Although Marek's disease virus was located in the epithelial cells in the kidney before cultivation, the infection did not produce colonies of transformed epithelial cells. Conditions under which HTV can induce a proliferative rather than cytocidal response are unknown.

Herpes-type virus particles similar to those seen in Marek's disease have been described in many human cell cultures that originated from African Burkitt lymphoma (*13*), American lymphoma (*14*), human leukemia (*15*), and "normal" individuals (*16*). There is also serological evidence that the HTV found in Burkitt lymphoma cultures could be a cause of infectious mononucleosis in man (*17*). Generally, HTV cannot be visualized in tissue until after cultivation (*18*). However, HTV has been observed directly in the Lucké adenocarcinoma in the frog during hibernation (*19*); and it has also been observed in one case of Burkitt lymphoma (*20*).

Our positive observations have followed negative attempts by us (*3*) and others (*2*) to visualize virus particles directly in Marek's disease tumors; perhaps this indicates the low incidence of virus in such tissues. Because Marek's disease has a high degree of contagiousness and successful experimental passage has been reported with oral and nasal washings (*21*), the epithelial cells of oral and respiratory passages appear likely areas for discovering herpesvirus multiplicating sites in birds infected with Marek's disease. Another point is that our failure to observe microscopically the HTV particles in lymphoid tumor cells does not exclude the possibility that the virus may induce a neoplastic transformation in lymphoid cells. In such cells it may persist as a viral genome that normally fails to code for all elements of the viral particle when the cell is under the physiological influences operating within organized tissues of the intact chicken.

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Proteins Synthesized before and after Fertilization in Sea Urchin Eggs

Abstract. *Acrylamide-gel patterns of proteins made before and during the first 30 minutes after fertilization were the same. The patterns of gels containing proteins from eggs whose rate of protein synthesis was stimulated reversibly by prior anaerobiosis or removal of carbon dioxide were also the same as that of the mature unfertilized egg. Changes were detected in the pattern of proteins synthesized in gastrulas as compared to proteins made in unfertilized eggs or zygotes.*

Until recently it has been widely accepted that the unfertilized sea urchin egg is incapable of protein synthesis. However, several groups (*1*) have reported that unfertilized eggs are active in protein synthesis, and we (*2*) have evidence that the basal incorporation of protein precursors represents the rate of protein synthesis in mature eggs.

We reported evidence (*3*) suggesting that the chain-initiation step is limiting for protein synthesis in unfertilized sea urchin eggs. This view is in contrast to the "masked message" concepts proposed by others (*4*) as an explanation for the repressed state of unfertilized sea urchin eggs. A question central to this problem is whether fertilization initiates transcription of messenger RNA (mRNA) not previously in use.

The rate of synthesis in unfertilized eggs can be reversibly increased (but not in fertilized eggs) by lowering the partial pressure of CO_2 in the medium (*3, 5*). The rate of protein synthesis increases in eggs made anaerobic with N_2 and allowed to recover under aerobic conditions. Neither treatment prevents fertilization, and the combined treatments result in a rate of protein synthesis equal to that for which fertilization is responsible.

We now report results of compari-

Table 1. Extraction of protein for electrophoresis. Eggs were labeled for 3 hours with C^{14}-amino acids and homogenized in electrophoresis buffer containing urea as indicated. Equal volumes of homogenates and 150,000*g* supernatant were precipitated with trichloroacetic acid and counted to obtain the data for disintegrations per minute (dpm) in the supernatant and the homogenate. Data for disintegrations per minute in the lower gel was obtained by using a sample of supernatant for electrophoresis. The lower gel was digested and counted after electrophoresis. Efficiency is the ratio of disintegrations per minute in lower gel to disintegrations per minute in the homogenate, and represents the fraction of radioactivity in the homogenate which actually enters the lower (running) gel. Higher concentrations of Brij were not more effective in extraction of radioactivity.

