Drugs which selectively inhibit this high-affinity choline uptake may be valuable tools in the study of cholinergic nerve transmission. Moreover, this high-affinity choline uptake system may furnish a heuristic approach to the labeling of cholinergic neurons in the brain and the peripheral nervous system.

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Adenosine 3′,5′-Monophosphate: Regional Differences in Chick Embryos at the Head Process Stage

Abstract. *The concentrations of adenosine 3′,5′-monophosphate and adenosine triphosphate and the activity of phosphodiesterase were determined in different regions of chick embryos at the head process stage. Adenosine 3′,5′-monophosphate, adenosine triphosphate, and phosphodiesterase were estimated to be higher in the mesoderm-forming portions of the hypoblast than in portions that form neural structures from Hensen's node or the epiblast.*

We have examined a few of the practical problems involved in the search for morphogenetic substances in embryonic development. By means of the recently proposed models for developing systems (*1*), such a search probably would require measuring relative differences in concentrations of compounds (morphogens) in the molecular weight range of 300 to 500. Concentration gradients of these morphogens would be set up within a time range of 3 to 6 hours over distances of 50 to 100 cells.

What differences in quantities of a presumed morphogen would constitute a functional gradient, and how can concentrations of morphogens be measured if turnover and metabolism of isotope compounds has taken place in only 3 to 6 hours? A variety of experiments could be designed for each type of presumed morphogen to answer the numerous technical questions involved.

Another approach to the problem of morphogen identification would be to study some of the effects of these substances. If adenosine 3′,5′-monophosphate (cyclic AMP) can be considered a second messenger for morphogenesis, as it is in numerous other biological messenger systems (*2*), then the finding of regional differences in cyclic AMP concentrations in chick embryos may indicate the possible action of a presumed morphogen. Thus, the investigation of regional differences in cyclic AMP and adenosine triphosphate (ATP) concentrations and phosphodiesterase activity provides a reasonable basis from which to begin the more laborious search for specific morphogens.

As a test system we have studied the conversion of [^{14}C]adenine into ATP and cyclic AMP in selected regions of explanted chick embryos. Since the tissues that develop from these regions have been determined (*3, 4*), differences in the concentration of cyclic AMP among these regions may indicate differences in morphogenetic action.

The embryos were removed from White Leghorn eggs (Truslow Farms) incubated 16 to 24 hours. The vitelline membranes to which the embryos remained attached were secured to a glass ring and suspended over a pool of albumen by means of the technique of New (*5*). Two groups of embryos were labeled by placing [8-^{14}C]adenine (0.5 μc in 0.1 ml of physiologic saline solution) on the hypoblast surface; these embryos were then incubated again for 2.5 to 4.5 hours, until they had reached the head process state (*6*), and were washed six times with 1 to 2 ml of saline at room temperature (*4*). Explanted embryos in a third group were again incubated to the head process stage and used to determine phosphodiesterase activity.

Selected portions of each embryo (Fig. 1) were dissected free with a tungsten needle (*4*). These portions consisted of a few thousand cells; many were 4 to 5 cells thick and 50 to 100 cells wide. The fragments were pooled in test tubes cooled with liquid nitrogen to which a known amount of 10 percent trichloroacetic acid was added to precipitate the protein.

The following tissues were represented in the embryonic fragments:

1) *Endoderm.* Almost all of the endoderm in fragment A was destined for the ventral portion of the foregut, for example, liver diverticula and a variable portion of the yolk sac (*3, 4*). Fragments B to H also contained some endoderm.

2) *Lateral plate mesoderm.* The splanchnic mesodern in fragments A to C was destined for heart and lung, and the somatic mesoderm was destined for limb and body wall. Kidney-forming, liver, limb, and area vasculosa mesoderm were found primarily in fragments D and E (*3, 4*).

3) *Paraxial mesoderm.* Fragment F eventually would form head mesoderm, anterior somites, and nephrotome. Fragment G would form more posterior portions of paraxial mesoderm, that is, the posterior somites (*4*).

4) *Hensen's node.* Fragment H consisted of cells that would form paraxial mesoderm, notochord, dorsal gut endoderm, and ventral portions of the neural tube (*4*).

5) *Ectoderm.* Fragment I contained material destined entirely for the neural tube. Fragment J represented material primarily destined for epithelium but might have contained some material destined for the dorsal portion of the neural tube (*4*).

The embryo fragments were thawed and centrifuged at 4°C. The acid supernatant fractions were processed as described for samples of cultured muscle cells (*7*). Each determination represents agreement between two or more samples. The ATP was measured with the firefly luciferin-luciferase assay (*8*) and cyclic AMP was estimated by the protein kinase method of Gilman (*9*). Protein determination was performed by the method of Lowry *et al.* (*10*). The phosphodiesterase activity was determined by the linear agreement of two methods, with the use of boiled samples for zero-time corrections (*11, 12*).

