manifested by septicemia, meningitis, and pneumonia caused by *Staphylococcus aureus*. This infection, as well as a reported attack of "thrush" at 3 weeks of age, and a subsequent cutaneous abscess at 6 months, were thought to be related to a striking paucity of granulocytes in her peripheral blood. The initial blood analysis revealed hemoglobin 10 g/100 ml of blood; total leukocytes, 10,000/mm$^3$; 2 percent neutrophiles, 3 percent stab cells, 54 percent lymphocytes, and 41 percent monocytes. During the illness the hemoglobin value fell to 6.6 g/100 ml, presumably because of infection, since after a blood transfusion and antibiotic therapy the anemia subsided and did not recur. Neutropenia and monocytosis persisted, and in many subsequent blood counts the granulocytes remained few or were completely absent. At the same time the percentage of monocytes and lymphocytes varied reciprocally from about 30 to 65, in a total leukocyte count of 6000 to 15,000/mm.$^3$ Examination of the bone marrow revealed that maturation of the granulocytes was arrested at the myelocyte stage. Hematologic studies of both parents failed to disclose any abnormality, and both siblings (one of whom has only hemoglobin A) are healthy. There is no family history of a comparable disorder. Kostmann (*12*), describing a similar type of agranulocytosis in 14 children from a genetically isolated population in Sweden, found that an extreme susceptibility to infection made the condition lethal, and suggested that it was caused by a recessive gene in the homozygous state. Since the three other persons with hemoglobin G$_{\text{Coushatta}}$ are healthy, the grandfather being 66 years old, the agranulocytosis appears unrelated to the presence of the hemoglobin variant.

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## Mutarotase Inhibition by 1-Deoxyglucose

Abstract. *The Michaelis-Menten constant for glucose and the inhibitor constant of 1-deoxyglucose for the intestinal mutarotase, glucose, 1-deoxyglucose system are consistent with the properties of the glucose transport system of intestine. The alleged sharing of the intestinal glucose transport system by l-deoxyglucose does not contradict the mutarotase theory.*

1,5-Anhydro-D-glucitol (1-deoxyglucose) has been reported to be transported against a concentration gradient in everted hamster intestinal sacs (*1*) and by hamster intestinal segments (*2*). That 1-deoxyglucose may presumably share a common pathway with glucose in intestinal transport (*1, 3–5*) has been widely used as a central argument against the mutarotase theory (*6*) since 1-deoxyglucose cannot mutarotate.

This report shows that the mutarotase of hamster intestine is inhibited by 1-deoxyglucose and exhibits data from which the Michaelis-Menten constant ($K_m$) for hamster intestinal mutarotase acting on glucose and the inhibitor constant ($K_i$) for its inhibition by 1-deoxyglucose may be calculated. These $K_m$ and $K_i$ values serve as a basis for the accurate prediction of the degree of inhibition of transport of glucose in the presence of 1-deoxyglucose, but not for a correct prediction of the degree of inhibition of 1-deoxyglucose transport by glucose. The data of Crane (*3*) on mutual inhibition of transport in studies of glucose and 1-deoxyglucose in hamster intestinal segments are shown to be in accord in these respects with the data for mutarotase.

The entire small intestines of 12 hamsters were removed and 0.025*M* phosphate buffer (*7*) was used to rinse out the lumen. The intestines were weighed, and a tissue extract was made, as described previously for lens (*8*), with 2 ml of buffer per gram of intestine. The hamster intestinal extract was fractionated as follows: For each milliliter of extract, 0.22 g of ammonium sulfate was added and the extract was centrifuged. The precipitate was discarded and 0.18 g of ammonium sulfate was added for each milliliter of supernatant and the mixture was centrifuged. The precipitate was allowed to drain and then it was dissolved in about 5 ml of buffer and dialyzed against water for 1 hour.

Mutarotase was assayed polarimetrically (*8*). The degree of inhibition, the fractional residual activity, $K_m$ and $K_i$, were also calculated as previously reported (*8*).

The portion of the rate of the mutarotation reaction due to catalysis by the enzyme is $V = (k_e - k_s)S$, where $S$ is the substrate concentration and $k_e$ and $k_s$ are the mutarotation coefficients of the reactions carried out in the presence and absence of the enzyme, respectively. The mutarotation coefficient is the sum of the rate constants of the forward and backward components of the reversible mutarotation reaction and is equal to $0.693/t_{\frac{1}{2}}$; $t_{\frac{1}{2}}$ is equal to the half-time of the reaction.

The average value of several determinations of $t_{\frac{1}{2}}$ for the spontaneous rate of mutarotation was 7.4 minutes (*8*). The value for spontaneous $t_{\frac{1}{2}}$ was independent of the glucose concentration in the range measured.

