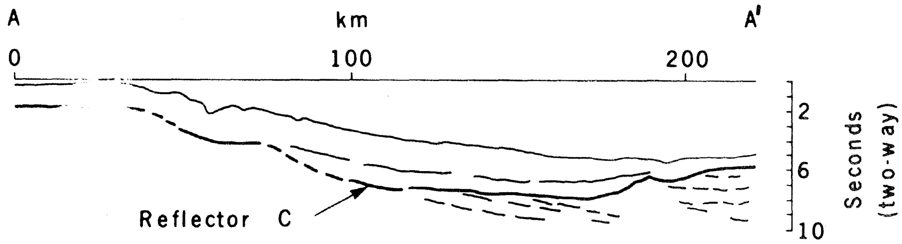

Fig. 5. Line drawing of a multichannel reflection seismic profile extending from the southern Grand Banks to the J-anomaly ridge (see location in Fig. 1).

slabs (*14*) with a poorly preserved microfauna, interpreted by one of us (F.M.G.) as probably Early Cretaceous in age. These carbonates are composed of a mixture of volcanic grains and highly micritized skeletal carbonate grains and are shallow marine in origin. An Albian-Cenomanian coarse, skeletal limestone is present in the Amoco Imp Heron H-73 well (Fig. 1) on the southern Grand Banks margin (*15, 16*).

It would appear that the limestones at site 384 are part of a middle Cretaceous carbonate bank complex which developed along the southern and eastern periphery of the Grand Banks. These carbonate banks were discontinuous and localized in a shallow marine environment in areas of low terrigenous sediment supply near the shelf's margin.

Seismic data provide additional evidence for the relation between the southern Grand Banks margin and the J-anomaly ridge. Figure 5 is a line drawing of a 24-channel seismic reflection profile extending from the southern Grand Banks to the northern flank of the J-anomaly ridge (Fig. 1). A strong reflector, C, can be traced from the Grand Banks, where it has been identified from well control as corresponding to the Early Cretaceous unconformity (*3–5*), to the southern end of the seismic line at a water depth of approximately 3700 m. Sediments overlying reflector C at the southern end of the profile are about 0.6 second thick. Midway along the profile to the north, the sedimentary section over reflector C exceeds 3.0 seconds in thickness. On the southern half of the profile a succession of weak but continuous reflectors expressing apparent southerly dip is visible beneath reflector C. These events are disrupted and show change in attitude beneath the flank of the J-anomaly ridge, but are present to at least 3.0 seconds below reflector C. By extrapolation from the Grand Banks, these events are interpreted as reflections from sedimentary strata, Early Cretaceous or older in age. Assuming that this seismic profile defines the primary nature of the J-anomaly ridge, this feature would appear to be a foundered fragment of the Grand Banks structural block. It is suggested, therefore, that site 384 was drilled to the Early Cretaceous unconformity, where it bottomed probably in a basalt flow rather than in true oceanic basement. This basalt flow might be coeval with mafic extrusions on the Grand Banks and Labrador Shelf (*6, 8*).

The comparable subsidence of the southern Grand Banks margin and the J-anomaly ridge, the presence of coeval shallow marine limestones in both regions, and the presence of seismic reflectors on the flank of the J-anomaly ridge below the strong seismic reflector C lead us to propose as an alternative hypothesis that the J-anomaly ridge was a part of the Grand Banks continental block in (late) Early Cretaceous time. Among the broader implications of this hypothesis is that continental crust extends seaward to the J-anomaly ridge. If the J-anomaly ridge in the eastern Atlantic (*17*) is of similar origin, then less spreading occurred in this segment of the North Atlantic Basin than has generally been proposed (*2*). It would further imply that the southwestern margin of the Grand Banks was probably not the site of a major transform fault during this spreading.

These speculations appear to be in conflict with conventional interpretations of the magnetic anomalies west of the J-anomaly ridge (*2, 18*) and highlight the need for further comparison of the sedimentary and igneous stratigraphy of the southern Grand Banks with that of the adjacent floor of the ocean.

