MASTER

The Effect of Protein Deprivation on RNA and Protein Breakdown
in Subcellular Fractions of the Cultured Chick Embryo

Eileen D. Hickey and Norman W. Klein

Department of Animal Genetics

University of Connecticut

Storrs, Connecticut 06268

Running title: Protein Deprivation in Cultured Early Embryos

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED
leg

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

ACKNOWLEDGEMENTS

Scientific contribution No. _____ of the Agricultural Experiment Station, University of Connecticut. Contribution No. _____ of the Institute of Cellular Biology. This work was supported by the U.S. Atomic Energy Commission Contract No. AT(11-1)-3139. A preliminary report of this work has been presented (Hickey and Klein¹⁸).

ABSTRACT

During protein deprivation the brain, neural tube and extraembryonic membrane of the early chick embryo rapidly lose label from rRNA and protein. At the same time no decrease in the quantity of these macromolecules can be found in these regions. On the other hand, ribosomal turnover can not be demonstrated in the somites or heart of protein-deprived embryos nor in any region of growing embryos. During 36 h culture on complete growth medium, the brain, neural tube and membrane double their ribosome/cell ratio, while this ratio remains constant in the heart and somite regions. This ratio also remains constant in all regions during 36 h culture on protein-deprivation medium. The rapid ribosomal turnover seen in the sensitive regions of the starved embryo appears to be a mechanism whereby rRNA/DNA is held constant during growth inhibition of a previously rapidly multiplying cell population.

INTRODUCTION

Growth inhibition by starvation has been used frequently as a tool to study the intracellular mechanisms regulating growth in various tissues. HENSHAW ET AL¹ have found that in adult rat liver and muscle, fasting caused reduced protein synthesis whereas brain and testes were not affected. This change in synthesis was regulated by changes in numbers and activity of ribosomes and polyribosomes. MUNRO² and EMONWU ET AL³ have found that, in the livers of fasted rats, extensive RNA degradation occurs before a change in RNA synthesis is noted.

KLEIN ET AL⁴⁻⁵ have been able to consider the effects of protein deprivation in an early embryo in which the main organ systems are just becoming defined. Protein starvation of the early embryo has been shown to severely inhibit growth while permitting morphological changes to continue for at least 36 h. During this time protein starved embryos lost radioactivity from RNA and protein of the brain and neural tube, but not, or only slightly, from the somites and heart. There does not appear to be a corresponding difference in relative rates of protein and RNA synthesis among the tissues.

The present study considers the subcellular sites of the observed losses of radioactivity, as a further step towards an understanding of the balance of synthesis and degradation underlying the sensitivity of the developing nervous system to starvation.

MATERIALS AND METHODS

Culture and labeling

The methods for explantation, labeling, culture and dissection of

embryos, as well as for subsequent analysis of DNA, RNA, and protein content, and incorporation of label into the regions, has been previously described in detail⁴. The basic experimental design was as follows: Embryos were pulsed in liquid medium containing ¹⁴C-labeled uridine or valine. They were then cultured for 6 h to reduce the radioactivity of acid soluble material, and to allow maximum incorporation into RNA or protein. At this time, considered zero time, embryos were either transferred to growth (whole-egg homogenate) or starvation medium (whole-egg covered by dialysis tubing) for 36 h. At zero time and after 36 h of culture, embryos were dissected into head, heart, neural tube, somite, and extraembryonic membrane regions. They were then homogenized for fractionation and analysis.

Fractionation

The regions were homogenized 30 seconds at low speed in a motor driven teflon-glass homogenizer in 1 ml ice cold buffer. The buffer was that described by PERRY AND KELLEY⁶ containing 0.05% triton X-100, with the magnesium concentration raised to 5 mM. Two-tenths ml of homogenate were precipitated immediately in 5% trichloroacetic acid to estimate recovery of label and of RNA or protein following fractionation. Nuclei were pelleted from the remaining homogenate at 650 x g for 15 min and washed once in homogenization buffer. The triton X-100 concentration of the combined supernatants was raised to 0.5% to assure dissolution of membranes and yolk granules. Ribosomes were pelleted at 105,000 x g for 3 h. The nuclear and ribosomal pellets, and post ribosomal supernatant (soluble) fractions were precipitated in ice cold trichloroacetic acid. This fractionation yielded nuclei which were free of cytoplasmic contamination

as seen in the phase microscope and which contained 10-12% of the homogenate RNA. The ribosomal fraction contained 80% and the soluble fraction 8-10% of the homogenate RNA. The RNA or protein content and the incorporation of label were determined for each fraction⁴⁻⁵. Comparison of the sum of the values for the fractions with that of the whole homogenate indicated recoveries of 95-100% for protein, RNA, and radioactivity. The DNA was determined from the nuclear pellet. There was no detectable loss of DNA from the nuclei during fractionation.

