Table 2. Inhibition of stress effect (measured by "sleep time") on drug response by morphine treatment. Rats (seven in each group) were given 20 mg of morphine per kg of body weight for 4 days; 24 hours after the last morphine injection, hexobarbital (100 mg per kg) was administered intraperitoneally.

Group	Treatment	Hexobarbital sleep time (minutes ± S.E.)*
A	Saline (1 ml per kg)	25.7 ± 1.4
B	Morphine (morphine control)	47.8 ± 1.4
C	Morphine + 2.5 hr hind-leg ligation	45.2 ± 2.2
D	Morphine + ACTH (100 mU)†	31.6 ± 1.8

* Significance: A compared to B or C, $p < .0005$; B compared to C, $p > .15$; B or C compared to D, $p < .0005$. † Milliunits intravenously.

removed. Data showing a direct action of intravenously administered physiological doses of corticosterone indicate that the suggested alteration of drug metabolism or disposition or both is promoted in the stressed rat by corticosteroids.

The implications of these findings are that pituitary-adrenal activity exerts a regulating influence on drug responses and that drug-induced stimulation or depression (morphine) of this system (characteristically at the level of the pituitary or hypothalamus) can be expected to decrease or increase the duration of drug response. Experiments with barbital, SKF 525-A, and morphine suggest that the mechanism of the response is related to stimulation of drug metabolism in the stressed animal (*7*).

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Ribosomal RNA in the Developing Chick Embryo

Abstract. *Developing chick embryos from the stage of primitive-streak formation make ribosomal (28S and 16S) RNA. The size and composition of the RNA appears to be constant throughout the first 7 days of development.*

Recent advances in molecular genetics and biochemistry have outlined the major steps in the transcription of genetic information into specific protein molecules (*1*). The active unit of protein synthesis in many different types of cells is the ribonucleoprotein particle, the ribosome, or as has recently been described, groups of ribosomes called polyribosomes (*2*). The RNA molecule (messenger RNA) which serves the function of information transport is attached to the ribosomes, but is distinguishable from ribosomal RNA on the basis of size and base composition (*1*, *3*).

It can be anticipated that these new ideas and techniques dealing with the control of protein synthesis will be useful to the embryologist, since differentiation is accompanied by changing patterns of protein synthesis in cells all of which presumably contain the same genetic potential.

As a preliminary to the study of messenger RNA in the developing chick embryo, an examination of ribosomal RNA from embryos of different ages was carried out. The size and overall composition of ribosomal RNA is constant during embryogenesis and ribosomal RNA is synthesized from the earliest embryonic stages.

Newly synthesized RNA of embryonic cells was labeled with radioisotope by two different procedures. (i) The embryo was removed from the egg and pressed through a wire screen (80 mesh). The resulting single cells and cell aggregates were collected in warmed (37°C) Eagle's medium (*4*) containing 5 percent calf serum (5 ml/embryo), and were kept in suspension by a rotating magnetic stirring bar. Radioisotope (P^{32} carrier-free orthophosphate or uridine-2-C^{14}) was either in the medium at the time of disaggregation of the embryo, or was added shortly afterward. In experiments with P^{32}, Eagle's medium containing dialyzed serum was used, and the radioactive phosphate was substituted for the usual phosphate. At the conclusion of the labeling period, the cells were centrifuged (5 min at 600*g*), and washed twice in Earle's saline solution. (ii) Radioisotope was placed directly in the upper portion of the yolk sac. The embryo was subsequently removed from the egg and disaggregated and the cells were washed as described above.

The RNA was extracted either immediately after labeling or after storage of the cells at −70°C (*5*). The base ratios of RNA represent distribution of P^{32}-labeled nucleotide in alkaline hydrolysates (*5*). The DNA was isolated as described by Marmur (*6*), and base ratios were determined after acid hydrolysis (*7*).

Sucrose-gradient analysis (*8*) of the RNA from the chick embryo revealed two larger components (28*S* and 16*S*), and a smaller (4*S*) component (see OD_{260} in Fig. 1). This size distribution of RNA has been observed in many different kinds of cells, and the larger components have been shown to be derived from ribosomes. These will, therefore, be referred to as ribosomal RNA. The apparent large amount of 4*S* RNA in Fig. 1 was not always ob-

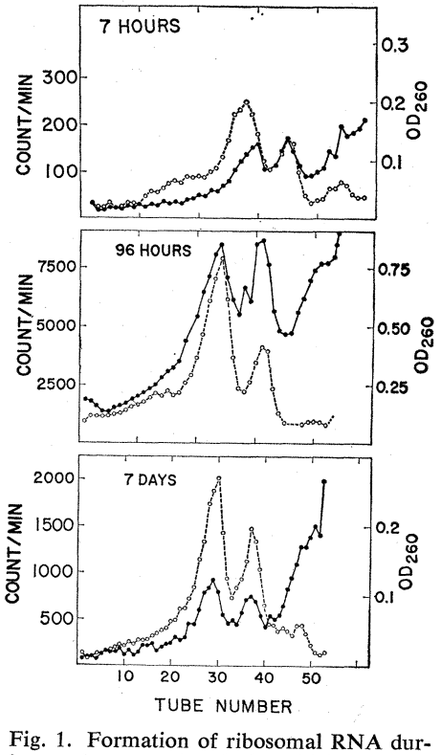

Fig. 1. Formation of ribosomal RNA during embryogenesis of the chick embryo. 200 μc P^{32} were placed into the yolk sac and RNA was extracted (*4*) from several embryos at each age along with extract from two 7-day chick embryos which served as the reference for the OD measurements. The 7-hour sample was derived from about ten embryos, 3-hours old labeled for 4 hours; the other samples were labeled for the 16 hours concluding at 96 hours and 7 days.