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References and Notes

1. B. Tucholke *et al.*, *Geotimes* **20**, 18 (December 1975).
2. W. C. Pitman III and M. Talwani, *Geol. Soc. Am. Bull.* **83**, 619 (1972).
3. Amoco Canada Petroleum Company Ltd. and Imperial Oil, Ltd., *Bull. Can. Pet. Geol.* **21**, 479 (1973).
4. F. M. Gradstein, G. L. Williams, W. A. M. Jenkins, P. Ascoli, *Can. Soc. Pet. Geol. Mem. 4* (1975), pp. 103–131.
5. L. F. Jansa and J. A. Wade, *Geol. Surv. Can. Pap. 74-30* (1975), pp. 2 and 51–105.
6. Well history reports, Amoco Imp Skelly Mallard M-45 and Amoco Imp Skelly Tern A-68, on file at the Department of Energy, Mines and Resources, Ottawa.
7. J. Bujak, F. M. Gradstein, L. F. Jansa, unpublished data.
8. J. R. H. McWhae and W. F. E. Michel, *Bull. Can. Pet. Geol.* **23**, 361 (1975).
9. R. Jackson, C. E. Keen, M. J. Keen, *Geol. Surv. Can. Pap. 74-51* (1975).
10. W. A. M. Jenkins, P. Ascoli, F. M. Gradstein, L. F. Jansa, G. L. Williams, *Geol. Surv. Can. Pap. 74-61* (1974).
11. F. M. Gradstein and W. A. M. Jenkins, unpublished data.
12. B. K. SenGupta and A. C. Grant, *Science* **173**, 934 (1971).
13. R. W. Schroeder (personal communication) determined *Orbitolina* together with *Choffatella decipiens* (Schlumberger) in the Flemish Cap dredge sample.
14. K. Sullivan and C. Keen, personal communication.
15. J. H. Swift, R. W. Switzer, W. F. Turnbull, *Can. Soc. Pet. Geol. Mem. 4* (1975), pp. 181–194.
16. F. L. Williams and F. M. Gradstein, unpublished data.
17. J. A. Ballard, P. R. Vogt, J. Egloff, *Eos* **57**, 264 (1976).
18. J. M. Auzende, J. L. Olivet, J. Bonnin, *C. R. Acad. Sci. Ser. D* **271**, 1063 (1970).
19. We thank Seiscan Delta Ltd., Calgary, for permission to publish an interpretation of Grand Banks seismic data, and C. Keen, B. Tucholke, B. Loncarevic, and R. Sheridan for criticism of the manuscript.

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Chemical Bioinduction of Rubber in Guayule Plant

Abstract. *The treatment of young guayule plants with 2-(3,4-dichlorophenoxy)-triethylamine stimulated the accumulation of polyisoprenoid rubber in the stem and root tissues. This finding suggests that rubber productivity can be improved by the use of chemical agents on guayule and other rubber-forming plants.*

Considerable effort has been directed toward the development of renewable energy sources to alleviate dependence on imported fossil fuel. Attention has focused on the direct production of hydrocarbons in plants such as *Hevea* (*Hevea brasiliensis*, Müll. Arg.), *Euphorbia tirucalli* L., and guayule (*Parthenium argentatum* A. Gray) (*1*). We present evidence for the bioinduction of polyisoprenoid rubber accumulation in guayule by 2-(3,4-dichlorophenoxy)-triethylamine (*2*) and suggest that the finding could make this Compositae shrub of the North American desert a viable domestic source of hydrocarbons, including natural rubber. The amount of rubber accumulated in the stems and roots of treated guayule was marked.

Earlier we reported the chemical bioinduction of tetraterpenoids in a wide range of carotenogenic tissues (*3*). We outline here further extension of our work on bioregulation of plant constituents.

We used cuttings (*4*) in initial experiments because entire plants were not available in the southern California area. Because the cuttings appeared to accumulate rubber when treated with 2-(3,4-dichlorophenoxy)-triethylamine (*5*), we tested entire seedlings grown in the greenhouse (*6*) (Table 1). Field-grown plants were tested in subsequent experiments (*7*) (Table 2). The data in Tables 1 and 2 show a 2.2- to 6-fold increase in the amount of rubber accumulated in the treated plants. In the initial experiments, cuttings were treated with a formulation that included 2-diethylaminoethanol. This compound alone did not cause rubber to accumulate, but appeared to influence the magnitude of the plant response to 2-(3,4-dichlorophenoxy)-triethylamine.

Curtis (*8*) reported that rubber accumulation was larger in older than in young plants and that commercially important quantities are found only in the barks of the stems and roots. Artschwager (*9*) showed that in plants older than 1 year the vascular rays of phloem and xylem carry the major portion of the rubber. Other parenchymatous tissues, the pith, primary cortex, epithelial cells of the resin canals, and xylem parenchyma, contain lesser amounts of rubber, and leaf parenchyma contains only trace amounts. In plants younger than 1 year old, however, these lesser sources contribute a relatively larger part of the total rubber. All of the plants used in our study were less than 8 months old.

We found the rubber of guayule to be present in parenchymatous cells as a latex, just as in lactiferous plants, in agreement with earlier observations (*10*).

Several strains of guayule were treated and observed for 10 months. The 2-(3,4-dichlorophenoxy)-triethylamine did not appear to harm either the foliage or the growth of the plants at the concentration [5000 parts per million (ppm)] used.

