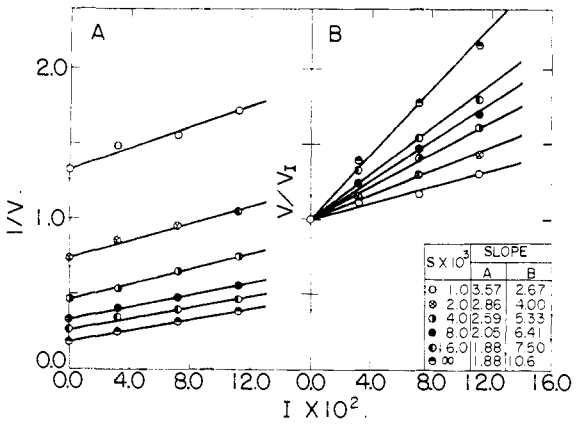

Fig. 6.—The influence of substrate concentration on Dixon (A) and Wilson (B) plots. The graphs represent different plots of the data appearing in Fig. 4 (diabetic). The values at infinite substrate concentration (S) were obtained from the ordinate intercept of Fig. 4. Similar plots may be obtained with microsomes from normal animals.

using v *vs.* v/s, they might be considered non-competitive. However, the plot of $1/v$ *vs.* $1/s$ according to Lineweaver and Burk[32] in Fig. 3, a plot of $1/v$ *vs.* I according to Dixon[48] in Fig. 6A and a plot of v/v_I *vs.* I according to Wilson[33] in Fig. 6B clearly show that our data cannot be classified as non-competitive inhibition. Equation 4 can be rearranged for the Dixon plot, equation 10, and the Wilson plot, equation 11

$$\frac{1}{v} = \left(1 + \frac{K_m}{s}\right)\frac{1}{V_m} + \left(1 + \frac{k_2}{k_1 S}\right)\frac{I}{K'_I V_m} \quad (10)$$

$$\frac{v}{v_I} = 1 + \frac{\left(1 + \frac{k_2}{k_1 S}\right)\frac{I}{K_I}}{1 + K_m/S} \quad (11)$$

As was previously pointed out, if k_2/k_1 equals K_m, equation 4 reduces to the equation for non-competitive inhibition, and of course this is equally true for alternative forms of equation 4 such as (10) or (11). However, k_2/k_1 equals approximately 8.3×10^{-4} M and K_m equals 6.1×10^{-3} M. This difference between k_2/k_1 and K_m is consistent with the disagreement between the plots in Fig. 6 and the plots expected for non-competitive inhibition. The magnitude of the disagreement is best seen in Fig. 6B where the substrate concentration should have no effect on the slope if the inhibition is of the non-competitive type.

K'_I for non-competitive inhibitors can be readily calculated from the Wilson plot since the slope equals $1/K'_I$, but the use of this method to determine K'_I for the inhibition of glucose-6-phosphatase by glucose would be in error except at high substrate concentrations where $k_2/k_1 \times 1/S \rightarrow 0$. Equation 11 and the Wilson plot (Fig. 6B) could be used for finite substrate concentrations if values for k_2/k_1 and K_m were included. The K'_I for glucose, reported in this paper, was calculated from the ordinate intercept in the Lineweaver–Burke plot, see Fig. 3, which, according to equation 4, requires only a knowledge of V_m and I to calculate K'_I.

The relatively specific inhibition of glucose-6-phosphatase by glucose could be of physiological importance. However, the K_I' for glucose is 0.088 M (0.088 M equals 1600 mg. %), and the normal blood and liver glucose concentration in the rat is *ca.* 80 mg. %.[49] The exchange or transfer activity has no known physiological function, but it should be pointed out that *in vivo* or in a complex *in vitro* system, the exchange activity would complicate the interpretation of experiments using isotopically labeled compounds. For example, Cahill, *et al.*,[50] have reported a series of studies where it was assumed that the conversion of glucose to G-6-P was carried out by an enzymatic phosphorylation using ATP. The application, insofar as possible, of the properties of microsomal glucose-6-phosphatase to hepatic carbohydrate metabolism and to the liver slice experiments of Cahill, *et al.*,[50] will be the subject of a future communication.

