The formation of D-fructose as a function of incubation time and initial D-glucose concentration is illustrated by Fig. 1. The data indicate that the affinity of the enzyme for D-glucose ($K_m = 0.5M$ at *p*H 8.0 and 40°C) is much lower than that reported for D-xylose ($K_m = 3 \times 10^{-3}M$ at *p*H 7.5 and 30°C, *3*). The *p*H and temperature optima determined at 0.2*M* D-glucose concentration are about 8.5 and 42° to 43°C, respectively. The conversion can readily be demonstrated in the presence of a variety of buffer systems; however, the addition of arsenate or fluoride, which presumably block competing reactions, leads to an increased accumulation of D-fructose. Present evidence suggests a requirement for either magnesium or manganese ions, as is the case with xylose isomerization (*1, 3*).

The formation of D-fructose in the system was confirmed by isolation and characterization of the product. In a typical experiment, 90 g of D-glucose was dissolved in 500 ml (final volume) of 0.03*M* arsenate buffer (*p*H 8.0) containing 2.5 mmole of $MgCl_2$ and 5.0 g of lyophilized, xylose-grown *Pseudomonas hydrophila* cells. After incubation in a closed flask for 48 hours at 40°C, the mixture was analyzed as described in the legend for Fig. 1 and found to contain 29.2 g of D-fructose. The mixture was then deproteinized with 100 ml of 0.5*M* $HClO_4$ and centrifuged. The supernatant was deionized by passage over columns of Nalcite HCR and of Duolite A-3 resins; the effluent (*p*H 5.0) was concentrated in a vacuum to approximately 30 percent dry substance. D-Fructose was isolated from the resulting syrup as the insoluble calcium complex. Calcium was removed as the oxalate, and the D-fructose was crystallized from aqueous ethanol. The product, obtained in 18-percent yield based on initial D-glucose, had the following properties: $[\alpha]_D^{20} = -91.8°$ (lit. $= -92.0°$); mp = 101 to 103°C (lit. = 102 to 104°C).

Although the role of xylose isomerase in the dissimilation of xylose has been recognized (*7*), present evidence warrants only speculation on the metabolic significance of the isomerization of other sugars by this enzyme. Further investigations are in progress on the levels of D-glucose isomerizing activity in other species of microorganisms, and on the substrate specificity of the enzyme (*8*).

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9. Increased precision in the analysis for fructose by the cysteine-carbazole test has been obtained by heating the reaction mixture for exactly 10 minutes at 60°C instead of permitting the color to develop at room temperature.

14 January 1957

Neurogenic Inhibition of Shivering

The shivering that follows a fall in environmental temperature is known to originate in a region of the central nervous system above the spinal cord. Sherrington (*1*) showed that, in the dog with transected spinal cord, shivering occurred in those parts of the body that were above the level of the lesion and did not take place below the level of the transection. Dworkin (*2*) found that transection of the brain stem of the rabbit at the level of the calamus scriptorius decreased the intensity and changed the character of shivering markedly.

Shivering has been inhibited by raising skin temperature, by anoxia (*3*), by insulin (*4*), and by stimulating the hypothalamus (*5*). We have recently found that shivering elicited by administering Nembutal and lowering the skin temperature may be inhibited by stimulating nerves from skin or muscle (*6*).

Dogs and cats were used. The animals were anesthetized with Nembutal (25 mg/kg) intraperitoneally. To register shivering movements, a hind limb was attached by a rubber band to a phonograph crystal pickup, and the output of the crystal was led to an ink-writing oscillograph. In most cases it was necessary to initiate shivering by placing ice around the trunk of the animal. With the elastic system used, frequency of shivering was between 7 and 12 per second. The inhibitory stimulus was a 1-msec pulse from a thyratron oscillator whose output voltage and frequency could be varied.

A typical, consecutive series of shivering responses is shown in Fig. 1, as obtained from one animal. The effect of a 60-cy/sec stimulus applied to the skin of the contralateral limb of a cat is shown in Fig. 1*a*. Inhibition began immediately but did not continue after termination of the stimulus. In all properties tested, this inhibition resembled that studied by Sherrington in the decerebrate and spinal dog. The inhibition could be graded. Decreasing the intensity of the stimulus resulted in diminished inhibition (Fig. 1*b*). The degree of inhibition also depended on the frequency of stimulation. At 40 cy/sec (Fig. 1*c*), the inhibition was less than that obtained at 70 cy/sec (Fig. 1*a*). However, inhibition was obtained with strong single shocks when an exposed nerve was stimulated.