Urea (*M*)	Detergent (1%)	Radioactivity (dpm)			
		Homogenate	Supernatant	Lower gel	Efficiency (%)
0		23850	2934	735	3
4		18954	5560	2553	14
8		4959	1873	1408	28
8	DOC Brij	4959	3123	2643	51
8	Brij	4959	3024	2623	50
8	NP40	4959	3206	2435	46
8	Triton X100	4959	2809	2196	44

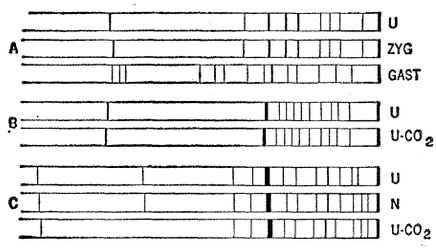

Fig. 1. Results of electrophoresis of protein labeled under a variety of conditions. Each rectangle represents the autoradiogram of a sliced, dried gel. Each line represents a band in the x-ray film which was visible as such to the eye. Exceptionally heavy bands are indicated by a thickened line. This is not intended to be a quantitative representation; the densitometer traces provide that. The lengths of gels are expanded by a factor of 2 (as they are in the densitometer tracings), and gels are arranged in three comparable groups.

Group A shows a comparison of labeled protein extracted from unfertilized eggs (*U*), zygotes (*ZYG*), and late gastrulas (*GAST*) with 8*M* urea and 1 percent Brij after a 30-minute exposure to label; the mixture was then exposed to 1 percent casamino acids for 30 minutes. Unfertilized egg protein (4300 dpm), fertilized egg protein (12,000 dpm), and gastrula protein (27,700 dpm) were applied to the gels. Gels were in contact with film for 3 months, 4 weeks, and 2 weeks, respectively.

Group B shows a comparison of labeled protein extracted from unfertilized eggs (*U*) and unfertilized eggs stimulated by CO_2 removal (*U-CO₂*). These eggs were labeled for 3 hours, and proteins were extracted with 8*M* urea and 1 percent Brij. Protein from control unfertilized eggs applied to the gel had 20,000 dpm, while protein from unfertilized eggs maintained in seawater free of CO_2 for 3 hours before labeling had 56,300 dpm. Gels were in contact with film for 1 week and 3 weeks.

Group C shows a comparison of labeled proteins extracted with 4*M* urea from unfertilized eggs which were stimulated by prior anaerobiosis (*N*) or by removal of CO_2 (*U-CO₂*) with control unfertilized eggs (*U*). Incubation in label was for 3 hours. To gel (*U*), protein with 14,300 dpm was applied, to get (*N*) 19,700 dpm was applied, and to gel (*U-CO₂*) 31,200 dpm was applied. For gel (*N*), eggs were made anoxic for 5 hours with nitrogen before labeling. For gel (*U-CO₂*), eggs were maintained in seawater free of CO_2 for 3 hours. Gels were in contact with film for 5 weeks (*U*), 4 weeks (*N*), and 18 days (*U-CO₂*).

sons of disc-electrophoretic data from proteins made before and immediately after fertilization and after treatment with agents which stimulate protein synthesis in the unfertilized egg.

Eggs of *Arbacia punctulata* (*3*) were labeled with C^{14}-labeled reconstituted protein hydrolyzate (Schwarz BioResearch). When short labeling periods were used the radioactive amino acids were chased with 1 percent casamino acids.

The standard system of disc electrophoresis on acrylamide gels (*6, 7*) with minor modifications was used. The stock solutions supplied by Canalco (Canal Industrial Corporation) were used throughout; reagent grade urea was added to these solutions to give the final concentrations of urea indicated below. After mixing the gel solution, one drop of 3-dimethylaminopropionitrile was added for each 10 ml of final gel solution to accelerate polymerization. Gels were supported in soft glass tubing (8 mm) in an apparatus described previously (*8*), and current was provided by a Buchler current-regulated power supply, set to deliver 3 ma per tube. Electrophoresis was carried out at 4°C. The red pigment of the *Arbacia* egg provides a suitable tracking dye (*9*). After electrophoresis, gels were removed from the tubes, stained, and fixed in 7.5 percent acetic acid containing 1 percent amino schwarz (Buffalo black, naphthol blue black). The stain was removed by treatment with 7.5 percent acetic acid, and slides were prepared for autoradiography (*10*). The 7.5 percent acryamide gels were cut into three longitudinal slices for drying; 5 percent acrylamide gels were used with 8*M* urea, and were cut into two equal longitudinal slices. Dried gels were exposed to x-ray film for periods corresponding to the amount of radioactivity applied. Autoradiograms were traced on a recording microdensitometer (Joyce, Loebel) (*11*) with a 0 to 2 or 0 to 1.6 absorbance wedge.