Bonner (*11*) reported that the accumulation of rubber ordinarily is small during spring and summer, the period of vigorous growth, and rapid from early fall throughout the winter. Low night temperatures and water stress favor rubber accumulation (*11*). At the time of treatment with 2-(3,4-dichlorophenoxy)-triethylamine, both the greenhouse- and field-grown plants were vigorously growing and were not subjected to water stress. The larger induction of rubber accumulation in the bare-rooted plant maintained in Hoagland's nutrient solution is due probably to conditions favorable to rubber formation but not to vegetative growth, although the rubber content of the control plant remained constant in the relatively short period after treatment. Conceivably, the magnitude of response in field-grown guayule plants will be greater when treated during periods of water stress or dormant growth than during periods of vigorous vegetative growth. Also, larger yields can be expected from older plants.

Guayule is native to the Chihuahuan Desert of north and central Mexico and the adjacent Big Bend region of Texas. The plant can be grown in arid and semiarid lands of the southwestern United States (*12*). During World War II, guayule was extensively investigated as a domestic source of natural rubber under the Emergency Rubber Project (*13*). The program, centered in the Salinas Valley of California, was terminated when synthetic rubber was developed. Our preliminary study suggests that the use of 2-(3,4-dichlorophenoxy)-triethylamine is a major step toward the feasible, economic production of rubber from guayule plants. Campos-Lopez (*13*) has suggested that the resins may be as valuable as the rubber because they contain important chemical intermediates, such as the lower-molecular-weight terpenes. The effect of the triethylamine on molecular weight distribution and on the composition of resin fraction still remains to be determined.

The response of guayule to 2-(3,4-dichlorophenoxy)-triethylamine should also enable examination of the gene control mechanism involved in the isoprenoid biosynthetic pathways. Further, the compound might also affect the isoprenoid biosynthetic pathways in other hydrocarbon-forming plants, such as *Hevea* (*Hevea brasiliensis* Müll. Arg.), Russian dandelion (*Taraxacum kok saghyz* Rodin) and its allies, and *Euphorbia tirucalli* L.

Table 1. Bioinduction of polyisoprenoid rubber in stem and root tissues of greenhouse-grown guayule seedlings (4 months old) by 2-(3,4-dichlorophenoxy)-triethylamine. The seedlings were treated with 5000 ppm of 2-(3,4-dichlorophenoxy)-triethylamine, 5000 ppm of 2-diethylaminoethanol, and 125 ppm of Ortho X-77 (wetting agent). Controls were treated with emulsion of 5000 ppm of 2-diethylaminoethanol and 125 ppm of Ortho X-77. Shortage of single-strain plants necessarily limited the number of plants used in each test. All the plants were harvested 3 weeks after treatment and were in a state of vigorous vegetative growth; the rubber content was then determined (*14*). Each result represents the mean of four plants.

Strain	Rubber content (mg/g, dry weight)	
	Control	Treated
235	9.5	32.2
245	15.0	41.0
244	13.0	28.0

Table 2. Bioinduction of polyisoprenoid rubber in stem and root tissues of field-grown guayule plants (8 months old) by 2-(3,4-dichlorophenoxy)-triethylamine. The plants were treated with 5000 ppm of 2-(3,4-dichlorophenoxy)-triethylamine, 5000 ppm of 2-diethylaminoethanol, and 500 ppm of Sweep 4F (wetting agent). Controls were treated with 5000 ppm of 2-diethylaminoethanol and 500 ppm of Sweep 4F. All plants were in the stage of good vegetative growth and remained so during the period of study. Three weeks after treatment, entire plants were harvested, and the rubber content was determined. Each result represents the mean for five plants except as noted.

Strain	Rubber content (mg/g, dry weight)	
	Control	Treated
233	8.2	24.0
240	10.0	36.5
240	11*	66*

*Entire plant was harvested and maintained bare-rooted in complete Hoagland's nutrient solution for 1 week. The plant was treated as described above. Each result represents one plant.