Table 1 shows the action and Fig. 1 the course of the action of hamster intestinal mutarotase on glucose and the inhibition of this action by 1-deoxyglucose. The course of the spontaneous mutarotation reaction is also shown. Figure 2 shows the determinations of the $K_m$ for hamster intestinal mutarotase by means of a Lineweaver-Burk (*9*) graph. This graph, which

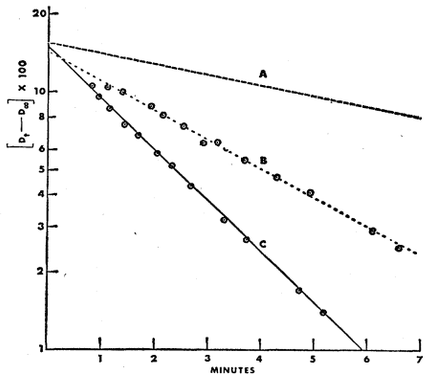


Fig. 1. Inhibition of the action of hamster intestinal mutarotase on glucose by 1-deoxyglucose. *A*, Course of the spontaneous mutarotation reaction of glucose (*8*); 42 mg of α-glucose in 8 ml of buffer; *B*, 3 ml of preparation HI, 5 ml of buffer; concentration of 1-deoxyglucose 0.064*M*; 42 mg of α-glucose; *C*, 3 ml of HI, 5 ml of buffer; 42 mg of α-glucose.

combines 14 separate determinations (see HP3, Table 1), yields 0.025*M* as the $K_m$ for glucose. The $K_i$ for 1-deoxyglucose was calculated to be 0.026±0.001*M* from the data of Table 1 (± refers to average deviation) (*8*).

Active transport in hamster intestinal sacs was not substantially inhibited by the presence of 1-deoxyglucose in amounts which theoretically should have almost entirely abolished the transport (about 96 percent inhibition calculated) (*10*). Jorgensen, Landau, and Wilson (*4*) essentially confirmed this finding since they reported only 23 percent inhibition by 1-deoxyglucose of active intestinal transport of glucose in hamster intestinal sacs where they predicted a theoretical inhibition of 90 percent and under conditions where these authors deemed that inhibitions below 20 percent are not significant (*11*). However, Wilson *et al.* (*11*) and Wilson (*5*) inferred that these data were indicative of support for the sharing of a common pathway by 1-deoxyglucose and glucose.

Crane (*12*) has pointed out that for the sacs the "rate and kinetics of absorption measured by these methods may be suspected of deviating from the true parameters of the epithelial cells." If Crane's view regarding the limitations of the sac technique is correct, then the failure to find significant competition in the everted sacs between 1-deoxyglucose and glucose (*4*, *10*) would have no bearing on whether there is, or is not, a common pathway for glucose and 1-deoxyglucose.

Crane (*3*) has been able to demonstrate the competition between various sugars, including the effect of 1-deoxyglucose on transport of glucose by means of the intestinal segment technique.

1-Deoxyglucose has a marked inhibitory action on mutarotase and has a value of $K_i$ very close to the value of $K_m$ for glucose. Crane (*3*) reported data which were stated to indicate a common pathway for transport of glucose and 1-deoxyglucose in hamster intestinal segments in vitro. His conclusion is based on a calculation of the degree of mutual inhibition of transport between glucose and 1-deoxyglucose from the apparent $K_m$'s of the actual transport processes for these substances. Crane found (*3*, Table 1) that inhibition of 1-deoxyglucose transport by glucose was 58 percent, and his calculated value was 62 percent. Our own recalculated values (*13*) show that his correct arithmetical prediction is 13 percent. The corresponding value for the inhibition of 1-deoxyglucose transport by glucose, calculated from our data on the $K_m$ and $K_i$ for mutarotase, is 2 percent. Thus neither Crane's values of the apparent $K_m$'s of the transport process in hamster intestinal segments for 1-deoxyglucose and glucose nor the

Table 1. Inhibitory action of 1-deoxyglucose on hamster intestinal mutarotase.

| Preparation No.* | Glucose (mole/liter) | 1-deoxyglucose (mole/liter) | Enzyme (ml) | Buffer (ml) | $t_{\frac{1}{2}}$ (min†) | Determinations (No.) |
|---|---|---|---|---|---|---|
| H1 | 0.0292 | 0 | 3 | 5 | 1.54±0.01 | 2 |
| H1 | 0.0292 | 0.064 | 3 | 5 | 2.63 | 1 |
| H2 | 0.0466 | 0 | 3 | 2 | 1.68±0.11 | 3 |
| H2 | 0.0466 | 0.0584 | 3 | 2 | 2.52±0.03 | 2 |
| HP1 | 0.0257 | 0 | 0.40 | 5 | 1.40±0.02 | 2 |
| HP1 | 0.0257 | 0.068 | 0.40 | 5 | 2.63 | 1 |
| HP2 | 0.0264 | 0 | 0.25 | 5 | 1.41±0.01 | 2 |
| HP2 | 0.0264 | 0.0465 | 0.25 | 5 | 2.29±0.11 | 2 |
| HP3 | 0.0154 | 0 | 0.40 | 5 | 1.31±0.03 | 2 |
| HP3 | 0.0309 | 0 | 0.40 | 5 | 1.69±0.02 | 6 |
| HP3 | 0.0618 | 0 | 0.40 | 5 | 2.36±0.04 | 6 |

* H, Extract of hamster intestine; HP, fractionated preparations. † $t_{\frac{1}{2}}$ for spontaneous mutarotation reaction = 7.5 minutes (*8*).