To check for contamination of the ribosomal fraction by other RNA species, and to determine the distribution of ¹⁴C-uridine among the species present, the ribosomal pellets were dissolved for 1/2 h at room temperature in EDTA buffer containing 0.5% sodium dodecyl sulfate⁷. They were layered on a 13 ml, 10-30% sucrose density gradient containing the dodecyl-sulfate-EDTA buffer, and centrifuged at 36,000 rpm for 7 h in a Spinco SW-36 rotor. While 0.4 ml fractions were collected, the optical density at 254 mμ was recorded with an LSCO model 222 ultraviolet analyzer. The fractions received 50 ug carrier bovine serum albumin before precipitation in ice cold 5% trichloroacetic acid. They were then collected on Millipore filters for measurement of radioactivity in a Nuclear Chicago gas flow low-background counter.

RESULTS

Embryos explanted on growth medium showed survival, as determined by the presence of a beating heart, of close to 100%, and growth and morphological development which lagged only slightly behind that of an embryo of the same age in ovo⁸. The viability of the protein-starved embryos was 70% or better, but only embryos showing a beating heart were selected

for analysis. These embryos maintained their DNA, RNA, and protein contents at slightly above the zero time level (Tables Ib, IIb). Their morphological development has progressed considerably, despite the reduced growth⁴.

Protein breakdown during starvation

Two separate experiments were run in which embryos were pulsed with ¹⁴C-valine and cultured on protein-starvation medium for 36 h. The results of these experiments were similar enough to allow combination of the data, and statistical analysis (Table I a and b). A differential sensitivity of the regions to starvation was clearly shown in these experiments. The only regions to show significant increases in protein content, the heart and somites, (Table Ib) also showed the smallest losses of radioactivity from protein when the radioactivity of the fractions were summed. The heart maintained its total radioactivity at the zero time level, and the somites lost only 9% of the original protein radioactivity (Table Ia). The brain and neural tube lost 35 and 41% of the zero time radioactivity respectively, and the membrane showed a less severe loss, of 23% of the radioactivity initially present.

At zero time, the distribution of protein radioactivity and protein content among the subcellular fractions was similar, the nuclei containing 20-30%, the ribosomes 30-35%, and the soluble fraction containing 40-50% of both protein and radioactivity of the homogenate. In the sensitive regions, the major losses of radioactivity occurred in the ribosomal and soluble fractions, each fraction accounting for about 40-45% of the total loss. The remaining 10-20% of the loss occurred in the nuclear fraction. The slight loss of radioactivity from the somite region in contrast, was confined to the nuclear fraction (Table Ia).

The results of a representative parallel experiment with growing embryos is shown in Table II. The protein content of the regions increased 4- to 10-fold, except in the membrane, where it only increases slightly. The radioactivity in all fractions was maintained, or increased slightly, with the exception of a consistent loss from all fractions of the membrane region. The slight losses of counts from the nuclear fraction of the brain and neural tube were probably not significant.

RNA breakdown during starvation

The changes in RNA content and radioactivity in the regions of protein-starved embryos were investigated in four separate experiments, which have been combined and analyzed statistically (Table III a and b). Essentially the same picture of regional sensitivity to starvation emerged. The RNA content of all regions ^{of the embryo proper} was maintained, and a significant increase was seen in the heart. A small increase in RNA occurred in the soluble fraction of the brain and neural tube, but did not account for more than 5% of the total RNA of these regions at 36 h. The heart, in contrast, has more than doubled its total RNA. The membrane shows a slight decrease in RNA content. The pattern of loss of total radioactivity (sum of the fractions) from RNA showed the same differential sensitivity among the regions as was seen in the protein. The brain and neural tube lost 32 and 24% respectively, and the membrane lost 50%, of their total RNA radioactivity. The somites have lost only 14%, and the heart gained in radioactivity.

