of normal serum, there was no impairment in phagocytic activity, but, rather, a hyperphagocytic state was manifested. When liver slices derived from normal animals were incubated in the presence of serum obtained from puromycin-treated blockaded rats, there was an 82 percent reduction in the uptake of the emulsion. In contrast, liver slices from the blockaded puromycin-treated group, when incubated in normal serum, demonstrated no inhibition of particle uptake when compared to normal slices, evidence of intact hepatic macrophage activity.

These experiments suggest that the retardation of RES recovery from blockade by puromycin is attributable to an effect of puromycin upon synthesis of opsonic protein in the serum and not upon alterations of the phagocytic cell. Our data, in conjunction with that of Saba and Di Luzio (*1*), demonstrate that reticuloendothelial blockade is due to a depletion of opsonic activity in the serum. Furthermore, blockade recovery is attributable to a formation of opsonic protein, the synthesis of which can be readily impaired by puromycin.

The inability of Fred and Shore (*3*) to demonstrate an effect of puromycin upon clearance rates was probably due to the temporal relations between blockade recovery and opsonic restoration. The ability of liver slices from blockaded and from blockaded puromycin-treated animals to perform in the same manner as liver slices from normal animals demonstrates that blockade of the RES is not due to a cellular saturation (*2*) or to a receptor-site deficiency (*3*), but to a loss of opsonins which act as recognition factors (*4*) in the phagocytic event.

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7. A portion of these studies were conducted at the University of Tennessee Medical Units. Supported by the AEC and PHS grant 05367.

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Estrogen-Dependent Increase in Transfer RNA during Differentiation of the Chick Oviduct

Abstract. *Estrogenic hormones induce morphologic and biochemical differentiation in the oviduct of the immature chick. Concomitant with the hormone-stimulated tissue growth, there was an increase in 4S RNA, as judged by polyacrylamide-gel electrophoresis, and a corresponding increase in cellular transfer RNA activity, as measured by the amino acid acceptor capacity. This system may be suitable for studying the relation of hormones to transfer RNA in a differentiating tissue.*

Certain transfer RNA (tRNA) molecules may be involved in the regulation of protein synthesis and cell differentiation at the level of translation of messenger RNA (mRNA) (*1*). Changes have been reported in the relative amount of specific tRNA's of bacterial cells during sporulation (*2*), in phage-infected bacteria (*3*), in virus-infected animal cells (*4*), in differentiating wheat seedlings (*5*), and in developing chick erythrocytes (*6*), but there has been no report of a system in which a specific chemical stimulus induces synthesis of functional tRNA out of proportion to all other types of stable cellular RNA.

We have described the effects of estrogens such as diethylstilbestrol on morphologic and functional differentiation in the immature chick oviduct. After estrogen stimulation, three distinct types of epithelial cells differentiate from the apparently homogeneous population of primitive cells of the mucosa; and two of these cell types synthesize the cell-specific proteins ovalbumin and avidin which can be used as markers for differentiation (*7*). For these reasons, we have studied RNA patterns during different stages of hormone-induced biochemical and cytological differentiation in higher animals.

Three-day-old female Rhode Island Red chicks were injected daily with 5 mg of diethylstilbestrol subcutaneously for various periods up to 20 days. The animals were killed, and nuclear and cytoplasmic RNA fractions were prepared from the immature and estrogen-stimulated (differentiated) chick oviducts. The patterns were obtained with a recently described, sensitive technique for polyacrylamide-gel electrophoresis (*8*). The gels were stained with methylene blue and scanned with a densitometer (Photovolt). There was a fivefold increase or more in 4*S* RNA in both the nuclear and cytoplasmic RNA preparations of diethylstilbestrol-stimulated oviducts (Fig. 1). There were no qualitative changes in ribosomal RNA (30*S* and 18*S*) or other low molecular weight RNA's, and there was no evidence of degradation. Subsequent administration of progesterone

