extracellular space and which appear to have a modified plasma membrane; and (v) a relatively low density in the cytoplasm.

The ribosomes in active cells are not only clustered but appear generally in aggregates larger than five or six, the size predominating in cell-free preparations. Furthermore, the polyribosomes are often of the "open" configuration (*9*). Rifkind *et al.* (*10*) have also noted such large ribosomal aggregates. In inactive reticulocytes or those occasional cells with low activity (that is, one-fifth to one-tenth the activity of most active cells), ribosomes are also found and frequently at relatively high concentration. The ribosomes in inactive cells are usually not clustered; but we regularly found such cells, in which most ribosomes were present as polyribosomes (*11*). However, these polyribosomes are usually dimers or trimers.

Mitochondria are invariably found in active cells. These mitochondria have a rather dense matrix and are relatively small—0.1 to 0.2 μ in length—although occasionally longer ones may be found. In sections from cells that have been extracted with acidic acetone after fixation and dehydration, dense particles are often seen in mitochondrial matrices. Mitochondria are generally absent in inactive cells but are occasionally seen in those inactive cells containing a high concentration of ribosomes.

Sections of active cells often show deep invaginations of the plasma membrane and large vacuoles. Serial sections indicate that these invaginations are finger-like and that some of the large vacuoles are, indeed, vacuoles and not cross-sectioned invaginations. Our results are consistent with the observations of Bessis and Bricka (*12*) who described young reticulocytes as motile cells which exhibited amoeboid-like movements.

Small infoldings showing structural modifications of the plasma membrane are found almost exclusively in active cells. Tubular and vesicular elements are always found in active cells, but also appear frequently in inactive cells with many ribosomes. By serial sectioning it was found that at least some vesicular elements were continuous with the cell membrane.

Attempts have been made, in studies on the maturation of reticulocytes, to correlate loss of protein-synthesizing activity with loss of ribosomes (*13*). However, our results clearly demonstrate that many totally inactive cells contain large numbers of ribosomes, and, in some cases, polyribosomes. Ribosomes, and, indeed, polyribosomes, do not appear to be rate-limiting. Biochemical experiments which complement these autoradiographic studies strongly suggest that primary control of hemoglobin synthesis may very well reside in mitochondria or in elements of the plasma membrane or in both (*14*).

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Polysome Morphology: Evidence for Endocrine Control during Chick Embryogenesis

Abstract. *Surgical removal of the pituitary gland had little apparent effect on the distribution or morphology of skin and feather polysomes in chicken embryos incubated for 12 days. However, polysome patterns obtained from 15-day-old hypophysectomized embryos differed markedly from those of their controls. In addition, a tetrad-shaped polysome, characteristic of the 158S peak of the 12-day-old embryo but not of the 15-day-old control, is still retained in the operated embryo at 15 days. Therefore, it appears that after day 12 of embryogenesis the structure of the four-ribosome polysome from skin and feathers is contingent on a functional hypophysis.*

During early development (day 0 to day 12), growth and general differentiation of the chick embryo are not markedly influenced by either extirpation of the pituitary or blocking of the thyroid gland by goitrogenic agents (*1*). However, sometime during the 2nd week of incubation, normal growth becomes dependent on the function of these glands. In the course of this time period, changes have been noted in the sucrose density gradient patterns of skin and feather polysomes obtained from the intact or normal embryo. Before day 13 of incubation the gradient profile is dominated by a four-ribosome peak which has been observed to be short lived, inactive in protein synthesis, and resistant to the action of ribonuclease. It has been suggested that the inactivity and ribonuclease resistance of the four-unit polysomes are the result of their being mainly composed of tight symmetrical squares. After day 13, the polysomes comprising this peak are longer lived, capable of carrying on protein synthesis, and no longer ribonuclease resistant (*2*, *3*). These changes appear to be related to their configuration, which after day 13 is elongated in the manner characteristic of functional units.

In considering the above information it appeared reasonable that the changes noted in the structure and function of skin and feather polysomes during differentiation might have been effected through endocrine mediation. The following experiments were designed to determine whether altered endocrine operation influences polysome structure and function during embryogenesis of the chick.