Fractionation showed that at zero time, the RNA radioactivity was distributed among the subcellular components corresponding to their RNA content. Thus, the nuclei contain 10-12%, the ribosomes 75-80%, and the soluble fraction 8-10% of both RNA and RNA radioactivity of a homogenate.

Of the RNA radioactivity lost from the sensitive regions, 60-70% was derived from the ribosomal fraction. The loss from the somites was here, as with protein, restricted to the nuclear fraction.

The parallel experiment with growing embryos presented in Table IV shows no significant loss of radioactivity from any region or fraction, with the exception of the nuclear fraction of the membrane. The RNA content of all regions increased about 4-fold.

Changes of radioactivity of the ribosomal fraction

Although loss of radioactivity from the RNA of the ribosomal fraction accounted for most of the radioactivity lost from the sensitive regions, it was not certain that this represented loss of rRNA. The ribosomal pellet is crude, and may contain contaminating species sufficient to account for the observed loss. Therefore, the distribution and radioactivity of the separate RNA species present in the crude pellet were determined after re-suspension and centrifugation on a sucrose density gradient containing sodium dodecyl sulfate. For regions at zero time and at 36 h, the ratio of the counts/min in the two major rRNA peaks to the total radioactivity of the gradient was determined (Fig. 1). If any species were selectively degraded, this ratio should change, and the direction of the change should indicate whether the loss is from the rRNA or the contaminating species. At zero time, the high molecular weight rRNA peaks accounted for about 70% of the radioactivity of the ribosomal fraction. This ratio, however, did not change either in sensitive or insensitive tissue following starvation. Thus the component comprising the largest proportion of the radioactivity of the fraction, the high molecular weight rRNA, must have accounted for the largest proportion of the radioactivity lost. Thus the major loss of

radioactivity from RNA in sensitive regions was derived from rRNA.

Little contamination by protein was found in the ribosomal fraction. The 260/280 absorption ratio was calculated for the ribosomal fraction after correction of the absorption values at 260 and 280 mμ for light scattering as measured at 320 mμ. Further purification by sedimentation of ribosomes through a sucrose cushion, or in buffer containing 0.5 M KCl did not change this ratio, or alter the quantitative recovery found in the original pellet.

DISCUSSION

During protein starvation, the brain, neural tube, and extraembryonic membrane of the early chick embryo rapidly lose radioactive label from RNA and protein, while the quantity of these macromolecules per region remains constant. Such degradation can neither be demonstrated in the somites or heart of starved embryos, nor in regions of growing embryos, with the possible exception of the membrane. The fractionation studies described in this paper show that the loss of label mainly represents ribosomal degradation, and breakdown of soluble proteins. The dodecyl sulfate gradient separation of the RNA present in the ribosomal fraction indicates that there is little contamination with non-ribosomal RNA, and that no one fraction of RNA is preferentially degraded. Therefore the greatest amount of label lost during starvation has been derived from rRNA. The protein lost from this fraction may also be considered to be ribosomal in origin.

These data are in agreement with results on rat liver systems^{1,2,3}, showing that starvation leads to ribosomal degradation before a significant decrease in RNA synthesis can be demonstrated. Although the nature of the response of early chick tissues to starvation agrees with that seen in adult tissues, the regional specificity of the response is in striking

contrast to that reported for adult and neonatal mammals. Whereas the embryonic brain is extremely sensitive, the adult rat brain has been shown to be the tissue most resistant to starvation damage^{9,10,11}.

The basis for the different response of embryonic and adult neural tissue to protein deprivation is quite possibly related to their different levels of proliferation. The adult brain has completed cell division shortly after birth^{12,13}, and shows a very slow rate of protein turnover. In contrast, the growing embryonic brain, at the time of our experiments, is accumulating DNA at a faster rate than any of the other regions¹⁴. This rapid growth is accompanied by a doubling in the proportion of ribosomes per cell (rRNA/DNA) (Table V). This increase in the rRNA/DNA ratio during growth is characteristic of those tissues (brain, neural tube, and membrane) which have been shown to be most sensitive to protein deprivation. The heart and somites on the other hand, maintain a constant proportion of ribosomes per cell during the growth period. During starvation, the rRNA degradation, measured as loss of counts, and whatever RNA synthesis is occurring, act together to maintain the rRNA/DNA ratio constant at the zero time value in the sensitive tissues. This reflects a constancy of both DNA and rRNA content. An increase in DNA content of the resistant regions, with no change in rRNA content, underlies the slight reduction of the ratio in these tissues.

