 21st passage some of these cells were transferred to minimal medium containing 10 percent *A. pernyi* hemolymph instead of the substitute factors. They failed to grow and underwent degeneration over a period of a month. When offered bovine and egg factors, these cells resumed their vigorous growth and normal morphology.

Three other strains of *Antheraea* cells were induced to grow in minimal medium containing either 5 percent lobster hemolymph or 5 or 10 percent rabbit serum. The first was maintained for 13 months (24 passages) and the last two for 58 passages (17 months) and 34 passages (6½ months), respectively. Growth rate of these strains, however, was never as rapid as with the above-mentioned subline, and routine maintenance of the cultures proved difficult. A gradual accumulation of

unidentified crystalline precipitates that were inseparable from the cells occurred and rate of cellular degeneration increased with successive passages. When it was found that enrichment of the medium with 0.25 to 1.0 percent *A. pernyi* hemolymph would prevent degeneration and crystal formation, it was assumed that these strains were poorly, if at all, adapted to the substitutes lobster hemolymph or rabbit serum.

Growth of cells from the subline (20th passage) adapted to bovine and egg factors was studied at the Insect Pathology Pioneering Laboratory (USDA) in comparison with that of cells grown continuously on 3 percent *A. pernyi* hemolymph. Stock cultures of both adapted and unadapted lines were maintained in 12 ml of the appropriate medium in 250-ml, disposable, plastic tissue-culture flasks. After 1 week, 6 ml of fresh complete medium was added to each culture, and 4 or 5 days later the cells were used to seed cultures for determination of growth. Although suspended cells predominate in both the *Antheraea* cell line and the adapted subline, some cells adhere to the vessel walls. Therefore, suspensions were prepared for use as inoculum by loosening adhering cells with a 1.5- by 15-mm Teflon-coated stirring bar (Microspinbar; Bel-Art Products) and a magnetic stirrer.

When necessary, suspensions from two or more flasks were pooled and cell concentration was determined by counting the cells in a sample of the suspension with an electronic counter (Coulter Electronics, Inc.). Sufficient amounts of suspension were added to fresh medium to give a final cell concentration of from 40,000 to 60,000 cells per milliliter, an approximate 1:15 dilution of the original cell suspension. Cells were kept uniformly dispersed by continuous gentle stirring with the Microspinbar. Cultures used in determinations of growth were prepared by pipetting 4 ml of the dilute cell suspension to each of a series of 30-ml, plastic, tissue-culture flasks. These were incubated at 26° to 28°C without addition of fresh medium for the duration of each experiment.

Beginning with day 0 and daily thereafter, three cultures were selected at random for determination of cell number. Cells attached to the floor of the flask were removed by agitation with the Microspinbar, and 0.5 ml of the suspension was removed for counting. Five counts were made on each sample, and the mean was used to calculate the cell number for that sample. The mean cell number for each day was derived from the means of each of the three samples. Daily counts were made for at least 14 days. Two replicate tests each were done for the adapted subline and for the unadapted line.

Typical growth curves are shown in Fig. 1. The cells of the seed-inoculum were presumed to be in the late log phase of the growth cycle. Therefore, the cultures had a lag phase of approximately 3 days following transfer to fresh medium. After this, a period of logarithmic growth occurred, which differed for the two sublines. The adapted subline maintained logarithmic growth for 9 to 10 days, as compared with 7 to 8 days for the unadapted line. Population doubling time, calculated from the slope of the curve during the log phase, was 2.5 days for the former culture and 4.2 days for the latter.

Thus, an insect cell line is now available whose use is independent of insect hemolymph and whose growth characteristics equal or surpass those of the parent cell line grown in insect hemolymph.

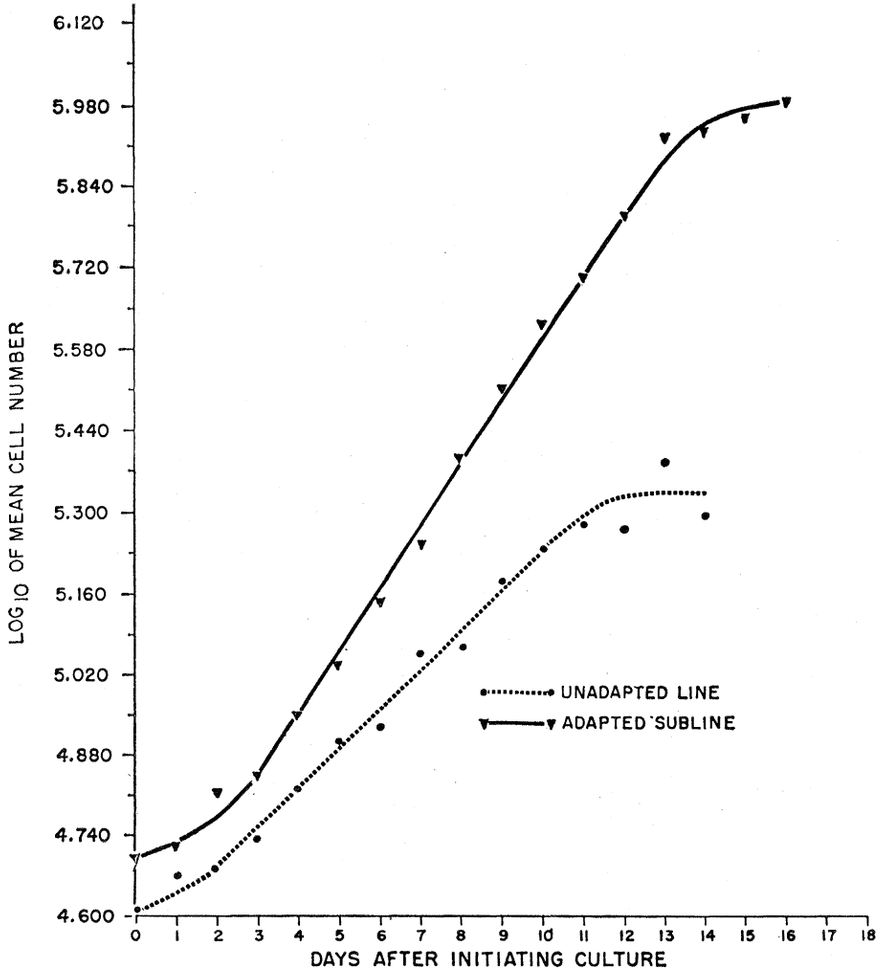

Fig. 1. Typical growth curves for Grace's *Antheraea* cell line in medium containing insect hemolymph and for a subline adapted to a medium containing fetal bovine serum, egg ultrafiltrate, and bovine plasma albumin in lieu of insect hemolymph.

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