one time of the day, to avoid or minimize the effect of rhythms, without knowledge of the circadian time structure, is not acceptable.

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Cyclic Adenosine Monophosphate Stimulation of Axonal Elongation

Abstract. *Elevated concentrations of adenosine 3',5'-monophosphate induce a variety of cell movements. The role of adenosine 3',5'-monophosphate in promoting those movements associated with growth prompted our study of in vitro microtubule-dependent axonal elongation. Ganglia treated with adenosine 5'-monophosphate show no enhancement over controls; treatment with adenosine 3',5'-monophosphate or its dibutyryl derivative significantly enhances elongation, as measured by increases in both axonal numbers and length. Our study suggests that adenosine 3',5'-monophosphate promotes elongation by stimulation of microtubule assembly.*

It has been suggested that many cellular events influenced by adenosine 3',5'-monophosphate (cyclic AMP) depend on intact microtubules; that is, aggregation (*1*), contact inhibition (*2*), growth (*2*), pigment migration (*3*), and secretion (*4*). In order to examine the possible relation between intracellular concentrations of cyclic AMP and microtubule assembly, a system with an easily measurable morphological feature dependent on microtubules is essential. Since cultured dorsal root ganglia undergo colchicine-sensitive axonal elongation that is dependent on microtubules (*5*), they provide an ideal system for this study. Test agents can be incorporated into the media, and the degree of resultant axonal elongation can be measured readily and can be compared to appropriate controls.

Dorsal root ganglia from 8½-day-old chick embryos (White Leghorn) were cultured on collagen-coated cover slips as double cover-slip preparations in classical Maximow culture chambers. The test agents were incorporated into basal medium 199 (Grand Island) and supplemented with 10 percent fetal calf serum (heat inactivated) (Colorado Serum). All cultures were incubated for 48 hours at 37°C, and then each culture was rated with regard to neurite length (measured with a micrometer to nearest 25 μm) and number (per quadrant). Representative cultures were then fixed overnight in Bouin's solution and photographed for permanent records. Various experimental groups were compared to controls with the aid of the *t*-test for comparison of two sample means. An IBM 1130 computer was used to calculate the mean and standard deviation of each group. Graphs were drawn to the Gaussian distribution by the IBM 1130 with the use of an IBM 1627 Calcomp plotter.

Explanted fragments of embryonic chick dorsal root ganglia exhibit an initial period of relative quiescence, usually 4 to 6 hours, followed by glial and connective tissue migration and then a period of axonal elongation. The time course of these events was variable and therefore large numbers of explants for statistical evaluation were used.

Table 1. The effects of test agents on axonal elongation.

Test condition	Axons per quadrant (No.)	Axonal length (μm)
Control	86 ± 50	178 ± 114
5'-AMP	50 ± 32	156 ± 112*
Cyclic AMP	120 ± 68	370 ± 264
Dibutyryl cyclic AMP	165 ± 68	686 ± 350

* Not significantly different from controls. All other values were significantly different when compared to controls, $P < .05$.

The effects of 48 hours of exposure to medium enriched with 5 m*M* concentrations of 5'-AMP, cyclic AMP, and dibutyryl cyclic AMP (all obtained from Schwarz Biological Research) on axonal length are shown in Fig. 1A. Treatment with 5'-AMP has no significant effect on axonal length, whereas treatment with either cyclic AMP or its dibutyryl derivative produces considerably longer axons (Table 1). Axons grown in media enriched with cyclic AMP or dibutyryl cyclic AMP are longer and are more numerous (see Fig. 1B). In contrast, explants exposed to 5'-AMP show a slight reduction in the total number of extended axons (Table 1).

Ganglia cultured in media containing 5'-AMP exhibit only slight differences from controls, whereas those ganglia treated with cyclic AMP or dibutyryl cyclic AMP show large increases in axonal numbers and lengths, two parameters that are a direct result of elongation. It is evident that cyclic AMP and dibutyryl cyclic AMP stimulate axonal elongation. The fact that dibutyryl cyclic AMP produces a higher degree of axonal elongation than cyclic AMP is not surprising, because the former is known to more readily penetrate the plasma membrane (*6*). Dibu-

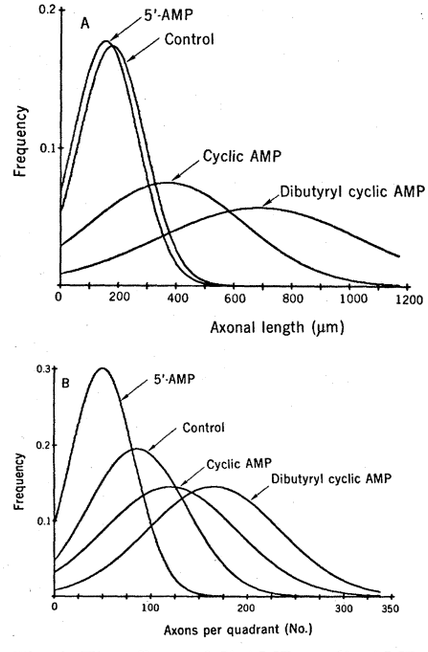

Fig. 1. The effects of 5'-AMP, cyclic AMP, and dibutyryl cyclic AMP on axonal elongation are summarized in these normalized frequency distribution histograms. (A) Axonal length. (B) Axonal number.

tyryl cyclic AMP may also be more effective in promoting elongation because it is less susceptible to phosphodiesterase activity (*6*) and thus may attain a higher intracellular concentration. This possibility is at present under investigation.