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The Condensation of D-Galactose with Nitromethane

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The condensation of D-galactose with nitromethane in alkaline methanol provides the corresponding, epimeric deoxynitroheptitols in 50% combined yield. The configurations of the nitroalcohols were established by converting them to the corresponding aldoheptoses (D-*glycero*-L-*manno*-heptose and D-*glycero*-L-*gluco*-heptose).

The nitromethane synthesis[1] has been applied with varying degrees of success to certain aldose sugars of the tetrose, pentose, hexose, heptose and octose[2] series. In the aldohexose series, D-mannose combines with nitromethane in alkaline methanol to yield 55% of the corresponding deoxynitroheptitols,[3] whereas D-glucose, under similar reaction conditions, combines to only a very limited extent.[4]

(1) J. C. Sowden, *Adv. in Carbohydrate Chem.*, **6**, 291 (1951).

(2) The condensation of D-*erythro*-L-*manno*-octose with nitromethane will be described in a forthcoming communication.

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(4) J. C. Sowden and H. O. L. Fischer, *ibid.*, **69**, 1048 (1947).

The synthesis has now been applied to a third aldohexose, D-galactose.

When a suspension of D-galactose in methanol and nitromethane containing sodium methoxide is stirred, heat is evolved, the reaction mixture remains heterogeneous, and the suspended sugar is gradually replaced by the precipitated sodium salts of 1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol and 1-deoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol. Removal of sodium from the latter by ion-exchange provides the mixed deoxynitroheptitols in 50% yield. These are readily separated by fractional crystallization, first from water to obtain 1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol monohydrate (m. p. 158–159°), and than from ethanol to obtain 1-deoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol (m.p. 152–153°). The former product loses its water of crystallization at reduced pressure, gradually at room temperature or rapidly at 110°, to give the anhydrous deoxynitroheptitol (m.p. 165–166°).

Application of the Nef reaction[5] to the sodium salts of the two deoxynitroheptitols yields D-*glycero*-L-*manno*-heptose (isolated as the phenylhydrazone) and D-*glycero*-L-*gluco*-heptose, respectively. The preparation of the two aldoheptoses by the nitromethane synthesis appears to be much more convenient than is their preparation by the cyanohydrin method.[6,7]

Acetylation of the two deoxynitroheptitols produces the corresponding, crystalline hexaacetates. Treatment of either of the latter in benzene solution with sodium bicarbonate yields D-*galacto*-3,4,5,6,7-pentaacetoxy-1-nitro-1-heptene.

Experimental

1-Deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol and 1-Deoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol.—To a stirred suspension of 50 g. of D-galactose in 100 ml. of absolute methanol and 130 ml. of nitromethane was added a cold solution containing 13 g. of sodium in 300 ml. of absolute methanol. The temperature of the reaction mixture rose spontaneously to about 50°. After 24 hours of stirring, the mixture was cooled to −20° and the precipitated sodium salts of the deoxynitroheptitols were filtered and washed with cold methanol. The moist salts were dissolved in 500 ml. of water and the solution was passed immediately over Amberlite IR-100[8] to remove sodium. Concentration of the effluent at reduced pressure yielded successive crops of crystals. The final sirup yielded additional crystalline material after dilution with ethanol. Recrystallization of the more insoluble fractions from water yielded 13.3 g. (18.4%) of 1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol monohydrate, m.p. 158–159°. Upon drying over phosphorus pentoxide at reduced pressure, this product lost its water of crystallization in 3 hours at 110° or in 24 hours at room temperature. The resulting anhydrous 1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol showed m.p. 165–166° and $[\alpha]^{20}D$ +6.3° in water, *c* 4.