Fertilized chicken eggs (White Leg-

horn strain) were placed in an incubator (37.4° to 38.6°C) until the 12- to 14-somite stage was attained. At this time the hypophyseal placode is identifiable as a thickened ectodermal plate. This placode was removed surgically under sterile conditions (*4*), and the resulting embryo is referred to as being "hypophysectomized." In order to accomplish the hypophysectomy at this stage of development, it was also necessary to remove much of the head, including both eyes. The operated eggs and their sham-treated controls (those in which the head of the embryo was damaged with a surgical needle but the placode left intact) were then sealed with transparent tape and returned to the incubator. On day 12 and day 15, hypophysectomized embryos and their respective controls were killed, and the skin and feathers were collected into cold hypotonic buffer (*2*). The polysome distribution patterns of this tissue were obtained by reading the optical density at 260 mμ after sedimentation at 0°C in a linear sucrose gradient (15 to 30 percent) for 2 hours.

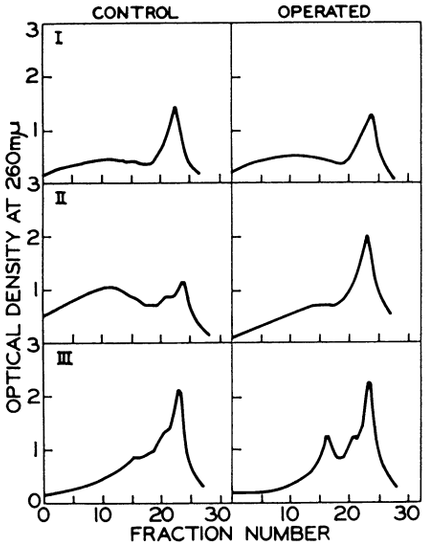

Fig. 1. Sucrose gradient patterns, optical density at 260 mμ, of skin and feather extracts from 12-day (I), 15-day (II), and actinomycin D–treated (III) chick embryos. Extraction of the actinomycin D–treated skin and feathers followed incubation of the skin and feathers in shaker flasks for 20 hours in Waymouth's medium containing 60 μg of actinomycin D per milliliter. Within each group, the gradients were normalized by applying the same amount of polysomal material to each gradient tube (measured by optical density at 260 mμ). The peak tube of the four-ribosome polysome region of the gradient corresponds to tube 14 (I), 15 (II), and 16 (III) after centifugation at 25,000 rev/min in a Spinco SW 25.1 Rotor (model L2 Spinco) for 2 hours. The peak tube varied slightly between runs because of slight variations in the fullness of the gradient tubes.

Polysome patterns of hypophysectomized 12- and 15-day-old embryos along with their respective controls are shown in Fig. 1. As indicated by the polysome profiles, extirpation of the pituitary gland had little apparent effect on skin and feather polysomes in the 12-day embryo. In contrast, the polysome patterns obtained from skin and feathers of operated and control embryos incubated for 15 days differed markedly from each other in appearance. The polysome pattern obtained from control tissue showed a decided shift from the monosome peak apparent in the 12-day profile to the heavier polysome region, whereas the optical density patterns obtained after gradient analysis from the tissue extracts of hypophysectomized embryos were similar to those of 12-day-old embryos.

As mentioned above, a four-ribosome polysome in the form of a tetrad characterizes the 158*S* peak before day 12, whereas an elongated four-ribosome unit predominates in this peak after day 12 (*3*). In order to observe the effect of hypophysectomy on polysome structure, samples from sucrose gradients of operated and control 12- and 15-day embryos were prepared for electron microscopy. A drop of the ribosome suspensions was placed on a carbon-coated grid, fixed in 10-percent Formalin, dehydrated in ethanol, dried from amyl acetate, and shadow cast with carbon-platinum. Photographs were taken at original magnifications of × 8000 with a Siemens electron microscope Elmiskop I, equipped with a pointed filament, double condenser, 100-μ condenser aperture, and a 50-μ objective aperture (*5*). Polysomes from 15-day hypophysectomized and control embryos were also compared after the skin and feathers were incubated for 20 hours in Waymouth's medium which contained 60 μg of actinomycin D per milliliter.