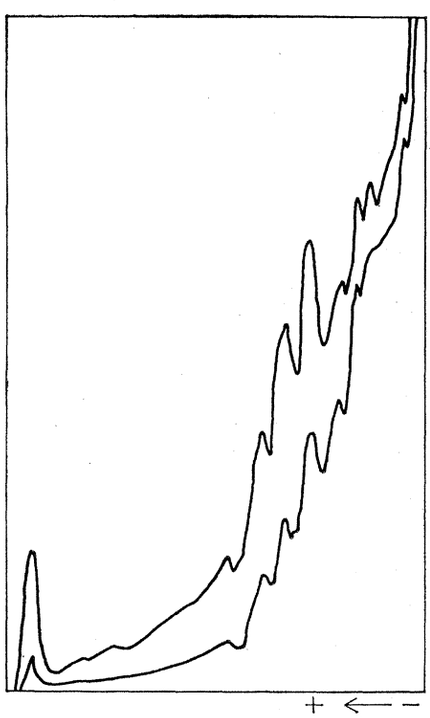

Fig. 2. Fertilized (lower curve) or unfertilized (upper curve) *Arbacia* eggs were pulse labeled for 30 minutes with 2 μc/ml of C^{14}-amino acids, and were then chased for 30 minutes with seawater containing 1 percent casamino acids (Difco technical) adjusted to *p*H 8. Eggs were extracted with 8*M* urea and 1 percent Brij. Fertilized eggs were labeled starting at the time of sperm addition. Gels run with unfertilized egg preparations were in contact with film for 3 months. Those run with fertilized egg preparations were in contact with film for 4 weeks. Fertilized egg protein (12,000 dpm) and unfertilized egg protein (4,300 dpm) were applied to the gels.

To prepare extracts for electrophoresis, we homogenized eggs (dounce homogenizer) in five volumes of the desired medium [tris(hydroxymethyl)-aminomethane–glycine electrophoresis buffer (*7*) containing urea and detergents as required]; the homogenates were centrifuged for 1 hour at 150,000*g* (Spinco SW-39). Appropriate volumes of the supernatants were applied to the gels; sample size was chosen so that equal amounts of protein, usually 200 to 400 μg as determined by the Lowry reaction (*12*), were applied to each gel in a series.

After the destained gel was digested overnight with hydrogen peroxide, the efficiency of entry of labeled material into the gels was determined by trapping radioactive protein in base (hyamine hydroxide) (*13*). The material was counted in Bray's scintillation fluid; efficiency of counting was determined by addition of an internal standard. This radioactivity was compared to acid-insoluble radioactivity in volumes of the homogenate and supernatant equal to the volume of sample applied to the gel.

Migration of soluble RNA in the gels was apparently very rapid, as the only toluidine-blue staining band in the extracts migrated with the tracking dye.

If "unmasking" of mRNA is responsible for the increased rate of protein synthesis which occurs after fertilization in sea urchin eggs, it would be expected that new species of protein might be detectable in electrophoregrams of homogenates of these eggs. The finding that new species of proteins are made as a consequence of fertili-

zation would lend support to the masked mRNA hypothesis, whereas a contrary finding would reinforce the hypothesis that mRNA already in use in the egg becomes more fully saturated with ribosomes when fertilization occurs and is the factor primarily responsible for the increased rate of protein synthesis.