The amounts of ATP per milligram of protein were highest in regions of the embryos that are the most susceptible to inhibitors of mitochondrial oxidation and phosphorylation (*13*), that is, regions destined to contribute to heart, lung, and paraxial mesoderm (fragments B, D, G, and H). The amounts of ATP were lowest in regions least affected by agents such as antimycin A or oligomycin (*13*), that is, in fragments that would form neural tube and epithelium (I and J) (Fig. 1 and Table 1). The specific activity of ATP (counts per minute per picomole) did not vary more than twofold among different regions of the explants. The lower specific activity was found at Hensen's node (fragment H) and the higher specific activity in the anterior hypoblast and ectoderm fragments (A, B, I, and J) (Table 1) where it may reflect increased utilization and turnover in these regions (*14*).

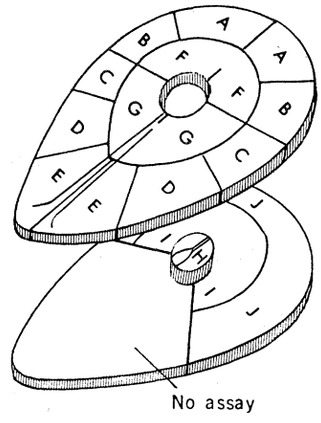

Fig. 1. Cyclic AMP, ATP, and phosphodiesterase assays were obtained for each of ten regions (A to J) of the chick embryo at the head process stage. Fragments A to G were excised from the hypoblast, fragments I and J were from epiblast, and fragment H included all of Hensen's node. The diagram shows an inverted embryo as explanted by the technique of New (*5*) with the hypoblast accessible for initial dissection.

Differences in amounts of cyclic AMP per milligram of protein among the various embryonic fragments were more prominent. Cyclic AMP amounts in areas containing precardiac mesoderm (fragments C and D) were 20 to 30 times higher than those in neuroepithelial ectoderm (fragments I and J). The sensitivity of the Gilman method (*9*) indicates that these differences may be significant. The specific activity of cyclic AMP in areas containing precardiac mesoderm was only 3 times higher than in the ectodermal fragments but was 15 to 20 times higher than in areas containing kidney-forming and liver mesoderm, and 7 times higher than in Hensen's node (Table 1 and Fig. 1) (*14a*).

Phosphodiesterase activity was higher in lateral plate mesoderm (fragments that would form heart, kidney, limb, liver, and so on) than in ectoderm (Table 1 and Fig. 1). The lowest activity was exhibited by the Hensen's node region.

Our study, by showing regional differences of cyclic AMP in chick embryos at the head process stage, suggests that differences in amounts of such compounds also might be detected at other stages of development. For example, the mesoderm cells in fragments B and C had, in the 12 to 18 hours prior to study, existed as typical epiblast cells; had then changed into the bottle-shaped cells found at the primitive streak, with precisely aligned microtubules and microfilaments (*15*); and subsequently had changed into the typical mesoderm cells of the lateral plate.

Since the cyclic nucleotides AMP and guanosine 3′,5′-monophosphate increase activity of protein kinase at concentrations well below 1 nmole/ml (*16*), the kinase activity as well as protein methylase activity (*17*) in different regions of the embryos can be measured to assess the activity of presumed morphogenetic substances (*18*). The meaning of the differences in activities

Table 1. Determinations for cyclic AMP, ATP, and phosphodiesterase in the fragments from explanted chick embryos at the head process stage. The later morphogenetic development of the fragments is indicated. Two determinations (I and II) were made for cyclic AMP and ATP. The asterisk indicates samples for cyclic AMP that were lost. Phosphodiesterase activity is expressed in nanomoles of cyclic AMP hydrolyzed per milligram of protein per minute.

Embryonic fragment	Cyclic AMP				ATP				Phosphodiesterase activity	Later morphogenesis
	Amount (pmole/mg of protein)		Specific activity (count min^{-1} $pmole^{-1}$)		Amount (nmole/mg of protein)		Specific activity (count min^{-1} $pmole^{-1}$)			
	I	II	I	II	I	II	I	II		
A	1.7	3.4	2.5	2.3	12.0	11.0	0.68	0.58	22.0	Ventral foregut
B	10.0	20.0	9.3	2.3	16.6	13.0	.71	.44	22.0	Heart, lung, body wall, limb
C	26.9	24.2	10.3	10.0	7.9	12.0	.40	.57	13.5	
D	16.0	18.2	0.4	3.8	11.1	15.2	.42	.78	9.8	Kidney, liver, limb, area vasculosa
E	5.8	5.8	0.6	0.6	5.1	5.0	.38	.58	16.3	
F	13.9	5.7	1.5	0.4	9.8	12.1	.42	.61	9.8	Head mesoderm, nephrotome, somites
G	6.5	*	1.5	*	14.0	14.3	.42	.62	8.3	
H	7.5	*	1.4	*	17.2	12.6	.35	.58	4.9	Dorsal gut, neural tube, paraxial mesoderm, notochord
I	1.1	*	3.0	*	3.6	7.6	.77	.86	7.4	Neural tube
J	0.8	*	2.4	*	2.4	5.3	.80	.66	10.0	Epithelium, neural tube

