explanation. The substances restricted the growth of the plants, thus presumably altering their metabolism and creating conditions conducive to flower initiation. Growth suppression should result in accumulation of photosynthate which may favor flower initiation, but the action mechanism is probably more involved since pruning of the plants also caused temporary growth restriction but did not result in flower initiation after growth was resumed. The potential importance of controlling flower initiation is obvious and points the way to further investigations (*6*).

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References and Notes

1. H. A. Borthwick, M. W. Parker, L. Rappleye, *Florists' Rev.* **108**, 29 (1951).
2. H. Skinner, *Proc. Am. Soc. Hort. Sci.* **37**, 1007 (1940).
3. R. J. Downs, unpublished data.
4. S. H. Wittwer and N. E. Tolbert, *Am. J. Botany* **47**, 560 (1960).
5. P. W. Zimmerman and A. E. Hitchcock, *Contribs. Boyce Thompson Inst.* **12**, 491 (1942).
6. Phosfon was supplied by the Virginia-Carolina Chemical Corp., Richmond, Va., and CCC by the American Cyanamid Co., Stamford, Conn.

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Inhibiting Effect of Tobacco Smoke on Some Crystalline Enzymes

Abstract. Tobacco smoke absorbed in phosphate buffer at neutral *p*H inhibits irreversibly the enzymes rabbit muscle glyceraldehyde-3-phosphate dehydrogenase and yeast alcohol dehydrogenase, whereas lactic dehydrogenase and glutamic dehydrogenase are not inhibited. A transient inhibition of beef liver catalase occurs. Indirect evidence suggests that the observed enzyme inhibition is caused by peroxides present in the smoke.

In spite of the current interest in the biological effects of smoking, almost no work seems to have been done on the biochemical effects of tobacco smoke. The present finding that tobacco smoke is capable of inhibiting various enzymes may therefore be of interest.

The main-stream smoke from cigarettes, cigars, or pipe tobacco was sucked through 5 ml of phosphate buffer at *p*H 7.4 in a gas absorption flask, and one volume of the smoke solution was subsequently mixed with an equal volume of the enzyme to be tested. In Fig. 1, the effect of cigarette smoke on the SH-enzyme rabbit muscle glyceraldehyde-3-phosphate dehydrogenase is shown. In these experiments the smoke from one nonfilter cigarette was used. It is apparent that the smoke solution is capable of reducing the activity of this enzyme by approximately 65 percent in the course of 30 min. When a 1000-fold molar excess of cysteine was added, a moderate reactivation (approximately 15 percent) was obtained. Similar results were found with cigars or pipe smoke.

Ordinary cigarette filters (cotton-cellulose) were unable to reduce the enzyme inhibition. On the other hand, when the smoke was inhaled and then blown through the absorbing buffer, the inhibition was strongly decreased. The small inhibition obtained in this case (approximately 15 percent) was completely reversed by the addition of cysteine. Thus, the data suggest that the inhibition is caused by two factors. The factor responsible for the irreversible inhibition is apparently removed or destroyed by inhalation.

Similar experiments were carried out with yeast alcohol dehydrogenase, lactic dehydrogenase, glutamic dehydrogenase, and beef liver catalase. Yeast alcohol dehydrogenase ($1.5 \times 10^{-7}M$) was inhibited by approximately 50 percent in the course of 30 min. Lactic dehydrogenase and glutamic dehydrogenase were unaffected. With catalase ($7 \times 10^{-7}M$) a transient inhibition was observed, and its maximum (about 20 percent) occurred after 30 min incubation with the smoke solution. The inhibition was spontaneously and completely reversed within 1 hr.

Previous experiments in this laboratory have demonstrated (*1*) that, when serum albumin is irradiated with x-rays and subsequently added to unirradiated solutions of the SH-enzymes here studied, both rabbit muscle glyceraldehyde-3-phosphate and yeast alcohol dehydrogenase are slowly and irreversibly inhibited, whereas neither lactic nor glutamic dehydrogenase is affected. The inhibition observed under these circumstances has been shown to be due to radiochemically formed peroxides (*2*). The striking similarity between the latter results and those reported above suggested the possibility that the present results might be explained by the presence of peroxides in smoke. Ingram (*3*) has demonstrated the presence of free radicals in smoke condensate, and conceivably, peroxides may be formed upon the reaction of such free radicals with oxygen. However, attempts to determine peroxides by standard micromethods were unsuccessful. The smoke solution formed precipitates with the reagents of the ferric-thiocyanate method (*4*) and the titan-sulfate method (*5*). With the iodine method (*6*) no peroxides could be demonstrated, even when bottle hydrogen peroxide was added to the smoke solution. The possibility that the added peroxide was dissipated in reactions with constituents of the smoke solution was excluded by the findings that the addition of bottle hydrogen peroxide led to the expected increase in the inhibition of the rabbit muscle dehydrogenase, and that catalase readily abolished this additional inhibition. Presumably iodine formed in the oxidation by peroxides is consumed in reactions with unsaturated hydrocarbons in the smoke solution.