Inspection of the polysomes from the 12-day control and operated birds reveals that the polysomes comprising the 158*S* peak are similar in these groups, the characteristic tetrad being the predominant feature in this region of the gradient. After a minimum of 1000 ribosomes in each group were counted, the tetrads were found to comprise 24.9 and 18.0 percent of the total ribosomes, respectively. However, electron micrographs of the 158*S* polysomes of 15-day embryos indicate that, as expected, the control polysomes (Fig. 2) are no longer present mainly as tight symmetrical units (now only 2.5 percent of the total). In contrast, those obtained from hypophysectomized embryos (Fig. 3) still retain many with the tetrad configuration (25.4 percent) characteristic of this peak in younger birds. Comparison of skin and feather polysomes of 15-day-old control and operated birds which were incubated in the actinomycin D revealed that the

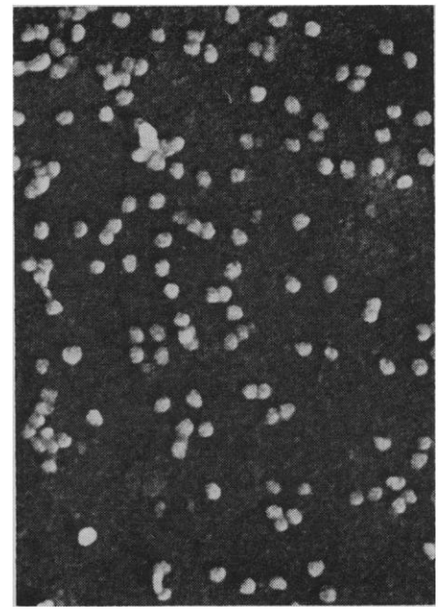

Fig. 2. Skin and feather polysomes from intact 15-day-old controls, from the four-ribosome region of a sucrose density gradient (× 75,000).

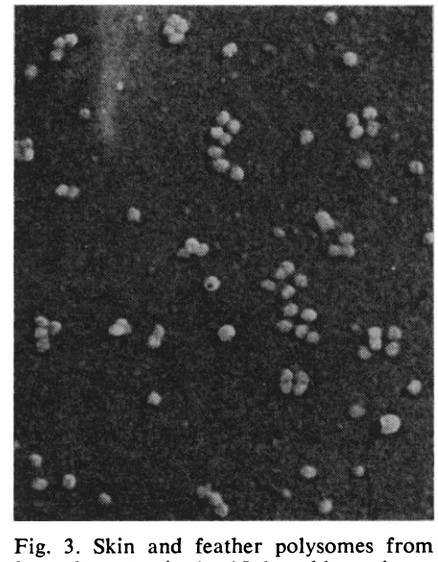

Fig. 3. Skin and feather polysomes from hypophysectomized 15-day-old embryos obtained from the four-ribosome region of a sucrose density gradient (× 65,000).

158*S* peak had a greater proportion of tetrads in both instances (18.7 percent in control, 64.5 percent in operated). It appears likely that the increased concentration of tetrads is the result of linear class polysome loss during incubation.

Thus skin and feather polysome distribution and structure are apparently little affected by removal of the pituitary gland in embryos incubated for 12 days. In contrast, there are clear differences in polysome structure and distribution when these operated and control birds are incubated for 15 days. It should also be noted that the gross appearance of the feathers of the 15-day-old hypophysectomized birds is very similar to that of the 12-day-old control embryos. This parity in gross feather development of 12-day-old control embryos and 15-day-old hypophysectomized embryos is not unexpected, since polysome morphology and distribution of these groups are equally parallel.

How configuration of the square-shaped polysome is related to inability to synthesize protein still remains to be elucidated, but it appears that translation for keratin synthesis requires that the tetrad open (*3*), which in turn, from my study, seems to be dependent on the presence of an intact pituitary gland. The requirement of an intact pituitary gland is inferred from the fact that birds with severely damaged heads but intact pituitary glands develop feathers and polysome patterns just as the unoperated controls do.