of cyclic AMP and ATP in different regions of these very early chick embryos cannot be easily determined. For example, further study is needed to indicate whether the concentration of cyclic AMP from fragments B and C (see Table 1) at the head process stage reflects the concentration of cyclic AMP and morphogenetic activity from an earlier stage, for example, at the primitive streak. The effects of cell movements and trauma of embryonic dissection are but two problems that have to be considered. Our study does represent a novel approach at examining specific embryonic regions of known morphogenesis obtained from eggs of a single flock sampled at a single stage of development.

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7. After centrifugation the supernatant was cleared of acid by four washes with ether. Portions were removed for ATP assays and polyethyleneimine cellulose (PEI) chromatography. The remainder was adsorbed on charcoal and the cyclic AMP was eluted with 2 percent ammoniacal alcohol. After removal of the alcohol the remainder of nucleotides were assayed for cyclic AMP by PEI chromatography and by Gilman's method (*9*). Between 15 to 20 embryos were pooled for each dissected fragment in each experiment and represented from 20 to 80 μg of protein for sampling 0.2 to 4 pmole of cyclic AMP. In experiment 2, cyclic AMP (0.5 pmole) was added to each sample as internal standard. Samples for cyclic AMP, fragments G to J, were lost in this experiment. The protein kinase from rat muscle was used for these assays. The assay was linear between 0.1 to 40 pmole of cyclic AMP. The major portion of the acid supernatant was used for determination of radioactivity by PEI chromatography as detailed by M. Reporter [*Biochem. Biophys. Res. Commun.* **48**, 598 (1972)]. We had obtained a similar distribution of nucleotides in cruder cuts of embryos explanted by the method of Spratt as well as in organ cultures from precardiac head fold and somite regions of the chick blastoderm.
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Proparathyroid Hormone: Biosynthesis by Human Parathyroid Adenomas

Abstract. *Biosynthesis of a precursor (proparathyroid hormone) to human parathyroid hormone was demonstrated during incubation of tissue from parathyroid adenomas. The proparathyroid hormone is labeled more rapidly than parathyroid hormone during incubation with amino acids labeled with carbon-14 and is progressively converted to the hormone. Apparent differences in the relative rate of conversion of precursor to hormone found in different tumors suggest that proparathyroid hormone may accumulate in some of the tumors and be secreted into the circulation.*

It has become evident that many proteins are initially synthesized by the cell as larger precursor molecules that are subsequently cleaved to form the final active protein. This phenomenon is not restricted to any particular functional class of protein since the existence of precursors has been demonstrated or suggested for enzymes (*1*), structural proteins (*2*), and polypeptide hormones (*3*). We have identified a precursor to parathyroid hormone (PTH) in bovine tissue (*4*), and independent evidence in agreement with this has also been obtained by Hamilton *et al.* (*5*). Immunological and clinical studies have indicated that certain adenomas of human parathyroids may secrete a form of parathyroid hormone with a molecular weight higher than that of the hormone extracted from the adenoma tissue (*6*). We now report the identification of a biosynthetic precursor to human PTH that is analogous to the precursor to bovine PTH. Furthermore, a survey of several parathyroid adenomas indicates considerable variation in the rate of conversion of the precursor to PTH among the tumors. This suggests that a variable loss of this cleavage function may occur as a result of transformation from normal to tumor cells.

Parathyroid adenomas were obtained from six patients, and parathyroid tissue showing the histological criteria of "clear cell hyperplasia" (*7*) was obtained from a seventh patient. At the time of surgery, the excised tissue was immediately chilled and portions of minced tissue were incubated at 37°C in medium containing ^{14}C-labeled amino acids. After incubation, acid-urea extracts (*4*) of the tissue were analyzed by electrophoresis on 10 percent polyacrylamide gels containing 8*M* urea at *p*H 4.4 (*8*) or containing 8*M* urea at *p*H 7.2 in 0.1 percent sodium dodecyl sulfate (SDS) (*9*). Slices of the gels were assayed for radioactivity (*4*) and for immunoreactive PTH by means of a competitive-binding radio-