For analysis of the proteins made under various conditions, it seemed desirable to find a method for extracting as large as possible a fraction of the sea urchin egg and embryo protein, especially because extracts containing less than 5 percent of the proteins of the egg had been previously used (*9, 14*). We did not use buffers of high salt concentration to solubilize the protein, even though they might be expected to work well with cells of the sea urchin, which is a high salt organism (*15*), because use of disc electrophoresis constrained us to the use of gels of low salt concentration. Use of urea and nonionic detergents, however, proved an effective way of solubilizing the sea urchin proteins. Table 1 shows the efficiency of entry into the gel of radioactive material in the homogenate under a variety of extraction conditions, the optimum yielding an efficiency of about 50 percent.

The pattern of stained protein in the gels did not vary perceptibly from one group of eggs to another. They did vary depending upon the procedure used for extraction of proteins and the conditions of labeling (short or long exposure, or exposure to isotopic precursor followed by exposure to unlabeled precursor) so that legitimate comparisons can be made only among gels of the same group. Because of the high density of bands it was not practical or meaningful to assign specific peaks of radioactivity to specific stained bands. Instead, autoradiogram tracings were compared directly. In three groups of gels (Fig. 1) the spectrum for the unfertilized egg is similar to that for the newly fertilized egg or that of the unfertilized egg in which protein synthesis had been stimulated. The patterns are complex, but each peak in one spectrum has a corresponding peak or shoulder in every other.

Comparisons of proteins made in unfertilized and newly fertilized eggs are shown by actual tracings of autoradiographs of the gels (Fig. 2). Significant differences cannot be observed, nor are they apparent when comparing autoradiographs of gels containing proteins from eggs stimulated by CO_2 removal or by prior anaerobiosis. However, proteins from gastrula-stage embryos extracted in 8*M* urea (1 percent Brij) do differ substantially from proteins in the unfertilized egg or in the zygote (Fig. 1); this demonstrates that our extraction procedure does not extract only some fundamental class of proteins which are synthesized at all stages and which constitute a substantial fraction of the total protein synthesized.

The possibility that new proteins are in fact made immediately after fertilization in small quantity cannot be ruled out by the foregoing experiments, even if coincidence of peaks is taken to imply identity of proteins. The actual peaks represent no more than 10 percent of the total synthesis which occurs in these eggs, the remainder of the 50 percent of the radioactivity of the homogenate which entered the gel being distributed as radioactivity not resolvable as bands throughout the gel. The possibility that new proteins are represented cannot be excluded except by an exhaustive fractionation and high-resolution analysis of all the protein of the egg and embryo. That major changes in the pattern of protein synthesis have not occurred is consistent with the view that peptide chain initiation is responsible for most of the rate of change at fertilization. Monroy (*16*) finds no change in the average size of peptide chains made before and after fertilization in *Paracentrotus lividus* (comparison based on density-gradient centrifugation in sodium dodecyl sulfate sucrose) which is also consistent with this view, as are the findings of Whiteley, McCarthy, and Whiteley (*17*) who show by molecular hybridization that mRNA's from unfertilized eggs and blastulas are not distinguishable.

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Ionic Mechanisms Controlling Behavioral Responses of Paramecium to Mechanical Stimulation

Abstract. *A mechanical stimulus applied to the anterior part of* Paramecium *causes a transient increase in membrane permeability to calcium. This permits a calcium current to flow into the cell, causing the membrane potential to approach the equilibrium level for calcium. The transient depolarization which results elicits a reversal in the direction of ciliary beat. When the organisms are free-swimming this is seen as the reversed locomotion of Jennings' "avoiding reaction." In contrast, a mechanical stimulus applied to the posterior part results in increased permeability to potassium ions, and hence an outward potassium current. The hyperpolarization which results causes an increase in the frequency of ciliary beat in the normal direction. In free-swimming specimens this is seen as an increase in the velocity of forward locomotion.*

The varieties of locomotor behavior exhibited by *Paramecium* depend on relative changes over the cell surface in the frequency, strength, and orientation of ciliary motion (*1–3*). When the paramecium encounters a noxious chemical or a mechanical stimulus with its anterior end, it transiently reorients (reverses) its cilia, which causes it to swim backward for a short distance.