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References and Notes

1. M. Calvin, *Photochem. Photobiol.* **23**, 425 (1976); Anonymous, *Chem. Eng. News* **54**, 24 (1976).
2. 2-(3,4-Dichlorophenoxy)-triethylamine synthesized from 2-diethylaminoethylchloride and 3,4-dichlorophenol according to R. D. Scheutz and R. A. Baldwin [*J. Am. Chem. Soc.* **80**, 162 (1958)].
3. C. W. Coggins, G. L. Henning, H. Yokoyama, *Science* **168**, 1589 (1970); W. J. Hsu, H. Yokoyama, C. W. Coggins, *Phytochemistry* **11**, 2985 (1972); S. M. Poling, W. J. Hsu, H. Yokoyama, *ibid.* **12**, 2665 (1973); W. J. Hsu, H. Yokoyama, S. M. Poling, *ibid.* **13**, 415 (1974); E. Hayman and H. Yokoyama, *J. Bacteriol.* **120**, 1339 (1974).
4. Cuttings with attached foliage were obtained from 4-year-old plants (strain 593) at the University of California Botanical Gardens, Riverside, in February 1976, and were maintained in complete Hoagland's nutrient solution with added sodium acetate for 10 days.
5. Initial results indicated a 4.4-fold increase in rubber yield (milligrams per gram, dry weight): control 57, treated 229. The result represents a mean of three cuttings, excluding leaves and remnants of flower peduncles.
6. Selected strains of greenhouse-grown seedlings (10 cm high) were obtained from the Los Angeles County Arboretum in June 1976.
7. A limited program of propagating guayule plants in a small field plot was undertaken by the Los Angeles County Arboretum in January 1976. Selected strains were made available from the field-grown plants in August 1976. Shortage of single-strain plants limited the number of plants used in each experiment.
8. O. F. Curtis, Jr., *Plant Physiol.* **22**, 333 (1947).
9. E. Artschwager, *U.S. Dept. Agric. Tech. Bull. No. 842* (1943).
10. F. E. Lloyd, *Plant Physiol.* **7**, 131 (1932).

11. J. Bonner, *Bot. Gaz.* **105**, 233 (1943).
12. *Arid Lands Resource Information Paper No. 7* (Office of Arid Lands Studies, University of Arizona, Tucson, 1975).
13. *Final Report, First International Conference on the Utilization of Guayule* (Office of Arid Lands Studies, University of Arizona, Tucson, 1976).
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,

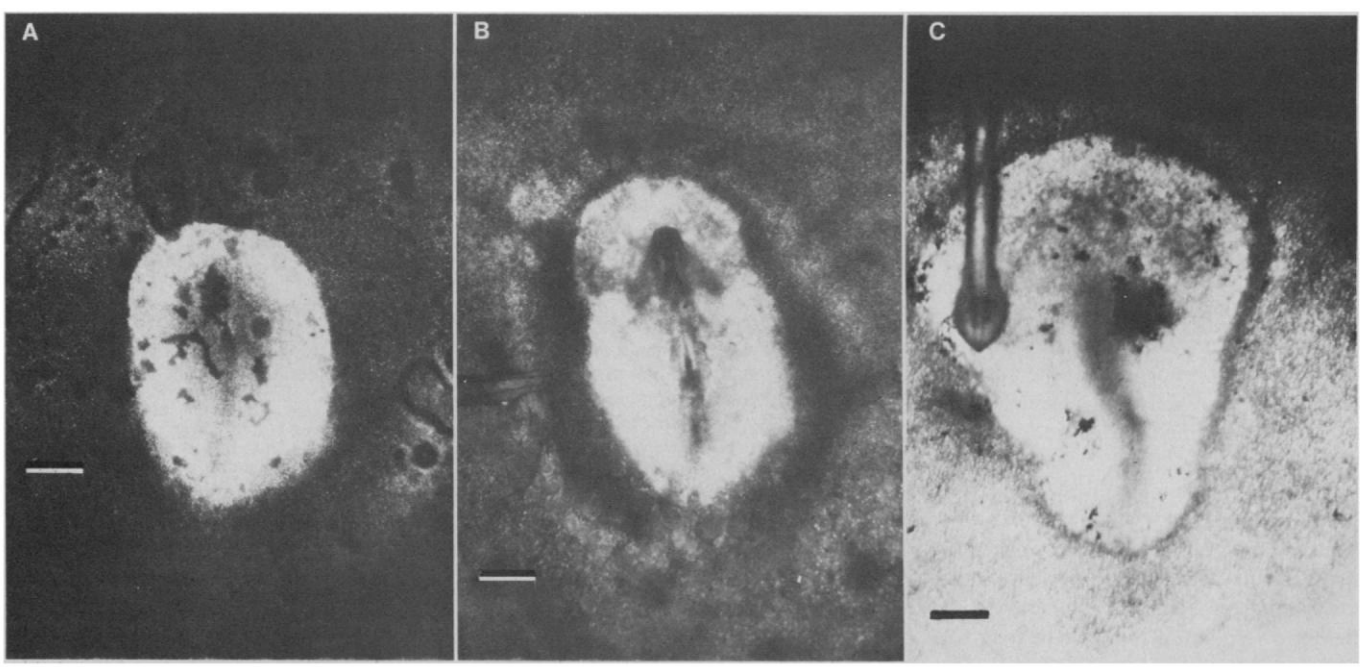

Fig. 1. (A and B) Control embryo at (A) Hamburger-Hamilton stage 5 and (B) stage 8 after a further 18 hours of incubation. The electrode [seen in (B)] was placed at the margin of the embryo opposite Hensen's node. There is no subsequent bending toward the electrode. (C) Stimulation with $10^{-3}M$ cyclic 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.