shivering commenced again as though the inhibitory system had been exhausted.

Inhibition of shivering was not limited to the segment where the stimulation was applied. No matter what region of body surface was stimulated, shivering stopped over the entire musculature of the animal. This can be observed visually or felt by the experimenter when a forelimb is held while the hind limb is stimulated.

The evidence indicates that inhibition of shivering takes place in a region of the nervous system located above the level of the spinal cord: (i) The simultaneous inhibition of the entire musculature suggests that the activity of a central region is being inhibited. (ii) Cutting the dorsal funiculi of the spinal cord at L2 resulted in an inhibition lasting for about 1 minute over the entire body. Thereafter shivering reoccurred. Severing the tracts acted as a stimulus which inhibited the central shivering mechanism. (iii) In several experiments, the dorsal region of the cord was cut at L2 so that shivering still occurred in regions of the body below the level of the section. Upon stimulation of the skin of a hind limb, however, inhibition of shivering did not take place either above or below the level of the section. Our interpretation of this result is that the centripetal passage of inhibitory impulses was blocked by dorsal section of the cord.

L. L. Boyarsky
Lauraine Stewart
Department of Anatomy and Physiology, University of Kentucky, Lexington

References and Notes

1. C. S. Sherrington, *J. Physiol.* 58, 26 (1924).
2. S. Dworkin, *Am. J. Physiol.* 93, 227 (1930).
3. A. C. Burton and O. G. Edholm, *Man in a Cold Environment* (Arnold, London, 1955), p. 154.
4. W. H. Finney, S. Dworkin, G. J. Cassidy, *Am. J. Physiol.* 80, 301 (1927).
5. A. Hemingway, P. Forgrave, L. Birzis, *J. Neurophysiol.* 17, 375 (1954).
6. This work was assisted by a grant from the University of Kentucky Research Fund.

22 January 1957

Mitosis in Adult Cartilage

Nowikoff (*1*) concluded from a study of cell division in amphibian cartilage that mitosis is the method of cell division during development but is entirely superseded by amitosis in the adult. Elliott (*2*) could not demonstrate mitosis in the articular cartilage of the extremities in the adult dog and rabbit; however, he described what he believed to be amitotic figures, which were presented in the form of a few questionable drawings. Clark and Clark (*3*) studied the formation of new cartilage in a transparent chamber that was installed in the ear of a rabbit and found that fully differentiated chondrocytes did not divide when they were observed for several months. Most of the recent editions of American histology textbooks either state or intimate that division of fully differentiated chondrocytes, although quite rare, may occur. However, undoubtedly because of the controversial experimental evidence, nearly all fail to state whether cell division, if it does occur, is mitotic or amitotic.

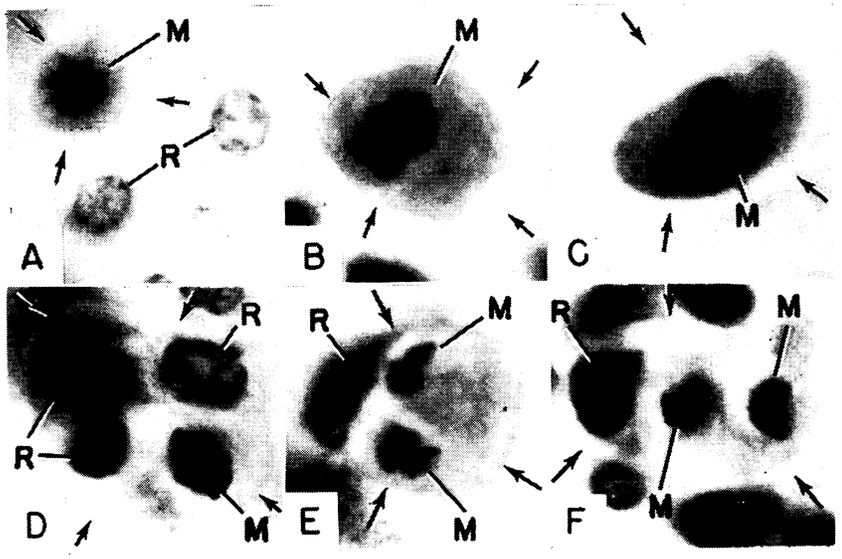

Fig. 1. Chondrocytes of adult symphysial cartilage in mice following injections of estrogen, relaxin, and colchicine. Hyaline cartilage: *A, D,* and *F* (× 1000); fibrocartilage: *B* and *C* (× 1500), *E* (× 1000). *M,* mitotic cell; *R,* "resting" cell; arrows, lacunar wall.

