rocytes were tested with 16 hemagglutinating units of type 2 reovirus in the usual hemagglutination titration but no inhibition of hemagglutination was observed. *N*-acetyl-D-glucosamine did not attach to the red blood cells in the inhibition reaction.

2) The converse experiment was performed to test the attachment of *N*-acetyl-D-glucosamine to the virus. Five hundred hemagglutinating units of reovirus type 2, strain 988 were incubated in 2 ml of Earle's saline containing 5000 μmole of *N*-acetyl-D-glucosamine for 1 hour at 23°C. An equal portion of the virus preparation was incubated in Earle's saline alone as a control. Each was layered separately onto 15 to 65 percent sucrose gradients, and centrifuged at the same time in the Spinco model-L (SW-25 rotor) ultracentrifuge at 24,000 rev/min for 210 minutes at 4°F.

Fractions were collected in 20-drop portions, and each portion was tested for hemagglutination at a dilution of 1 to 10 (Fig. 1). The amount of reovirus recovered in the sample treated with *N*-acetyl-D-glucosamine was about half of that in the control. Moreover, the treated virus banded in a slightly less dense region of the gradient in several duplicate runs. *N*-Acetyl-D-glucosamine, therefore, attaches directly to the reovirus, but not to the red blood cells, in inhibiting hemagglutination.

The terminal nonreducing ends of the antigenic determinant of blood group substances A, B, and H are *N*-acetyl galactosamine, galactose, and α-linked fucose residues (*3*). It cannot be stated definitely that the reovirus–erythrocyte union occurs through the attachment of the protein of the virus capsomeres to the *N*-acetyl-D-glucosamine which is present in ample amounts on the surface of the red blood cells (*2*). This is indeed a cardinal possibility, but the inhibition of reovirus hemagglutination by *N*-acetyl-D-glucosamine could be a steric effect as well (*6*).

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7. Aided by grants (1 T1 AI 261-01) and (AI-05721-01A1) from the National Institutes of Health. Address reprint requests to A.M.L.

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Messenger RNA Utilization during Development of Chick Embryo Lens

Abstract. *A marked increase in synthesis or availability of messenger RNA which can accommodate eight to ten ribosomes was observed in the chick embryo lens between 11 and 12 days of development. The increase is seen in a shift from polyribosomes consisting of predominantly two to five single ribosomes to polyribosomes of predominantly eight to ten ribosomes in lens cells. Short-lived (half-life, 3 hours) and long-lived (half-life, > 30 hours) messenger RNA's coexist in whole lenses on the 14th day of development.*

A change in the kinds of messenger RNA's which occur during differentiation of a specialized tissue should be detectable by a change in the polyribosome distribution of its cells, particularly if the tissue begins to synthesize a large amount of a few species of message. Humphreys, Penman, and Bell (*1*) have reported that during feather development a marked increase in the number of polysomes containing four and especially of those containing five and six ribosomes occurs between 13 and 14 days of incubation. Long-lived messenger RNA's were associated with these ribosomal complexes.

A more remarkable change, described below, in the distribution of polyribosomes occurs during development of the lens in the chick embryo. We also present further evidence for coexistence of short and long-lived messenger RNA's in the crystalline lens. It has been shown (*2*) that some lens proteins are made on polyribosomes which have a long half-life.

As late as the 10th day of incubation, the lens polyribosome profile displayed in a sucrose density gradient (Fig. 1) is similar to that seen in 3- or 5-day whole embryos (*2*), in which polyribosomes containing two to five ribosomes predominate. By the 11th day a slight shoulder appears in the profile, indicating a new class of larger polyribosomes. These larger polyribosomes increase in number during the next 24 hours until the profile is dominated by a sharp high peak in the region of eight- to ten-member polyribosomes. This peak also characterizes the 13-, 14-, and 15-day profiles. Polyribosomal protein synthesis was observed in all lenses after a 2-minute exposure to radioactive algal protein hydrolyzate. The lens polyribosomes