This pattern of ribosomal turnover upon growth inhibition of a previously rapidly growing tissue finds parallels in cell culture systems, in particular, WEBER¹⁵ has studied the stability of rRNA in exponentially growing and contact-inhibited chick fibroblast cultures. During growth no ribosomal turnover can be demonstrated either with reutilizable or

non-reutilizable label. On the other hand, confluent cultures are characterized by rapid rRNA turnover.

The RNA and protein turnover demonstrated in our chick embryo cultures does not appear, in light and electron microscopical studies^{14,16}, to reflect cell death and cell turnover. It has also not been possible to detect a concomitant change in relative rates of protein or RNA synthesis among the regions during starvation. However, studies are underway which will allow more precise determination of protein synthetic rates in regions of growing and protein-starved embryos. It remains to be seen whether ribosomal synthesis eventually decreases in sensitive regions during starvation, so that the rRNA/DNA ratio is maintained constant without such a high rate of degradation. The present studies indicate that the primary mechanism for maintaining constant cellular ribosomal content, during the change from rapid cell multiplication to a non-growing state, is an increase in ribosomal breakdown.

REFERENCES

1. E.C. Henshaw, C.A. Hirsch, B.E. Morton, and H.H. Hiatt, J. Biol. Chem., 246 (1971) 436.
2. H.N. Munro, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 29 (1970) 1490.
3. C. Ewonwu, R. Stambaugh, and L. Sreebny, J. Nutr., 101 (1971) 337.
4. N.W. Klein, J.D. Yager, Jr., and K. Hagedorn, Dev. Biol., 24 (1971) 178.
5. N.W. Klein, E.D. Hickey, J.D. Yager, Jr., and S.J. Amott, unpublished.
6. R.P. Perry and D.E. Kelley, J. Cellular Physiol., 72 (1968) 235.
7. S.M. Heywood and M. Nwagwu, Biochemistry, 8 (1969) 2829.
8. N.W. Klein, E. McConnell, and D.J. Riquier, Dev. Biol., 10 (1964) 17.
9. H.N. Munro, in H.N. Munro and J.B. Allison, Mammalian Protein Metabolism, Vol. 1, Academic Press, New York, 1964, p. 393.
10. M. Winick and A Noble, J. Nutr., 89 (1966) 300.
11. M. Winick, I. Fish, and P. Rosso, J. Nutr., 95 (1968) 623.
12. A. Lajtha, J. Neurochem. 3 (1959) 258.
13. A Lajtha, S. Furst, A. Gersten, and H. Waelach, J. Neurochem. 1 (1957) 289.
14. N.W. Klein and L.J. Pierro, unpublished.
15. M.J. Weber, Nature (London), 235 (1972) 58.
16. A. Sawyer, unpublished.
17. K.W. Giles and D.A. Myers, Nature (London), 206 (1964) 93.
18. E.D. Hickey and N.W. Klein, Teratology, 4 (1971) 231.

Fig 1 Distribution of RNA and RNA Radioactivity of the Ribosomal Fraction

The ribosomal pellet was dissolved in dodecyl-sulfate-EDTA buffer, layered on a 15-30% sucrose gradient, and centrifuged for 7 h at 36,000 RPM in a SW-36 rotor. (- - -) optical density at 254 mμ. (———) counts/min/0.4 ml.

Fig 1

TABLE Ia

PROTEIN RADIOACTIVITY OF SUBCELLULAR FRACTIONS OF REGIONS OF PROTEIN-STARVED EMBRYOS

The values represent the mean of two experiments, giving determinations of 4 homogenates of 15 brains, somites, or neural tubes each, or of single membranes, and 2 homogenates of 30 hearts each. Counts/min have been corrected for background, self absorption and quenching.