In numerous studies on the pubic symphysis in mice, I have never observed a mitotic or amitotic figure in the interpubic chondrocytes of intact and untreated adult males and virgin females. However, the first changes which occur in the relaxation of the pubic symphysis, induced experimentally or occurring during pregnancy, are an increase in the number of chondrocytes within the individual lacunae of both the hyaline and fibrocartilage and a concomitant swelling of the matrix. Apparently no change occurs in other articular cartilages of the body at this time (*4*). The significance of mitosis in the proliferation of the fully differentiated interpubic chondrocytes has been unclear, because only a few mitotic figures have ever been observed in serially sectioned symphyses (*4, 5*).

In an attempt to elucidate the significance of mitosis, mice were treated as follows: five primiparous mice were sacrificed daily from the 12th day of pregnancy to term (19th to 20th day). Gonadectomized adult males and females received three daily injections of 1 μg of estradiol benzoate in sesame oil, followed by an injection of 100 G.P. units of relaxin (*6*) in benzopurpurine. Two of each sex were sacrificed at 2-hour intervals from 6 to 48 hours following the injection of relaxin. Each pregnant and gonadectomized mouse received an injection of 50 μg of colchicine in saline 6 hours before necropsy to halt mitotic activity occurring during this interval at metaphase. Mitotic figures were increased 10 times in the symphysial cartilage (9 to 10 average per section) when compared with identically treated mice that had not received colchicine (1 to 2 average per section). The mitotic activity was not localized in any particular area; however, as many as three mitotic figures were found in a number of individual lacunae of both the hyaline and fibrocartilage (Fig. 1). These findings cast great doubt on the occurrence of amitosis in cartilage, for nothing was found which I could even vaguely consider to be amitotic divisions.

E. S. Crelin
Department of Anatomy, Yale University School of Medicine, New Haven, Connecticut

References and Notes

1. M. Nowikoff, *Z. wiss Zool.* 90, 205 (1908).
2. H. C. Elliott, *Am. J. Anat.* 58, 127 (1936).
3. E. R. Clark and E. L. Clark, *ibid.* 70, 167 (1942).
4. E. S. Crelin and A. L. Haines, *Endocrinology* 56, 461 (1955).
5. K. Hall, *J. Endocrinol.* 5, 174 (1947).
6. Releasin ® courtesy of R. L. Kroc, Warner-Chilcott Laboratories.
7. This work was aided by U.S. Public Health Service grant No. RG-4433(C).

29 November 1956

Effect of Kinetin on Protein Content and Survival of Detached Xanthium Leaves

When a leaf is detached from a plant, its protein content undergoes a prompt and rapid decline (*1*), the chlorophyll

content decreases in close proportion (*2*), and the life-span of the leaf is markedly reduced. Detached leaves are capable of incorporating labeled nitrogen and carbon into their protein (*3, 4*). Thus, their ability to synthesize protein is not altogether lost. However, they seem to have largely lost the ability to synthesize certain amino acids (*4*), and the ratio between protein synthesis and breakdown is greatly shifted in favor of the latter. Although this characteristic pattern of protein metabolism in detached leaves has led to extensive experimentation, attempts to modify it experimentally have not been successful.

In an attempt to control experimentally the survival and protein balance in detached leaves, we studied the effect of some plant regulators on these processes (*5*). Auxin (indole-3-acetic acid) sometimes reduced protein loss, but the effect was slight and erratic. Kinetin (*6*), in contrast, reduced protein loss in a consistent and striking manner.

The opposite primary leaves of young, vegetative *Xanthium pennsylvanicum* (cocklebur) plants were used in all experiments. Leaves that had reached full expansion or were quite close to reaching it were cut off and inserted with the petioles either into aqueous solutions of kinetin or into water. They were kept in bright, diffuse daylight and in a nearly water-saturated atmosphere (in glass-covered enamel trays) at a temperature of 22° to 25°C. The cuts were renewed every other day; the solutions, every fifth day.

Figure 1 shows the condition of the leaves at the end of an experiment. The leaves that were kept on water lost most of their chlorophyll, but those supplied with kinetin retained their green color.

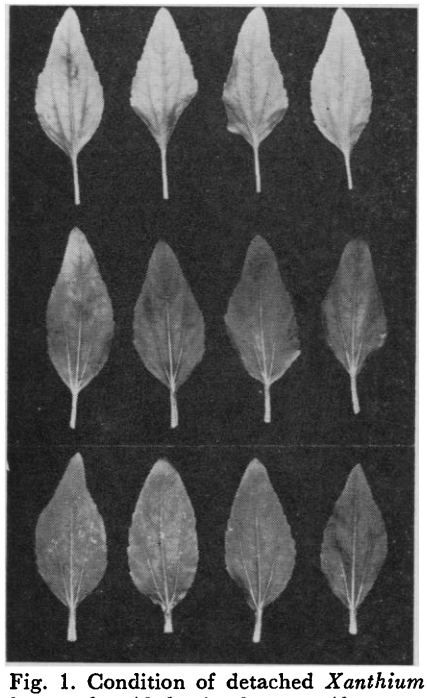

Fig. 1. Condition of detached *Xanthium* leaves after 10 days' culture on (from top to bottom) water, 1 mg of kinetin per liter, and 5 mg of kinetin per liter.