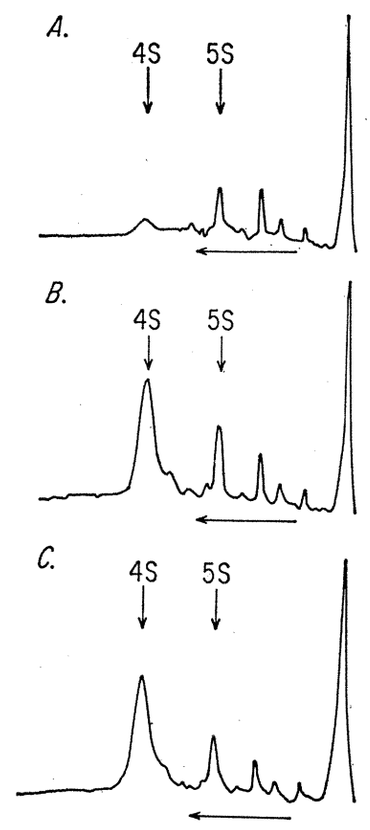

Fig. 1. Electrophoresis patterns of low molecular weight nuclear RNA extracted from oviducts of (A) immature chicks; (B) of chicks that received estrogen; and (C) of chicks that received estrogen and progesterone. The RNA samples were prepared as described (*8*); the direction of electrophoresis on 10 percent polyacrylamide gels (*8*) is designated by the arrow. The gels were stained with methylene blue (*8*) and scanned with a Photovolt densitometer.

did not significantly alter the pattern of nuclear RNA (Fig. 1C).

To determine whether this increased 4*S* RNA was accompanied by an increase in tRNA activity in the cell, the following studies were carried out. Acylating enzyme free of RNA was prepared from oviduct tissue (8 g) of chicks treated with the stilbestrol for 20 days. The tissue was homogenized in four volumes of buffer A (0.25*M* K_2HPO_4, 0.001*M* $MgCl_2$, 0.014*M* β-mercaptoethanol, 10 percent glycerol, 0.25*M* sucrose, *p*H 6.5), and the homogenate was centrifuged at 105,000*g*. The supernatant was applied to a DEAE-cellulose column (20 cm^3 of DEAE per gram of tissue) equilibrated with buffer A. The enzyme eluted in the first major protein peak. The preparation was concentrated (protein content, 15 mg/ml) and dialyzed against buffer B (0.01*M* phosphate, *p*H 7.0, 0.001*M* $MgCl_2$, 0.014*M* β-mercaptoethanol, 10 percent glycerol) for 2 hours; glycerol was added to a concentration of 50 percent, and the enzyme was stored at − 30°C. The incubation mixture consisted of 40 μg of RNA, a mixture of 15 amino acids ($1 \times 10^{-5}M$ each) uniformly labeled with ^{14}C (New England Nuclear Corp.); 0.1*M* potassium cacodylate, *p*H 6.9; 0.2*M* adenosine triphosphate (potassium salt); 0.01*M* $MgCl_2$; and 0.01*M* KCl in a total volume of 0.2 ml.

In the presence of an excess of all other reactants for the two-step amino acid acceptor reaction, available tRNA becomes the limiting compound in the formation of aminoacyl tRNA. At the end of the incubation period, RNA was precipitated with cold 10 percent trichloroacetic acid (TCA) for 1 hour; the precipitate was then collected on a Millipore filter. The filter was then washed three times with cold 5 percent TCA, dried, and counted in a toluene-phosphor counting solution; 30 to 40 μl of enzyme and a 15-minute incubation period were sufficient to bring the reaction to completion. Oviducts were removed at various stages of estrogen treatment and homogenized. The nuclei were isolated (*9*) and suspended in a bentonite buffer (0.01*M* sodium acetate, 0.01*M* EDTA, 0.05 percent bentonite, *p*H 5.1), and sodium dodecyl sulfate was added to 0.1 percent. The mixture was extracted with 90 percent phenol for 5 minutes at 23°C and 2 minutes at 60°C. Sodium chloride was added to the aqueous phase to a concentration of 3 percent, and the nucleic acid was precipitated with two volumes of 95 percent ethanol. The nucleic acid was washed with 95 percent ethanol and again extracted with phenol, precipitated with ethanol, and stored in water at − 30°C. The RNA was isolated from whole tissue homogenates in the same fashion. The RNA samples were then assayed for tRNA activity by the above method. The amount of ^{14}C-amino acids bound by RNA at the completion of the assay was considered to reflect amino acid acceptor activity.