Fraction		Nuclear			Ribosomal			Soluble		
Region	Culture hours	Counts/min region	% O	P**	Counts/min region	% O	P	Counts/min region	% O	P
Brain	0	185 $\pm$ 10*		0.02	480 $\pm$ 33		0.01	546 $\pm$ 25		0.01
	36	147 $\pm$ 7	81		279 $\pm$ 16	58		360 $\pm$ 32	66	
Somites	0	205 $\pm$ 13		0.02	352 $\pm$ 16		NS	416 $\pm$ 34		NS
	36	157 $\pm$ 7	78		322 $\pm$ 28	92		401 $\pm$ 38	96	
Neural tube	0	122 $\pm$ 9		0.01	249 $\pm$ 9		0.01	328 $\pm$ 15		0.01
	36	73 $\pm$ 3	60		142 $\pm$ 15	57		198 $\pm$ 16	60	
Heart	0	56 $\pm$ 8		NS	108 $\pm$ 10		NS	123 $\pm$ 3		0.05
	36	71 $\pm$ 3	127		141 $\pm$ 15	131		200 $\pm$ 27	163	
Membrane	0	1732 $\pm$ 187		0.05	2600 $\pm$ 244		NS	5033 $\pm$ 309		NS
	36	1202 $\pm$ 125	69		1899 $\pm$ 286	73		4241 $\pm$ 588	84	

* Standard error of the mean

** Probability of significance of the difference between the means Student "t" test. NS = P > 0.05, not significant

TABLE 1b

PROTEIN CONTENT OF SUBCELLULAR FRACTIONS OF REGIONS OF PROTEIN-STARVED EMBRYOS

For experimental details cf. TABLE 1a

Fraction		Nuclear		Ribosomal		Soluble	
Region	Culture hours	ug Protein/region	P	ug Protein/region	P	ug Protein/region	P
Brain	0	3.1 ± 0.2	NS	5.3 ± 0.4	NS	6.2 ± 0.6	NS
	36	3.6 ± 0.2		5.7 ± 0.4		6.7 ± 0.5	
Somites	0	3.0 ± 0.3	NS	3.6 ± 0.0	0.01	4.3 ± 0.4	0.01
	36	3.7 ± 0.3		6.4 ± 0.4		7.5 ± 0.6	
Neural tube	0	1.8 ± 0.1	NS	2.4 ± 0.1	NS	3.3 ± 0.1	NS
	36	2.0 ± 0.2		3.6 ± 0.3		4.0 ± 0.4	
Heart	0	0.8 ± 0.1	0.01	1.2 ± 0.2	0.01	1.2 ± 0.2	0.01
	36	1.7 ± 0.0		3.1 ± 0.3		3.8 ± 0.6	
Membrane	0	44.1 ± 8.2	NS	50.3 ± 4.8	NS	87.7 ± 10.0	NS
	36	30.3 ± 3.2		52.6 ± 7.6		93.7 ± 11.9	

TABLE II

PROTEIN AND PROTEIN RADIOACTIVITY OF SUBCELLULAR FRACTIONS OF REGIONS OF GROWING EMBRYOS

Fraction		Nuclear		Ribosomal		Soluble	
Region	Culture hours	Counts/min region	ug Region	Counts/min region	ug Region	Counts/min region	ug Region
Brain	0	214	3.8	430	6.8	500	5.3
	36	191	14.4	515	23.2	689	30.3
Somites	0	187	3.5	271	3.5	329	3.9
	36	192	15.6	355	16.9	483	20.1
Neural tube	0	137	2.3	181	2.6	234	2.9
	36	102	12.1	219	11.3	306	13.8
Heart	0	65	1.2	105	1.5	110	1.1
	36	91	6.3	259	11.9	309	10.9
Membrane	0	1935	44.0	3497	58.2	7054	117.5
	36	1207	77.9	2201	75.3	4582	148.8

TABLE IIIa

RNA RADIOACTIVITY OF SUBCELLULAR FRACTIONS OF REGIONS OF PROTEIN-STARVED EMBRYOS

The values represent the mean of four experiments giving determinations of a total of 8 to 10 homogenates of 30 brains, somites, or neural tubes each or of single membranes, and a total of 4 homogenates of 60 hearts each. Counts/min were corrected for background, self absorption and quenching.