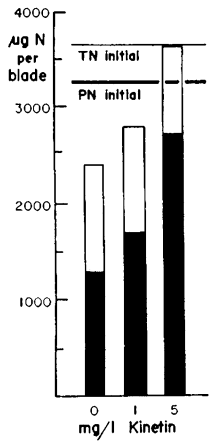

Fig. 2. Protein nitrogen (PN) and total nitrogen (TN) in detached *Xanthium* leaves (blades) after 12 days' culture on water and kinetin solutions. The total columns represent total nitrogen; the solid parts of columns represent protein nitrogen; and the horizontal lines show levels at the start of the experiment.

Figure 2 shows the protein nitrogen and total nitrogen content of the leaf blades after an experimental period of 12 days. The blades of the controls lost 60 percent of their initial protein content. In the blades of leaves that were kept on 5 mg of kinetin per liter, the loss amounted to only 15 percent and was of the same magnitude as it is in attached leaves of comparable age. Leaves kept on 1 mg of kinetin per liter lost 50 percent of their protein. As has been found before (*1*), the protein nitrogen lost by the blades appears as soluble nitrogen in the petioles and the major veins. The amount of soluble nitrogen (measured as difference between total nitrogen and protein nitrogen) in the blades was about the same in controls and treated leaves.

The effect of kinetin on condition and protein content of detached *Xanthium* leaves has so far been somewhat variable. In one experiment, the treated leaves were still fully green after a period of 20 days, while the controls were completely yellow and were dying at the tip and margins. In other experiments, the difference in the survival period was smaller. This variability, which seems to depend on the age of the leaves and on the growing conditions of the plant, should have further investigation. However, there is no doubt that kinetin is capable of reducing or preventing the accelerated protein loss that is typical of detached leaves; at the same time, it delays the loss of chlorophyll and extends the life-span of the leaf. The former effect is very likely the immediate cause of the two latter.

Kinetin was discovered as a regulator of cell division (*6, 7*). However, several authors (*8*) found that kinetin promotes the growth of leaf discs, and this effect was based solely on cell enlargement. In the blade of kinetin-treated, detached *Xanthium* leaves, new cell division cannot be observed either. Cell-division activity thus does not seem to be a premise for the effects of kinetin on the growth of leaf tissue and on its protein metabolism. Whether the last-named effect is an essential feature of the action mechanism of kinetin in growth responses will have to be decided in future work.

AMOS E. RICHMOND*
ANTON LANG

Department of Botany, University of California, Los Angeles

References and Notes

1. A. C. Chibnall, *Protein Metabolism in Plants* (Yale Univ. Press, New Haven, Conn., 1939), chap. 8; J. Bonner, *Plant Biochemistry* (Academic, New York, 1950), chap. 20.
2. G. Michael, *Z. Botan.* 29, 385 (1935).
3. A. C. Chibnall and G. H. Wiltshire, *New Phytologist* 53, 38 (1954).
4. D. W. Racusen and S. Aronoff, *Arch. Biochem. and Biophys.* 51, 38 (1954).
5. This work was in part supported by research grants from the National Institutes of Health, U.S. Public Health Service (RG-3939) and the University of California Cancer Research Coordinating Committee (grant Nr. 407).
6. C. O. Miller *et al.*, *J. Am. Chem. Soc.* 78, 1375 (1956).
7. N. K. Das, K. Patau, F. Skoog, *Physiol. Plantarum* 9, 640 (1956).
8. C. O. Miller, *Plant Physiol.* 31, 318 (1956); S. Kuraishi and F. S. Okumura, *Botan. Mag. (Tokyo)* 69, 300 (1956).
* Present address: P.O.B. 4816 (c/o Dr. Fenichel), Haifa, Israel.

17 January 1957

Temperature-Respiration Curve of Flour Beetles Exposed to Nonoptimal Temperatures

The ability of various species of poikilotherms to adapt to temperatures above or below their normal temperature range is well known (*1*). The adaptation may or may not be accompanied by a change in metabolic regulation (*2*). Bělehrádek (*3*) has shown that temperature coefficients often increase with protoplasmic adaptation to a higher temperature. Respiratory compensation in poikilotherms at subnormal temperatures is evidenced by a higher oxygen consumption, at any given temperature, compared with that of the organism at its normal environmental temperature. In supranormal temperatures, compensatory respiration is depressed (*1*).

The work described here represents a portion of a study made to determine the factors influencing the effects of temperature on adult flour beetles, *Tribolium confusum* Duval (*4*). The insects were taken from a culture kept at 30°C which has been maintained in our laboratory for 10 years. The food medium