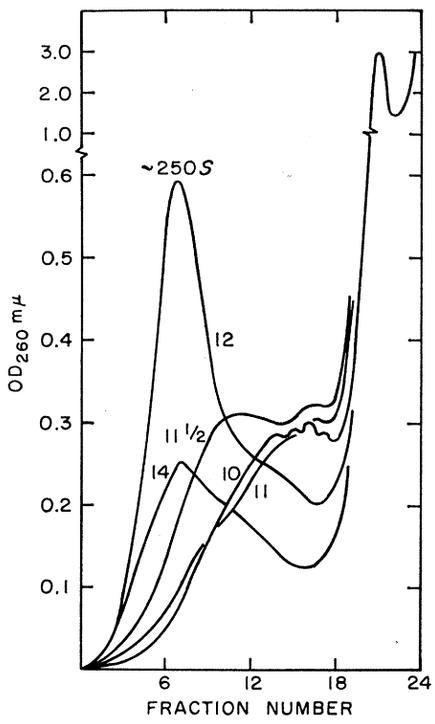

Fig. 1. Sucrose density gradient centrifugation of lens polyribosomes of 10- to 14-day chick embryos. One milliliter of homogenate (*2*) was layered on a 15- to 30-percent sucrose gradient (25 ml), centrifuged for 2 hours at 24,000 rev/min (SW-25 spinco rotor). The direction of sedimentation was to the left. The large peak at tube 21 represents single ribosomes. The peak at about 250*S* represents polyribosomes containing eight to ten ribosomes. Equivalent amounts of lens tissues were used in each experiment.

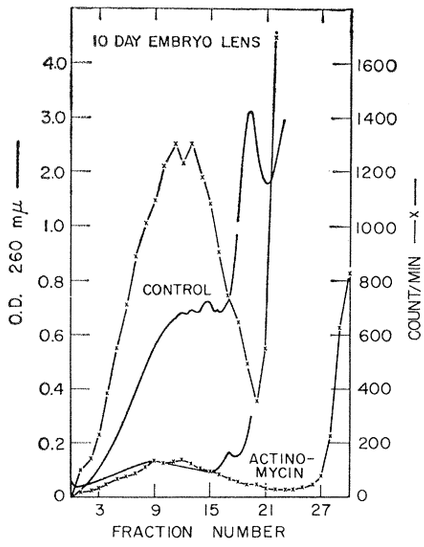

Fig. 2. The effect of actinomycin D (40 μg/ml) on polyribosomal protein synthesis in 10-day embryonic chick lenses. Lenses were incubated in Charity Waymouth (*11*) medium at 37°C with gentle rotation in an atmosphere of 5 percent CO_2. Penicillin (4 mg), streptomycin (2.5 mg), and phenol red indicator were included in each 20 ml of medium. The protein was labeled with C^{14}-algal hydrolyzate (1.4 mc/mg) for 2 minutes in buffered saline in a 37°C water bath. The reaction was stopped by washing the tissue in ice-cold saline.

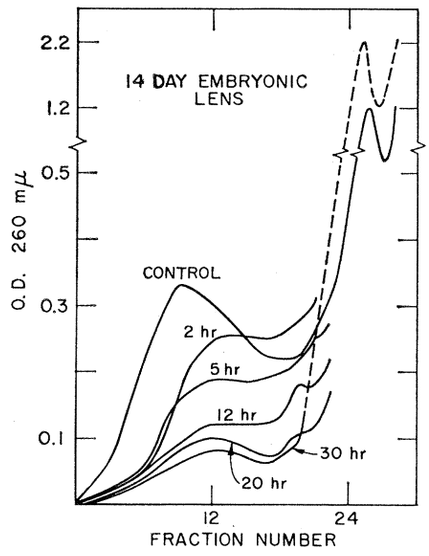

Fig. 3. The effect of actinomycin D on polyribosomes of 14-day embryonic lenses. Curves are numbered according to the number of hours that the lenses were incubated in nutrient medium containing actinomycin (40 μg/ml). The rate of disappearance of polyribosomes is taken as a measure of the disappearance of messenger RNA with which ribosomes are complexed. Protein synthesis on polyribosomes, as measured by incorporation of radioactive amino acid into nascent protein, occurred in lenses at all time intervals shown.

were dissociated by a brief exposure to ribonuclease (5 μg/ml) at 4°C, although ribosomal RNA was not degraded.

