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14. Rubber determinations were made on stem and root (swollen crown portions); dead twigs and branches, remnants of flower penduncles, and leaves were removed from stems. Material was dried rapidly at 65°C, comminuted, and ground in a hammermill. The ground material was extracted sequentially in the Polytron apparatus with water, acetone, and methylene chloride. Acetone extracted the resins, and the methylene chloride extracted the rubber. The solid residue from the methylene chloride extraction was precipitated once with acetone and dried in a vacuum to constant weight. The determination gave highly reproducible results; hence even low values could be compared. Bonner (*11*) had arrived at similar conclusions after using acetone and benzene. The solid residue was identified as *cis*-1,4-polyisoprene by ^{13}C nuclear magnetic resonance spectroscopy [M. W. Duch and D. M. Grant, *Macromolecules* **3**, 165 (1970)].
15. We thank Dr. Louis Erickson for the cuttings from strain 593 and Dr. George Hanson for the greenhouse- and field-grown guayule plants. We also thank Dr. Robert Holm for samples of Sweep 4F.

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Axial Bending in the Early Chick Embryo by a Cyclic Adenosine Monophosphate Source

Abstract. *A microelectrode continuously releasing cyclic adenosine monophosphate can divert the axis of the early chick embryo and attract cells on its ventral surface. Cell movement in the intact embryo may be controlled by a cyclic adenosine monophosphate signal.*

We present the results of experiments in which we stimulated early chick embryos with external sources of cyclic adenosine monophosphate (cyclic AMP). In an earlier paper (*1*) it was shown that a microelectrode releasing cyclic AMP could control aggregation of the cellular slime mold *Dictyostelium discoideum*. The technique was subsequently used to measure parameters of the aggregative signal (*2*) and the rate of differentiation of some of the competences required for aggregation (*3*, *4*). It seemed that such an approach might be useful in the study of embryonic development (*1*) in situations where cell movement is known to be important for morphogenesis and where the movement itself might be controlled by an extracellular signal (*5*). Since preliminary evidence had been obtained for the extracellular involvement of cyclic AMP in anuran (*6*) and chick (*7*) development, we first examined films of early chick morphogenesis to determine whether the wave propagation seen during slime mold aggregation had any counterpart in these much more complex multicellular organisms. We observed periodically propagating waves of refractive index change, sometimes clearly associated with cell movements, in our films and those of others (*8*). A full description of these waves is not germane to the present argument and will be published separately (*9*). In the experiments reported here we found that an electrode releasing cyclic AMP could attract embryonic cells and, when positioned appropriately, could lead to bending of the embryonic axis. The effects observed had a strong concentration dependence.

Fertile White Leghorn eggs were kept at room temperature (21°C) until incubation for 20 hours at 38°C. Embryos at Hamburger-Hamilton stages 4 and 5 (*10*) were explanted on their vitelline membranes, ventral side uppermost, into 55-mm plastic petri dishes containing a 2-mm layer of a 50:50 mixture of thin albumen in Ringer solution (*11*) and 2 percent Difco purified agar in Ringer solution. The vitelline membranes were held down with stainless steel rings and covered with oxygenated Klearol (*12*) mineral oil (Witco Chemical Co., New York) to prevent desiccation. Ten preparations at a time were photographed and placed on a special support with ten electrode holders. A microelectrode was positioned just above the ventral surface of the embryo, at its lateral margin opposite the anterior end of and 1.5 to 2 mm away from the primitive streak. The tip of the electrode was in the Ringer solution,