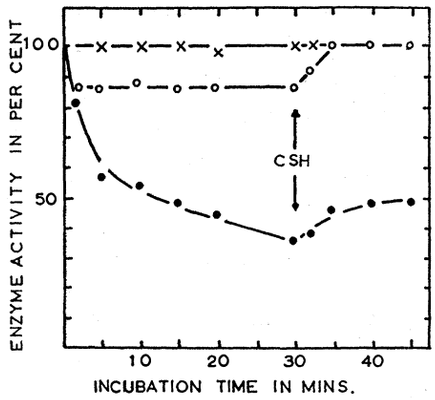

Fig. 1. Inhibition of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase by cigarette smoke. The enzyme ($10^{-6}M$) in $0.067M$ phosphate buffer, *p*H 7.4, at 0°C was mixed with an equal volume of a buffer solution of cigarette smoke. Cysteine (CSH) in a final concentration of $5 \times 10^{-4}M$ was added after 30 min. Solid circles: cigarette smoke absorbed directly in buffer; open circles: cigarette smoke inhaled prior to absorption in buffer; crosses: control (buffer).

Since chemical methods did not provide any direct proof for the existence of peroxides in the smoke solution, some experiments have been performed in order to obtain indirect evidence for the role of peroxides in the enzyme inhibition by tobacco smoke. Thus, when different compounds were added to the absorbing buffer *prior* to the smoking procedure, it was found that thiols in high concentration ($5 \times 10^{-3}M$) abolished completely the inhibition of the rabbit muscle dehydrogenase, whereas ethylene diaminetetraacetic acid had only a moderate effect. Furthermore, when the smoke solu-

tion was incubated for half an hour with catalytic amounts of catalase ($7 \times 10^{-10} M$) before mixing with the enzyme solution, no inhibition of the rabbit muscle dehydrogenase was observed. These results, which are in close agreement with those obtained when the inhibiting effect of irradiated protein solutions was studied, are clearly consistent with the proposed peroxide mechanism. The transient inhibition of catalase is also compatible with this mechanism, as a transient formation of inactive catalase-peroxide complexes is observed when catalase is added to an excess of perodixes (*7*).

It appears that, irrespective of the underlying mechanism, the present finding that tobacco smoke inhibits various enzymes, and that inhalation apparently removes or destroys the inhibitor, may be of biological interest (*8*).

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References and Notes

1. A. Pihl and R. Lange, *Acta Physiol. Scand.* **50**, Suppl. No. 175, 123 (1960).
2. ———, unpublished data.
3. D. J. E. Ingram, *Free Radicals as Studied by Electron Spin Resonance* (Butterworth, London, 1958).
4. A. C. Egerton, A. J. Everett, G. J. Minkoff, S. Rudrakanchana, K. C. Salooja, *Anal. Chim. Acta* **10**, 422 (1954).
5. G. M. Eisenberg, *Ind. Eng. Chem.* **15**, 327 (1943).
6. C. J. Hochanadel, *J. Phys. Chem.* **56**, 587 (1952).
7. B. Chance, *Nature* **161**, 914 (1948).
8. This work has been supported by grants from the Norwegian Cancer Society.

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Response of Condylar Growth Cartilage to Induced Stresses

Abstract. The endochondral growth apparatus of the mandibular condyles, in contrast to epiphyseal plates of long bones, reacts positively to mechanical stimulation. Roentgenographic and histologic analyses of the joint structures of two experimental rhesus monkeys, when compared with untreated controls, showed obvious morphologic and structural transformations of the condylar heads as the result of the instituted treatment. Corollary differences were found in the ontogenetic and histogenetic pattern, as well as in the hormonal control, of these condylar growth centers.