In order to study the fate of the polyribosomes and thus that of associated messenger RNA, actinomycin D treatment was utilized to halt the synthesis of lens RNA in vitro. To be certain that the actinomycin penetrated the cells and stopped RNA synthesis, RNA was extracted after labeling the actinomycin-treated lenses with radioactive uridine. Table 1 shows the effect of actinomycin (40 μg/ml). After 2 hours, the incorporation of precursor into RNA was less than 1 percent, and the incorporation remained low at 6, 18, and 24 hours. The synthesis of RNA may have been stopped before 2 hours, but the 2-hour labeling time precluded earlier measurements.

When 10-day lenses were treated with actinomycin for 20 hours (Fig. 2), there was still some incorporation of amino acid in the remaining polyribosomes. Although small polyribosomes predominate in the 10-day lens, a greater proportion of large polyribosomes persist after treatment with actinomycin D (40 μg/ml) for 20 hours. The peaks of the actinomycin-resistant polyribosomes in 10-, 12-, 13-, and 14-day lenses were in a similar position in the gradients and were displaced only slightly from the peak at 250*S* seen in the 12- to 14-day controls.

Table 1. Effect of actinomycin D on RNA synthesis in 14-day embryonic lenses. Uridine-2-C^{14} (about 25 μc/mmole) was added to 2 ml of Charity Waymouth medium in small petri dishes, and lenses were incubated at 37°C in 5 percent CO_2 for 2 hours with agitation on a rotary shaker. The reaction was stopped with ice-cold saline, and samples were frozen at −15°C until the RNA was extracted (*10*), and the radioactivity (per O.D. unit at 260 mμ) was determined. Data represent averages of duplicate extractions. C, control; A, actinomycin.

Period	Radioactivity (count/min)		
	C	A	Percent of control
2 hr	2978	12.5	0.42
6 hr	14,169	37.4	.26
18 hr	8167	29.9	.37
24 hr	1369	20.9	1.5

Figure 3 shows the alterations in the polyribosome profile after intervals of actinomycin treatment of 14-day embryonic lenses. The profile does not show persistence of any sharply defined peaks after exposure to actinomycin, as found in the polyribosomes of down feathers (*1*, *3*). In all experiments, radioactivity was incorporated into nascent protein on the polyribosomes after actinomycin D treatment.

The total polyribosome-bound radioactivity [associated with the optical density (O.D.) curves of Fig. 3] was plotted as a function of time in Fig. 4. The points taken begin at 2 hours when RNA synthesis was known to have been halted.

The data suggest exponential decay (*4*). The inflection of the curve (Fig. 4) at about 12 hours indicates that at least two classes of messenger RNA are present in the lens. One class has a half-life of about 3 hours and the other class has a half-life of at least 30 hours.

Our results suggest that a great increase either in synthesis or availability of messenger RNA which can accommodate eight to ten ribosomes occurs between 11 and 12 days of development in the chick lens. This event is reflected in a shift from polyribosomes of predominantly two to five single ribosomes to polyribosomes of predominantly eight to ten ribosomes.

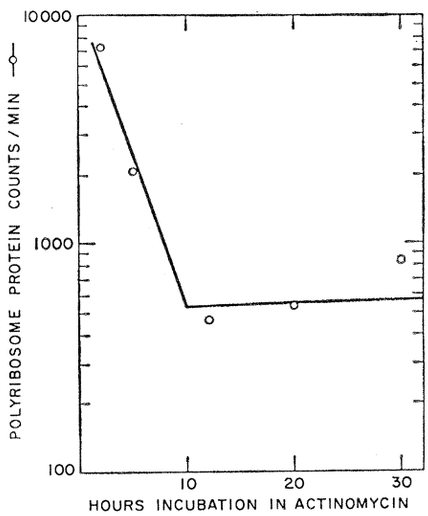

Fig. 4. Rate of disappearance of nascent protein in 14-day lenses treated with actinomycin D for different periods. The total radioactivity in nascent polyribosomal protein (count/min) is plotted on a log scale against time. Calculations were based on equal amounts of 14-day lenses.

The shift occurs at a time when lens morphogenesis is essentially completed except for the laying down of many fibers. The shift cannot be correlated with any specific morphogenetic event since the essential features of the lens are formed by 8 days (*5*). It does occur at a time when the lens undergoes a slight and transitory increase in growth rate (*6*). The shift must result in initiation or accentuation of synthesis of a class of lens proteins, possibly monomers of one of the crystallins.