Table 1. Base composition of chick embryo RNA and DNA. C, cytosine; A, adenine; G, guanine; U, uracil; and T, thymine.

Mol. size (S)	Age of embryo (days)	Base (% of total base) C	A	G	U(T)	G+C
		RNA				
28	7	28	19	34	18	62
	3	24	18	36	22	60
16	7	25	24	29	22	54
	3	25	24	30	21	55
40	7	25	23	28	25	53
		DNA				
	7	22	28	22	28	44

served, and the ratio of optical densities at 260 to 280 mμ indicated contamination with non-nucleic acid material in this region of the gradient.

Figure 1 shows that relatively long periods of exposure to isotope (P^{32} for 4 to 18 hours) result in incorporation of radioactivity into ribosomal RNA of similar size in embryos of from 4 hours to 7 days of age. This represents a striking difference in embryogenesis between the chick embryo and the frog embryo. Brown and Caston (*9*) have recently shown that only one type of ribosomal RNA is formed during the first 96 hours of development in the frog egg. Presumably one of the factors underlying this difference is that during early differentiation in the chick embryo the cell mass is increasing, while in the frog egg, individual cells are being formed within an already existing mass.

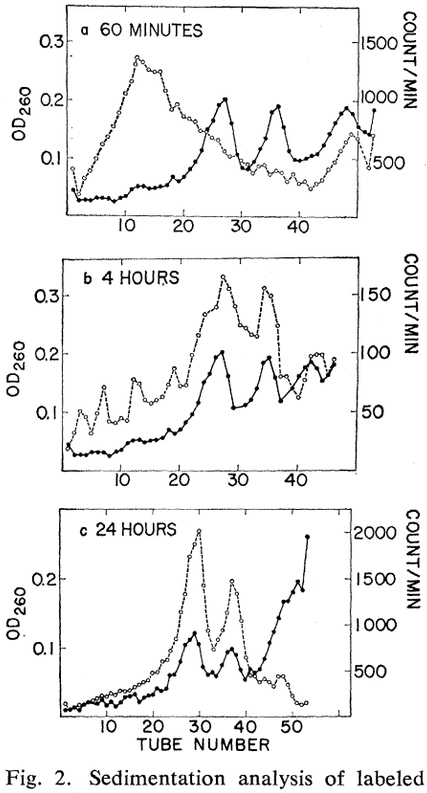

Fig. 2. Sedimentation analysis of labeled RNA from a suspension of 7-day chick-embryo cells. A suspension of cells from three 7-day embryos prepared as described were incubated with 6 μc C^{14}-uridine (33 μc/μmole) in 30 ml Eagle's medium in (*a*) 1 hour, (*b*) 4 hours, and (*c*) 24 hours. RNA was then extracted and analyzed on sucrose gradients (*5*). Solid lines ·—·, optical density; broken lines o---o, count/min.

In addition to similarity in size from day 1 to 7, ribosomal RNA was similar in composition when 3- and 7-day chick material was studied (Table 1). The 28*S* RNA from material obtained at both 3 and 7 days had identical base ratios, as did also the 16*S* RNA. There was a distinct difference, however, between the base composition of 28*S* and 16*S* molecules at both times. This agrees with the recent finding (*10*) that the two ribosomal RNA molecules in *Escherichia coli* are derived from separate loci on the DNA template.

When animal cells in culture are exposed to P^{32} orthophosphate or uridine-2-C^{14}, radioactivity accumulates in a precursor of high molecular weight (35*S* and 45*S*) before it appears in ribosomal RNA. Actinomycin D, which stops further RNA synthesis (*11*), has been used to follow the change of precursors from 45*S* and 35*S* to 28*S* and 16*S* RNA in the absence of further RNA synthesis (*5*).

With labeling periods of 30 to 60 minutes, material of large molecular weight (40*S*) is preferentially labeled also in the chick embryo (Fig. 2). The guanine-cytosine (G-C) content of the 40*S* material (Table 1) was similar to that of 16*S* ribosomal RNA and intermediate between that of 28*S* ribosomal RNA and DNA. Attempts to use actinomycin D to follow the fate of the 40*S* RNA in chick-embryo cells in suspension, as was done with HeLa cells, have been unsuccessful owing to the disintegration of the cells within 4 hours in medium containing actinomycin. Thus, although it is quite likely that some of the 40*S* material is ribosomal precursor RNA, the fraction remains uncertain.

The results of these experiments indicate a similarity of ribosomal RNA throughout embryogenesis. Although subtle changes in ribosomes during development could not be detected by our techniques, it seems reasonable to search for different messenger RNA molecules as the basis for the formation of different proteins which appear as development proceeds (*12*).

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Tremorine: Its Effect on Amines of the Central Nervous System

Abstract. *The administration of tremogenic doses of Tremorine, 1,4-dipyrrolidino-2-butyne, is followed by a significant decrease in the concentration of norepinephrine in the brain stem of three common laboratory species. The change in the concentration proceeds at a rate which coincides with the occurrence of the tremor in each of these species. In the rat, the change in norepinephrine is followed by a progressive increase in the concentration of 5-hydroxytryptamine in the brain stem. Bilateral adrenalectomy in the rat enhances the Tremorine-induced changes in the concentration of norepinephrine and antagonizes the increase in the concentration of 5-hydroxytryptamine.*

When Tremorine is administered to laboratory animals, it causes a condition which simulates many aspects of Parkinsonism, in that it produces tremor, rigidity, and a predominance of parasympathomimetic signs (*1*). It has been shown that this compound affects