Fraction		Nuclear			Ribosomal			Soluble		
Region	Culture hours	Counts/min region	% 0	P**	Counts/min region	% 0	P	Counts/min region	% 0	P
Brain	0	236 ± 24*		0.01	1232 ± 69		0.01	101 ± 17		NS
	36	126 ± 15	55		867 ± 75	70		105 ± 16	104	
Somites	0	246 ± 22		0.01	994 ± 136		NS	75 ± 9		NS
	36	134 ± 23	60		934 ± 135	94		69 ± 6	92	
Neural tube	0	98 ± 5		0.01	527 ± 36		0.02	41 ± 4		NS
	36	70 ± 5	71		405 ± 30	77		46 ± 5	111	
Heart	0	65 ± 15		NS	360 ± 37		0.01	26 ± 4		0.05
	36	83 ± 6	128		673 ± 52	187		60 ± 13	231	
Membrane	0	4018 ± 461		0.01	14,270 ± 962		0.01	1676 ± 116		NS
	36	1021 ± 126	25		7963 ± 938	56		947 ± 142	58	

*, ** see TABLE I

TABLE IIIb

RNA CONTENT OF SUBCELLULAR FRACTIONS OF REGIONS OF PROTEIN-STARVED EMBRYOS

For experimental details cf. TABLE IIIa

Fraction		Nuclear		Ribosomal		Soluble	
Region	Culture hours	RNA ug/region	P	RNA ug/region	P	RNA ug/region	P
Brain	0	0.28 ± 0.02	NS	1.76 ± 0.12	NS	0.14 ± 0.02	0.05
	36	0.34 ± 0.07		1.91 ± 0.32		0.24 ± 0.04	
Somites	0	0.23 ± 0.02	NS	1.50 ± 0.15	NS	0.13 ± 0.04	NS
	36	0.36 ± 0.06		2.01 ± 0.33		0.19 ± 0.03	
Neural tube	0	0.17 ± 0.02	NS	0.91 ± 0.09	NS	0.08 ± 0.02	0.05
	36	0.26 ± 0.05		1.00 ± 0.13		0.15 ± 0.02	
Heart	0	0.06 ± 0.00	0.01	0.37 ± 0.06	0.01	0.05 ± 0.02	NS
	36	0.18 ± 0.03		0.95 ± 0.13		0.08 ± 0.02	
Membrane	0	4.8 ± 1.1	0.05	16.9 ± 1.3	NS	2.2 ± 0.4	NS
	36	2.2 ± 0.5		13.8 ± 1.6		2.5 ± 0.3	

TABLE IV

RNA AND RNA RADIOACTIVITY OF SUBCELLULAR FRACTIONS OF REGIONS OF GROWING EMBRYOS

Fraction		Nuclear		Ribosomal		Soluble	
Region	Culture hours	Counts/min region	ug Region	Counts/min region	ug Region	Counts/min region	ug Region
Brain	0	230	0.31	1232	2.24	101	0.20
	36	321	1.33	2545	12.34	216	0.97
Somites	0	246	0.25	994	2.44	75	0.20
	36	237	0.87	1866	6.99	153	0.55
Neural tube	0	98	0.19	527	1.33	41	0.08
	36	112	0.56	851	4.21	78	0.30
Heart	0	65	0.26	360	0.46	26	0.03
	36	84	0.29	1139	2.37	75	0.14
Membrane	0	4018	5.2	14,270	16.0	1626	1.6
	36	1536	4.9	17,312	36.2	1646	3.6

TABLE V THE $\bar{r}$ RNA/DNA RATIOS OF REGIONS OF GROWING AND PROTEIN-DEPRIVED CHICK EMBRYOS

DNA was determined by Giles and Myers Modification of the Burton technique⁽¹⁷⁾. Each value represents 4 to 7 determinations. The values for $\bar{r}$ RNA were taken from Tables III and IV.

	Growing		Starved	
	0 Time	36 Hr.	0 Time	36 Hr.
Brain	5.7*	10.4	4.5	4.6
Somites	7.0	7.6	4.3	3.1
Neural Tube	6.3	12.4	4.3	4.0
Heart	9.2	9.5	7.4	5.9
Membrane	6.2	13.4	6.5	5.1

* $\frac{\text{ug } \bar{r}\text{RNA}}{\text{ug DNA}}$