A small increase in tRNA was observed in tissue from chicks that had been treated with diethylstilbestrol for 1 day (Fig. 2), and further increases were noted up to 20 days of treatment. Similar changes were noted when RNA extracted from oviduct nuclei was analyzed (Fig. 3). Oviduct morphological and biochemical differentiation is essentially complete by 18 days of the estrogen administration.

The mechanism and biological significance of this disproportionate increase in the amount of tRNA in the differentiating oviduct cell is not yet clear. The increase may be the result of a stimulation of nuclear transcription of tRNA, decreased tRNA degradation, or gene amplification. Stimulation of tRNA transcription could be the direct result of the estrogen, or it might be secondary to feedback stimulation from the concomitant increase in cytoplasmic protein synthesis; experimental evidence is lacking for the latter explanation. This alteration in the number of tRNA molecules in the developing cell could subsequently affect protein synthesis at the translation level. Synthesis of 4*S* RNA begins at a definite stage of echinoderm and amphibian development (*10*). However, we are not aware of another system in higher animals where a given chemical stimulus (hormone) causes a relative increase in functional tRNA.

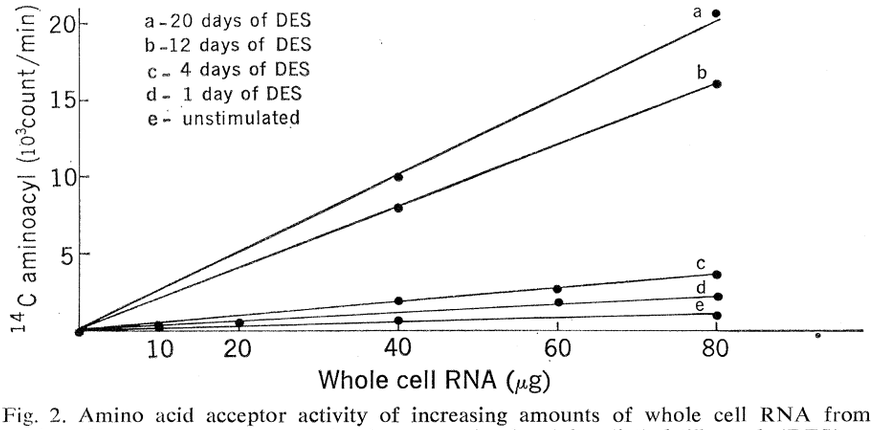

Fig. 2. Amino acid acceptor activity of increasing amounts of whole cell RNA from oviduct from various stages of development stimulated by diethylstilbestrol (DES).

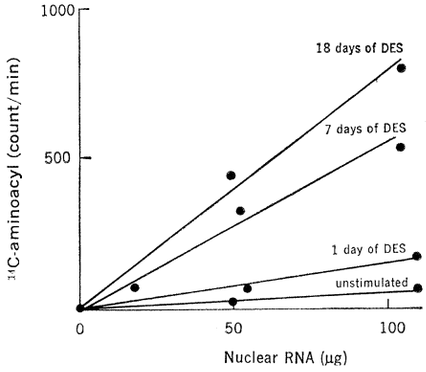

Fig. 3. Amino acid acceptor activity of increasing amounts of oviduct nuclear RNA from various stages of development stimulated by diethylstilbestrol.

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