My study does not indicate which hormone or hormones may be responsible for control of the structure of the tetrad-shaped polysomes or whether this control is exerted upon the cytoplasm or the nucleus. Nevertheless, it is interesting to note that polysome structure appears to be hormonally regulated.

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Quenchable High-Pressure Polymorph of Zinc Selenate

Abstract. *A new quenchable high-pressure form of zinc selenate ($ZnSeO_4$) was produced by subjecting the low-pressure modification to 40 kilobars at 400°C for 30 minutes. The new form is orthorhombic, space group* D_{2h}^{17}–Cmcm. *The cell constants at 29°C are:* a, *5.511 angstroms;* b, *8.110 angstroms; and* c, *6.585 angstroms. The calculated density is 4.70 grams per cubic centimeter in comparison with 4.61 grams per cubic centimeter for the low-pressure modification. This implies a volume change of 2 percent at the transition.*

Pannetier and Courtine (*1*) have found that anhydrous zinc selenate is orthorhombic and has the space group D_{2h}^{16}–*Pbnm*, with *a* equal to 4.905 Å, *b* equal to 9.012 Å, and *c* equal to 6.793 Å (*2*). This is the same space group as that of the low-pressure modification of $ZnSO_4$ (*3*). However, at higher pressures the low-pressure form of $ZnSO_4$ changes to a high-pressure form which is also orthorhombic, but which has the space group D_{2h}^{17}–*Cmcm* (*4*). Similar polymorphic behavior is encountered in a number of divalent sulfates (*4*) and selenates (*5*), and it was thought desirable to investigate $ZnSeO_4$ and to find out whether this substance could also exist in two different structure types.

Zinc selenate was prepared by heating $ZnSeO_4 \cdot H_2O$ to 250°C for 2 hours. The resulting material was subjected to 40 kb at 400°C for ½ hour in a piston-anvil high-pressure apparatus (*6*); the encapsulating technique (*6*) was used. The sample was cooled while at 40 kb. The x-ray powder diffraction pattern of the product was obtained at 29°C in a Philips high-angle diffractometer, with filtered $CoK\alpha$ radiation ($\lambda = 1.7889$ Å) (Table 1).

Table 1. X-ray powder pattern of high-pressure $ZnSeO_4$ at 29°C ($CoK\alpha$ radiation).

d-spacing		*hkl*	Intensity (*I*)
Obs. (Å)	Calc. (Å)		
4.55	4.558	110	25
4.05	4.055	020	20
3.745	3.748	111	100
3.449	3.453	021	50
2.753	2.755	200	25
2.669	2.669	112	75
2.560	2.556	022	20
2.430	2.427	130	35
2.149	2.154	221	15
2.026	2.028	040	10
1.934	1.930, 1.938	023, 041	10
1.872	1.874	222	15
1.727	1.726, 1.729	042, 311	12

The diffraction pattern was different from that of ordinary $ZnSeO_4$ (*1*). However, it was entirely similar to that of the high-pressure modification of $ZnSO_4$ (*4*). All the observed peaks could be explained on the basis of the following orthorhombic unit-cell dimensions: $a = 5.511 \pm .010$ Å; $b = 8.110 \pm .010$ Å; $c = 6.585 \pm .010$ Å.

The selection rules are consistent with a space group D_{2h}^{17}–*Cmcm*, as in the case of high-pressure $ZnSO_4$.

The calculated density of high-pressure $ZnSeO_4$ at 29°C, if $z = 4$, is 4.70 g/cm³. The low-pressure form has a density of 4.61 g/cm³ (*1*). This implies a volume change at the transition of 2 percent. The corresponding volume change in the case of $ZnSO_4$ is 3.4 percent.

It may be noted that the general rule (*5*) for the transition $D_{2h}^{16} \rightarrow D_{2h}^{17}$, namely, increase of *a*, decrease of *b*, and decrease of *c*, applies to $ZnSeO_4$ also. This is consistent with the structural differences between the two phases (*3, 5, 7*).

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