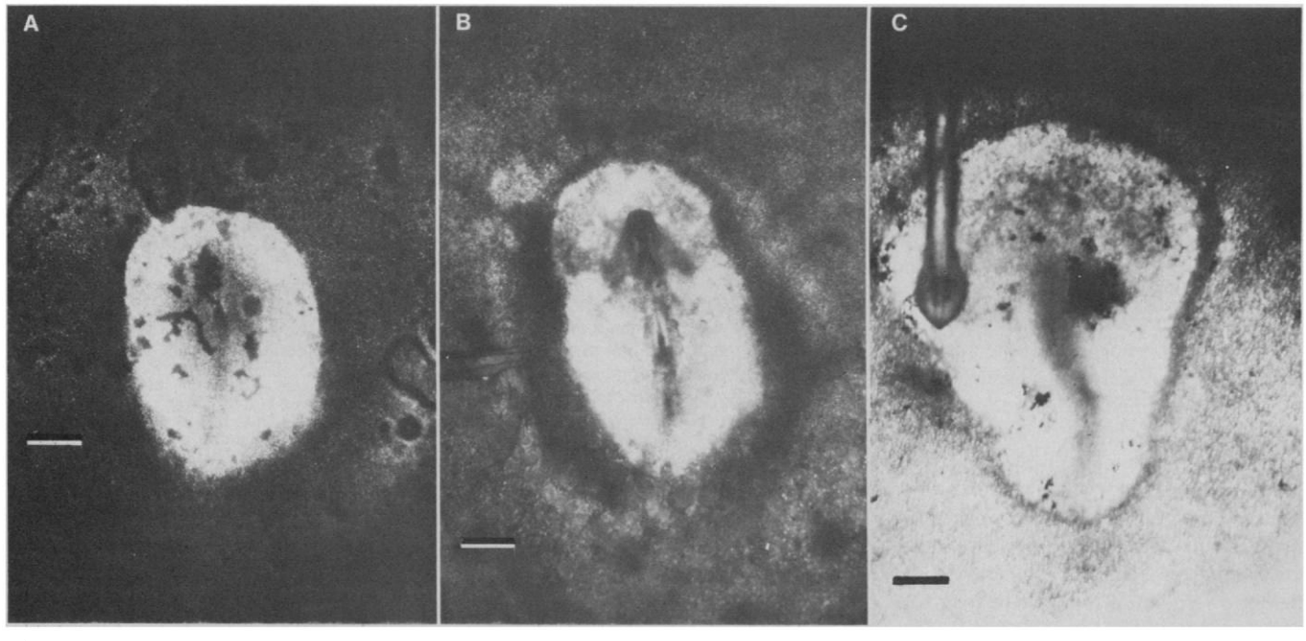

Fig. 1. (A and B) Control embryo at (A) Hamburger-Hamilton stage 5 and (B) stage 8 after a further 18 hours of incubation. The electrode [seen in (B)] was placed at the margin of the embryo opposite Hensen's node. There is no subsequent bending toward the electrode. (C) Stimulation with $10^{-3}M$ cyclic AMP in the electrode was started at stage 3 after 9 hours of incubation, and the embryo is shown 14 hours later. Current was on when the streak bent toward the electrode; the streak progressed anteriorly when the current was off. Scale bars: 1 mm for (A) and (B), 0.5 mm for (C). Dark spots in (C) are iron particles, which aid in tracing tissue movements.

through which electrical contact was made, and was approximately 50 μm above the embryo surface. The embryo was not touched by the electrode. The electrodes were glass micropipettes with tip diameters of 10 to 15 μm, filled with either Ringer solution alone (controls) or Ringer solution plus $10^{-3}M$ cyclic AMP solution (experimentals). The tips were sealed with molten agar to prevent loss of electrolyte by passive flow. A current of 40 na was passed through chlorinated silver wires placed in the electrodes. A return path was provided by a chlorinated wire in the agar supporting each embryo. Current was individually monitored with an electrometer (*13*). After incubation at 38°C for 18 hours, the final state of each preparation was photographed and the embryos were fixed and stained for subsequent histological examination. Thus a pair of "before and after" photographs was obtained for each embryo and scored for effects of stimulation.

