tions found in the virus dialyzed for 7 days against EDTA. Assuming a molecular weight for the virus of 50×10^6, calculation of the number of atoms of iron, copper, calcium, and magnesium per virus particle gave values of 20, 3, 52, and 45, respectively.

The fact that iron and magnesium were concentrated approximately 20-fold in the virus ribonucleic acid compared with the occurrence of about 5 percent nucleic acid in the virus indicates that these metals are located in the nucleic acid rather than in the protein. That their occurrence in the virus nucleic acid in the concentrations found may be of more general significance in relation to nucleic acid chemistry is indicated by the comparable amounts found in yeast ribonucleic acid. The relatively strong binding of the virus ribonucleic acid for iron and magnesium as shown by the dialysis experiments against EDTA (*5*) indicates the existence of metal chelates or relatively stable metal complexes in ribonucleic acid structure. While the function of the individual metals in the virus or in its nucleic acid is not clear at present, some of them may be involved in the binding of smaller nucleic acid subunits into the larger asymmetric particles characteristic of "native" and infectious nucleic acid (*14*). Since the tobacco mosaic virus undergoes a disintegration into relatively small units soon after inoculation (*15*), it appears that the larger nucleic acid may undergo a subdivision into smaller genetic subunits during the infection process in a manner similar to that shown for bacteriophage by Doermann (*16*) and by Visconti and Delbrück (*17*). A further logical hypothesis is that the genetic subunits, the "vegetative phase" of Visconti and Delbrück, are joined into the larger asymmetric particles, the "genetic recombinants" characteristic of "native" and infectious ribonucleic acid by metal chelate bonds. Although no analyses for the metallic components considered in this paper have yet been made on "native" deoxyribonucleic acid, it is known that magnesium is present (*11*). The occurrence of smaller deoxyribonucletic acid units joined by metal chelate bonds into the highly asymmetric particles characteristic of "native" deoxyribonucleic acid would appear to provide a rational explanation for many of the physical (*18*) and biological (*19*) properties of this nucleic acid also (*20*).

HUBERT S. LORING
RICHARD S. WARITZ*
Department of Chemistry, and
School of Medicine, Stanford University, Stanford, California

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* Present address: Grasselli Chemicals Dept., Experimental Station, E. I. du Pont de Nemours and Co., Inc., Wilmington, Del.

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Enzymatic Conversion of D-Glucose to D-Fructose

Xylose isomerase, which catalyzes the interconversion of D-xylose and D-xylulose, has been demonstrated in extracts of xylose-grown cells of *Pseudomonas hydrophila* (*1*), *Lactobacillus pentosus* (*2*), and *Pasteurella pestis* (*3*). Although it has been reported that other aldose-ketose isomerases (*4, 5*) isomerize series of structurally related aldoses, xylose isomerase has been described as unable to act on aldoses other than D-xylose. However, experiments in these laboratories have shown that sonic extracts and washed, lyophilized cells of xylose-grown *Pseudomonas hydrophila* (N.R.C. 491 and 492) (*1*) do in fact convert D-glucose to D-fructose. Similarly, 6-deoxy-D-glucose was converted to a sugar that readily reacted in the cysteine-carbazole test (*6*) and exhibited an R_f in paper chromatography substantially greater than that of 6-deoxy-D-glucose. It seems reasonable to assume that the sugar formed is 6-deoxy-D-fructose (*5*).

The ability of the enzyme preparations to isomerize D-xylose or D-glucose is a concomitant of growth in the presence of D-xylose as the major carbon source. Growth on D-glucose, D-fructose, or maltose gives rise to cells which are essentially devoid of isomerase activity for either D-xylose or D-glucose.

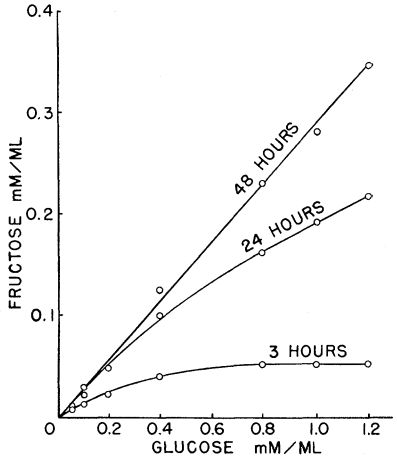

Fig. 1. Formation of D-fructose as a function of incubation time and initial D-glucose concentration. The final concentrations of the components of the incubation mixtures were as follows: arsenate buffer (*p*H 8.0), 0.05*M*; $MgCl_2$, 0.01*M*; washed lyophilized cells of *Pseudomonas hydrophila* (N.R.C. 492), 10 mg/ml; and D-glucose as indicated. Final volume was 2.0 ml, and the incubation temperature was 40°C. The reaction was stopped by withdrawing 0.25-ml aliquots into 4.75 ml of 0.5*M* $HClO_4$. After centrifugation and suitable dilution, fructose was estimated by a modification (*9*) of the cysteine-carbazole test. All values were corrected for the color contributed by D-glucose. The color contributed by the enzyme preparation was negligible.

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*).

RICHARD O. MARSHALL
EARL R. KOOI

George M. Moffett
Research Laboratories,
Corn Products Refining Company,
Argo, Illinois

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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.

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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,

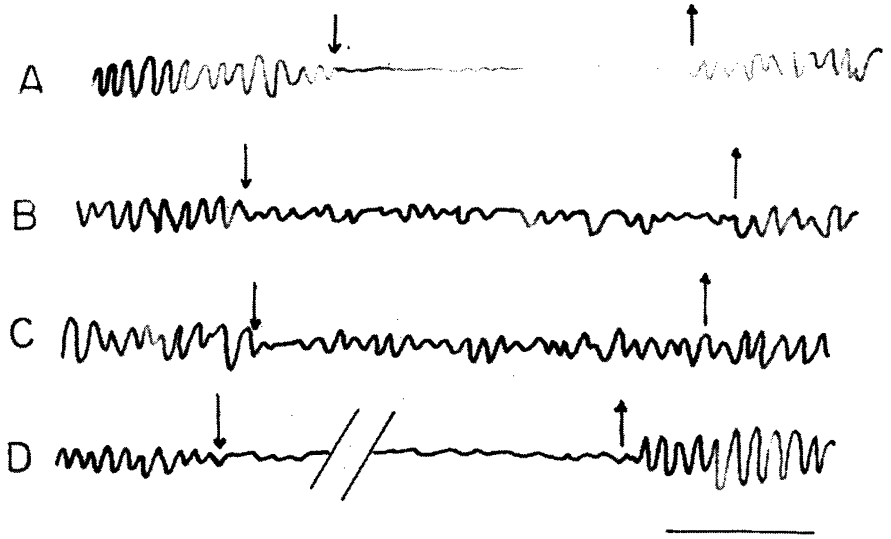

Fig. 1. Inhibition of shivering. Downward arrow marks onset and upward arrow marks termination of stimulus. Time (horizontal line) 1 second. Stimuli: (a) 70 v, 60 cy/sec; (b) 40 v, 60 cy/sec; (c) 70 v, 40 cy/sec; (d) 70 v, 60 cy/sec. Break indicates period of 3 seconds.