The work of Yamada (*7*) on regenerating amphibian lens points to the late appearance of γ-crystallin which is restricted to the fiber cells. If γ-crystallin is detectible before 11 days in the chick embryo lens (*8*, *9*) the polyribosomal shift possibly represents an increase in synthesis of γ-crystallin and not necessarily initiation of its synthesis. Also, it is not known whether the change in the distribution of polyribosomes between 11 and 12 days can be correlated with cytological maturation of lens cells which ultimately become filled with the crystallin proteins. Nuclei disappear during this process.

At 14 days many lens cell nuclei have not yet ceased to function. From the curve of polyribosome decay it is clear that at least two classes of messenger RNA's are being used by a relatively homogenous population of cells. One class has a half-life of about 3 hours and the other a half-life of longer than 30 hours. The presence of short-lived messenger RNA may be taken as evidence of nuclear function.

Even though short- and long-lived messenger RNA's coexist in the whole 14-day lens, which consists nearly entirely of cells that make crystallins, it is not yet clear that the two kinds of messenger RNA occur in the same cell at the same time. Either a cell uses short- and long-lived messenger RNA's continuously and concurrently, or it uses them sequentially, first translating short-lived messages and then translating long-lived messages. The transition from use of short- to use of long-lived messages would come at a time when nuclear function ceased. All proteins synthesized after that would be made on long-lived messenger RNA.

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Heparin Enhancement of Factors Stimulating Bone Resorption in Tissue Culture

Abstract. *The addition of small amounts of a commercial heparin solution, or a synthetic polysaccharide-sulfuric ester similar in structure to heparin, to the medium in which bone tissue is cultured markedly enhances the amount of bone resorption obtained with suboptimal concentrations of parathyroid extract or other factors which stimulate bone resorption. It is suggested that heparin be considered a "cofactor" which stimulates bone resorption and which may play an important role in physiological or pathological resorption.*

In a previous study (*1*) it was shown that the extent of bone resorption obtained in our tissue culture system was dependent upon the amount of resorption-stimulating factor added to the medium as well as the concentration of oxygen in the gas phase. Known factors which stimulate bone resorption include parathyroid extract, crystalline vitamins A, D_2, and D_3, and dihydrotachysterol. The experiments described here indicate that the amount of bone destruction obtained with suboptimal concentrations of these factors may be markedly enhanced by the addition of heparin to the medium.

According to our system of tissue culture, calvariae were removed aseptically from 4- to 5-day-old Swiss albino mice of the Webster strain (litters were pooled when more than 10 to 12 calvariae were needed); the occipital bone was removed, and the remaining portion (frontal bone and parietal bones) was attached to a rectangular coverslip by covering the tissue with a thin film of a mixture of chicken plasma and chick embryo extract (2 : 1). After clotting occurred, each coverslip was inserted into the "well" portion of a Leighton tube, covered with 2 ml of a supernatant fluid composed of heated horse serum (80 percent), Gey's balanced salt solution (10 percent), and 100 units each of penicillin and streptomycin in Gey's balanced salt solution (10 percent).

Water-soluble test substances were usually diluted in the balanced salt solution or in the serum, whereas fat-soluble substances were diluted in the horse serum component or first dissolved in a small volume of absolute alcohol before being diluted in the serum. The oxygen tension of the gas phase in the culture tubes was increased to 50 percent by introducing a pipette (leading from a tank containing a mixture of 50 percent oxygen and 50 percent nitrogen) into the bottom of each tube containing 2 ml of medium and raising gas bubbles until they completely filled the length of the tube and replaced all the air. The tubes were stoppered and then placed horizontally in a rotor at 37°C. The media were changed every 2 days, at which time the procedure for increasing oxygen tension was repeated. Microscopic

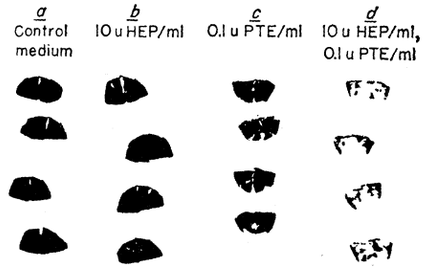

Fig. 1. Effect on bone resorption of heparin (*HEP*) in combination with a suboptimal concentration of parathyroid extract (*PTE*); the bone was cultured for 12 days and then stained by the Von Kossa reaction (*u*, units).