Axonal elongation is directly dependent on microtubule formation (*5*). This elongation occurs in the presence of inhibitors of protein synthesis (*7*), which suggests an intracellular subunit pool. We therefore believe that cyclic AMP and its dibutyryl derivative stimulate axonal elongation by initiating tubule assembly from a preexisting pool. Support for this hypothesis comes from the recent work of Gillespie (*8*), who has shown that cyclic AMP stimulates microtubule assembly in several rat tissues.

Another agent known to stimulate axonal elongation in similar cultures is nerve growth factor (NGF) (*9*). Using the techniques described above, we have begun to compare the growth-promoting activities of cyclic AMP with those of NGF. We have some evidence that leads us to the interesting possibility that NGF acts through cyclic AMP stimulation of microtubule assembly as a "second messenger system."

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Prehistory of the Formosan Uplands

Abstract: *Recent archeological surveys in the central mountain ranges of Formosa show human occupation of considerable antiquity. The pattern of ecological adaptation which dichotomizes Southeast Asia characterized prehistoric Formosan settlement.*

The cultural ecology of aboriginal Formosa shows the adaptive dichotomy between uplands and plains that is typical of Southeast Asia. The time depth of this pattern of adaptation was suggested by the results of archeological survey and trial excavations made in the high central mountain range of Nant'ou District (see Fig. 1). Shallow terrace formations along the slopes of the mountains which rise precipitously from the Ch'en Yu Lan River are dotted with surface concentrations of chipped, flat, stone "hoes" and eroded potsherds. In addition, the chipped stone hoes were found thinly scattered over the slopes for miles around. Three small excavations were made in the area of the five hamlets that compose the village of Tungp'u (altitude 1000 m). The density of artifacts suggests that these areas of concentrated debris were centers of occupation, although no indications of houses were discovered. One charcoal sample has been submitted for analysis; it derives from the upper portion of the occupation layer, which is about 95 cm thick. This sample (GX-1538) was dated by carbon-14 at 1165 ± 110 years ago (A.D. 785 ± 110).

The stone artifacts are made of locally derived material, mostly slate. They are all chipped along the lateral edges and some show use-polish on the flat surfaces. Several are notched at the butt end. The hoes are thin in section and average 15 cm in length. No other type of stone artifact was discovered.

The pottery is homogeneous in color and composition; it is all a low-fired brown ware with sparse, fine grit inclusions in the paste. Almost all the pottery has textured surfaces achieved during manufacture by beating the clay with carved wooden paddles. Check-stamping predominates, but some triangular and herringbone patterns occur. Less frequent were designs produced by use of the cord-wrapped paddle (see Fig. 2). Although no reconstructions are possible, rim sherds indicate that the most common shape of vessel was a wide-mouthed jar of medium size.

The function of the stone tools is suggested by the horticultural practices of the contemporary settlers in the region, linguistically identified as Bunun and Tsou. The primary working implement is a short-handled hoe with which the very steep slopes are carved into narrow terraces, protected from soil runoff by stone embankments set into the hillside. Millet and sweet potatoes are grown on these terraces after the natural vegetation has been burned off. The people in the villages today easily identify the stone hoes as tools used by their "ancestors."

The aboriginal groups living in the central mountains have a population density of less than 83 per square mile (32 per square kilometer) (*1*). The settlement pattern appears to vary in relation to altitudinal zones: the Bunun, who inhabit the zone above 1000 m (30 percent live above 1500 m) live in small hamlets of dispersed homesteads, while the Tsou, over half of whom live below 1000 m, have centralized villages with satellite hamlets. The Atayal, distributed in the same zones, have occupied the high river valleys, where small dispersed villages tend to have alliances within each drainage system. The subsistence diet consists of cultivated millet and tubers and some fruits, augmented by birds and small game. Recently abandoned village sites indicate a good deal of small-scale movement, while the annual routine of each family also involves migration through a considerable territory.

Observation of widespread archeological remains in the Tungp'u region suggests the antiquity of this adaptive pattern. This is reinforced by the radiocarbon date which verifies settlement of the uplands well before the Chinese migrated to the west Formosan plain. We can hypothesize that at an early, but as yet unknown, date, a people with a fully established upland style of life had covered the central mountain ranges with a thin blanket of population. They engaged in cultivation of millet and tubers and exploited the diversified natural resources of the environment. The dispersed settlement pattern was a function of their subsistence technology. There is no reason to postulate a prior existence on the plains which would have been charac-