Experiments by Strobino *et al.* on calf tibias (*1*) and the clinical experiences of Blount and Zeier (*2*) with unsuccessfully splinted long bones of children indicated that, contrary to Wolff's law and Roux's principle of functional bone transformation, epiphyseal cartilage plates remain highly unresponsive to mechanical stimuli. Fell (*3*) concluded from findings in tissue cultures that the "gross morphology of bone tissue depends much more upon environmental influences than does that of cartilage." On the other hand, we have presented histologic evidence that in an infant with micrognathia (Robin's syndrome) the condylar cartilage responded to functional treatment with a corrective growth spurt (*4*).

In view of reports of self-corrections of this condition (*5*), an experiment in three rhesus monkeys aged 44 to 50 mo was designed to assess condylar response to induced stresses.

Monkey I served as untreated control, and monkeys II and III as the experimental animals. Inclined bite planes were cemented on the upper and lower dental arches so that the mandible upon occlusion was guided into a forward position. The forces applied thus were physiologic but parafunctional. Monkey II was killed after 2½ mo of treatment; monkey III was autopsied after 4½ mo of the experiment.

Both experimental monkeys show obvious changes in the gross morphologic and roentgenographic appearance of the condylar head (Fig. 1). Histologically the structural arrangement of the condylar head, compared with the normal control (Fig. 2), shows increased cartilage proliferation in a backward and upward direction; endochondral ossification is most active in the posterior portion (Fig. 3). Since modeling resorption at the anterior surface lagged behind proliferation at the posterior surface, the condyles assumed a bilobed shape (Fig. 4). These growth processes result in a bend of the condyles toward the fossa as if they were returning to the position before treatment.

Thus, concrete evidence is obtained which shows that the condylar growth center responds to functional therapy. This unique behavior of condylar cartilage is accompanied by some peculiarities in development, histologic structure, endochondral ossification, and its hormonal control, which differ from the pattern of epiphyseal growth centers:

1) All epiphyseal plates of long bones are derivatives of the primordial cartilage skeleton; the condylar cartilage develops independently from it as a so-called secondary cartilage "grafted" upon the mandibular membrane bone (*6*).

2) Epiphyseal plates grow interstitially; condylar cartilage accrues by surface apposition in a peripheral fibrocartilage layer.

3) In contrast to other cartilage bones, no secondary ossification centers are formed in the condyles. Maturation

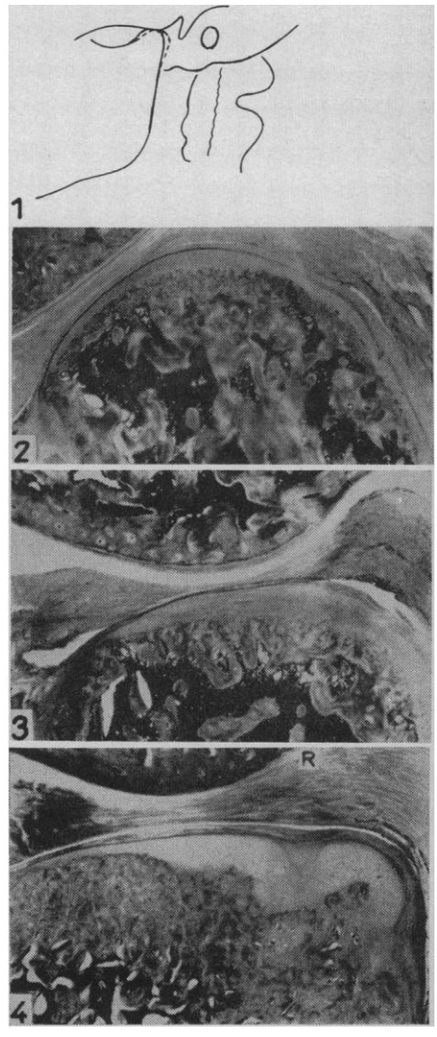

Fig. 1. Superposed tracings of the roentgenocephalograms of the temporomandibular joint structures of experimental monkey III at the beginning of experiment upon insertion of a fixed mandibular protractor appliance, and after 4½ mo of treatment (stippled), reveal that upon treatment the condyle has assumed an oviform shape and is dorsally more prominent with respect to the posterior border of the ramus. The circumferences of the mandibular angle and temporal joint structures are unchanged. Fig. 2. Sagittal section of joint structure of control monkey I. (Alizarin-S vital-staining had unfavourable effect upon histodifferentiation.) Fig. 3. The condyle of experimental monkey II after 2½ mo of treatment shows increased size through cartilage proliferation in a caudal direction. Fig. 4. The condyle of experimental monkey III after 4½ mo of treatment exhibits a prolonged, bilobed shape. *R*, resorbtion.