In several experiments, rebound occurred when the stimulus was terminated. Figure 1*d* shows the increased shivering that followed cessation of the stimulus. In most experiments, rebound, when present, was small.

Fatigue of the inhibitory system was also observed. When the inhibitory stimulus was prolonged for 10 to 20 seconds,

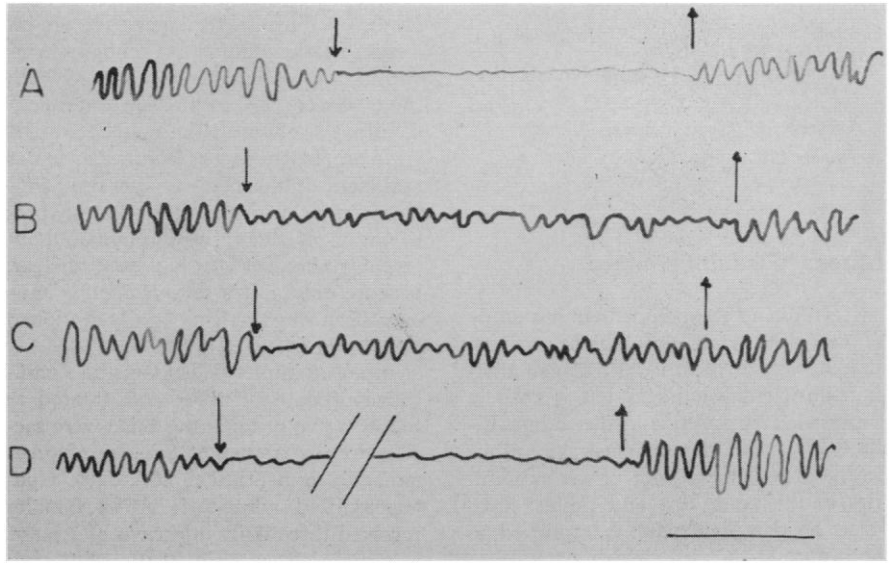

Fig. 1. Inhibition of shivering. Downward arrow marks onset and upward arrow marks termination of stimulus. Time (horizontal line) 1 second. Stimuli: (a) 70 v, 60 cy/sec; (b) 40 v, 60 cy/sec; (c) 70 v, 40 cy/sec; (d) 70 v, 60 cy/sec. Break indicates period of 3 seconds.

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.

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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.

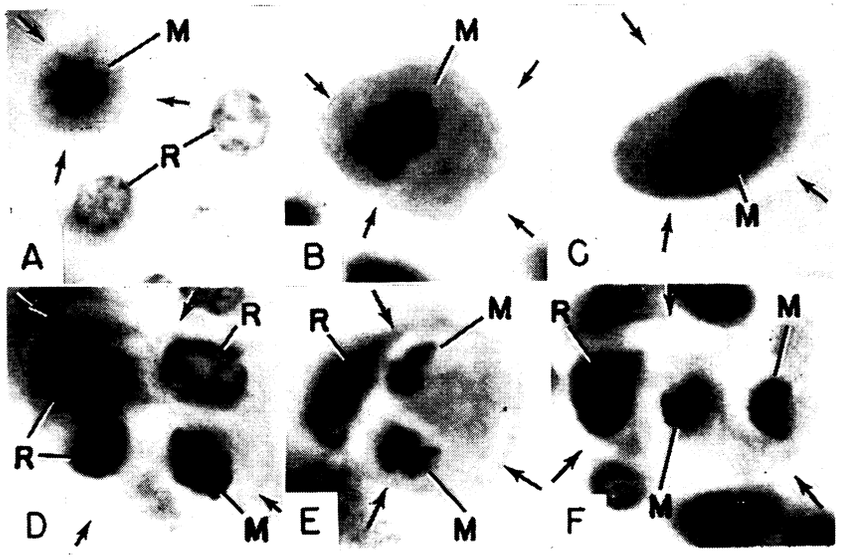

Fig. 1. Chondrocytes of adult symphysial cartilage in mice following injections of estrogen, relaxin, and colchicine. Hyaline cartilage: *A, D,* and *F* (× 1000); fibrocartilage: *B* and *C* (× 1500), *E* (× 1000). *M,* mitotic cell; *R,* "resting" cell; arrows, lacunar wall.

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.

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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