The results for 298 embryos are shown in Table 1. The top figure of each pair represents the total sample; the lower figure represents the score obtained when preparations with electrodes which had lost electrical contact overnight, or in which the embryo had broken up or detached from the vitelline membrane sufficiently to prevent scoring, were removed from the sample. Each preparation was scored for bending of the embryonic axis toward the electrode and for attraction of cells to the electrode; both effects were confirmed by histological examination and were thus not destroyed by fixation. Significant differences ($P < .01$) were found between experimentals and controls by the two criteria. The number of rejected embryos was similar for experimentals (70) and for controls (61). Both experimentals and controls showed delays of 1 or 2 stages (up to 5 hours) in development as a result of the experimental procedure, but there was no difference between experimentals and controls in this respect.

In addition, we made time-lapse films which confirm these observations and show that development was continuous, although biased, during the experiments. Figure 1C shows a photograph of an intermediate stage of such a filmed embryo stimulated with a microelectrode containing $10^{-3}M$ cyclic AMP released continuously by a current of 10 na. The electrode was switched off after 5 hours of filming and on again after a delay of 5 hours. The axis progressed anteriorly while the electrode was off but laterally while it was on, leading to the distinctively S-shaped primitive streak. We found that effects of stimulation were lost at electrode concentrations below approximately $10^{-8}M$ for axis bending and $10^{-9}M$ for cell attraction.

Table 1. The results of stimulation of early embryos with Ringer solution (controls) and Ringer solution plus $10^{-3}M$ cyclic AMP (experimentals). The top figure in each category shows the fraction of the total number of controls (150) and experimentals (148) scored as responding. The bottom figure in each category shows the fraction after failures were rejected from the sample, as described in text.

Effect	Fraction responding	
	Controls	Experimentals
Axis bending		
Entire sample	0.05	0.39
Failures deleted	0.08	0.76
Cell attraction		
Entire sample	0.08	0.31
Failures deleted	0.11	0.43

We tried similar experiments with cyclic guanosine, inosine, and uridine monophosphates; adenosine 5′-monophosphate; adenosine; serotonin; and L-glutamine. None showed comparable effects. We also incubated embryos with cyclic AMP phosphodiesterase, which slowed and at very high activities stopped development, and with acetylcholinesterase, which had no effect.

These experiments thus show that extracellular cyclic AMP signals can divert the axis of early chick embryos and can attract cells on the ventral surface. They imply that a systematic search for a cyclic AMP signaling mechanism within the undisturbed embryo might be profitable; we have undertaken this (*7*). To this end it has already been shown (*14*) that there are regional differences in cyclic AMP and phosphodiesterase content in chick embryos at stages 5 and 6. One interpretation of these findings and our own results is that an extracellular cyclic AMP signal may be used in the control of early chick development. If this were so, cyclic AMP might correspond to the heat-stable (animalizing) morphogen postulated by others and its phosphodiesterase to the heat-labile (vegetalizing) antagonist also postulated (*15*). Such an identification would resolve many of the inconsistencies arising from grafts of inducing regions into host embryos, but much more knowledge is needed before this interpretation can become more than a mere speculation.

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Rous Sarcomas in Chickens: Enhanced Growth Coexisting with Concomitant Immunity

Abstract. *Chickens bearing Rous sarcoma virus–induced tumors in one wing did not develop new tumors when subsequently inoculated with Rous sarcoma virus in the other wing. However, the second inoculation of Rous sarcoma virus caused accelerated growth of the established tumors. This phenomenon was found to be bursa-dependent. Paradoxically, established tumors in bursectomized chickens grew at a diminished rate if the chickens were reinoculated with Rous sarcoma virus.*

Animals bearing large progressively growing tumors are resistant to the development of new tumors following challenge with syngeneic or autochthonous tumor cells of the same type (*1*). This resistance to tumor growth has been attributed to an acquired immunity (concomitant immunity) to tumor antigens, and has been postulated to be a determining factor in controlling metastases