Anal. Calcd. for $C_7H_{15}O_8N$: C, 34.9; H, 6.27; N, 5.81. Found: C, 35.0; H, 6.41; N, 5.74.

Recrystallization of the more soluble crude fractions from aqueous ethanol and ethanol yielded 4.5 g. (6.6%) of mixed deoxynitroheptitols, m.p. 147–152°, and 16.6 g. (24.8%) of 1-deoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol, m.p. 152–153° and $[\alpha]^{20}D$ +7.8° in water, *c* 4.

Anal. Calcd. for $C_7H_{15}O_8N$: C, 34.9; H, 6.27; N, 5.81. Found: C, 34.7; H, 6.23; N, 5.97.

D-*glycero*-L-*manno*-Heptose Monohydrate.—To a stirred solution of 1.2 ml. of sulfuric acid in 1.6 ml. of water at 50° was added dropwise a solution of 2.0 g. of 1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol monohydrate in 10 ml. of 1 *N* sodium hydroxide. After complete addition, the reaction mixture was stirred for several minutes and then deionized over columns of Amberlite IR-100 and Duolite A-4.[9] The effluent and washings were concentrated at reduced pressure to a small volume and 1.2 ml. of phenylhydrazine in 2.8 ml. of 25% acetic acid was added. Precipitation of the phenylhydrazone began in a few minutes. After standing overnight, the product was collected and washed with cold water and ethanol. The yield of phenylhydrazone,[10] m.p. 191–192°, was 1.8 g. (78%).

Cleavage of the phenylhydrazone with benzaldehyde in the usual manner, and seeding[11] of the resulting sirup, yielded D-*glycero*-L-*manno*-heptose monohydrate,[7] m.p. 83–85°, $[\alpha]^{20}D$ −13.7° equil. in water, *c* 4.

D-*glycero*-L-*gluco*-Heptose.—Treatment of the sodium salt of 1-deoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol with sulfuric acid, as described above for its epimer, followed by deionization and concentration yielded a sirup from which D-*glycero*-L-*gluco*-heptose was crystallized directly in 20–25% yield by seeding.[11] It seems probable that this yield could be improved by isolating the sugar as an appropriate hydrazone. The heptose[7] showed m.p. 198–199° and $[\alpha]^{20}D$ −50.7° equil. in water, *c* 4.6.

D-*galacto*-3,4,5,6,7-Pentaacetoxy-1-nitro-1-heptene.—Acetylation of the epimeric deoxynitroheptitols with acetic anhydride containing a trace of sulfuric acid yielded the corresponding hexaacetates in 90–95% yield. **1-Deoxy-1-nitro-D-*glycero* L-*manno*-heptitol hexaacetate**: recrystallized from ethanol, m.p. 143–144°, $[\alpha]^{20}D$ +4.7° in chloroform, *c* 6. **1-Deoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol hexaacetate**: recrystallized from ethanol, m.p. 180–182°, $[\alpha]^{20}D$ +4.3° in chloroform, *c* 4.

Anal. Calcd. for $C_{19}H_{27}O_{14}N$: C, 46.2; H, 5.52; N, 2.84. Found: C, 46.3, 46.4; H, 5.39, 5.56; N, 2.80, 2.95.

The above hexaacetates (1.5 g.) were separately refluxed for 2 hours with sodium bicarbonate (1.5 g.) suspended in benzene (20 ml.). Filtration and concentration then yielded in each instance D-*galacto*-3,4,5,6,7-pentaacetoxy-1-nitro-1-heptene in 40% yield. After recrystallization from ethanol, the acetylated nitroölefin showed m.p. 190–191° and $[\alpha]^{20}D$ +20° in chloroform, *c* 4.

Anal. Calcd. for $C_{17}H_{23}O_{12}N$: C, 47.1; H, 5.35; N, 3.23. Found: C, 46.8; H, 5.35; N, 3.24.

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(11) The authors are indebted to Dr. H. G. Fletcher, Jr., N. I. A. M.D., National Institutes of Health, for seeding samples of the heptose sugars.