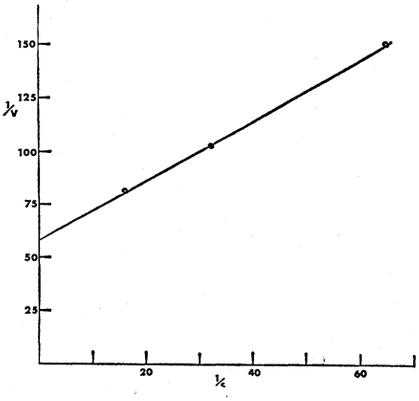

Fig. 2. Lineweaver-Burk graphs of the action of hamster intestinal mutarotase on glucose (data of Table 1, series HP3).

$K_m$ for glucose and $K_i$ for 1-deoxyglucose for hamster intestinal mutarotase successfully predict the actual inhibition (58 percent) of intestinal transport of 1-deoxyglucose by glucose.

With respect to the other two systems where inhibition of 1-deoxyglucose transport was cited by Crane (*3*), our calculations with the apparent $K_m$ (2 m*M*) (*14*) of the hamster intestinal transport process for 6-deoxyglucose and the apparent $K_m$ (7.4 m*M*) (*3*) of the transport process for 1-deoxyglucose likewise afford prediction of a degree of inhibition (13 percent) which is at variance with actual inhibition (45 percent) he found. Crane stated that there is a significant disparity between "calculated" and "found" values for the pair, galactose and 1-deoxyglucose, in his study. Thus, none of the three intestinal segment studies cited by Crane (*3*) on the inhibition of 1-deoxyglucose transport by glucose and the other sugars can be taken as indicative of the sharing of the glucose pathway by 1-deoxyglucose.

However, Crane (*3*) found 44 percent inhibition of glucose transport by 1-deoxyglucose, while the value which he calculated from the apparent $K_m$ data of the transport processes was 63.5 percent (*3*). The corresponding value calculated, from the mutarotase $K_m$ and $K_i$ data, is 48 percent. The $K_m$ and $K_i$ data for hamster intestinal mutarotase thus correctly predict the degree of inhibition of glucose transport by 1-deoxyglucose without requiring the assumption of a common pathway of transport for 1-deoxyglucose and glucose.

In broadest terms, the murarotase hypothesis (*6*) permits glucose (at some defined anomeric composition) to

be transported against an *apparent* concentration gradient into a region where glucose is present at a different anomeric composition. This hypothetical passage of glucose is actually passive but would appear to be active when viewed as total glucose without considering the anomeric compositions of the glucose in the two compartments. There is no bar in the mutarotase hypothesis to the passive transfer of a nonmutarotating inhibitor through the mutarotase system since an inhibitor could possess the structural requirements for passage via this system. Mutarotation would be required only for the type of "uphill" transport exemplified by glucose in this instance. The mutarotation of a substance, therefore, may not be necessary for its transport via the mutarotase system. Carrier mechanisms for transport have been proposed by which both substrates and inhibitors may be transported (*15*). Widdas (*16*) has postulated that the entrance into the cell and active transport may be the result of two different systems, with only the active transport being energy dependent. In accordance with this view, to actively transport a nonmutarotating substance by way of mutarotase, a second step dependent on energy would be required.

Thus, it is possible to state that the sharing of all or a part of a common pathway by glucose and by an inhibitor of mutarotase, cannot of itself be used as an argument to rule out participation of mutarotase in such a common pathway. Obviously, these considerations also apply to a compound such as $\alpha$-methyl glucoside which is actively transported (*17*) and inhibits mutarotase (*18*).

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## Heterotransplantation of the Kidney: Two Clinical Experiences

Abstract. *The field of heterotransplantation is now being explored because of success with immunosuppressive measures in homotransplantation, and because of the scarcity of suitable human organs. Two patients in terminal uremia, maintained on dialysis, received heterotransplants from nonhuman primates. In the first case a renal heterotransplant from a rhesus monkey implanted in a 32-year-old woman showed satisfactory immediate function but was removed after 10 days because of inadequate function. The second patient received a renal heterotransplant from a chimpanzee. A threatened rejection was reversed with immunosuppressive measures, but 2 months after transplantation the patient died of pneumonia. The transplanted kidneys showed no evidence of rejection.*

Heterotransplantation of the kidney was attempted early in this century (*1*) but no function of such grafts was documented. When the immunologic basis of the rejection process was defined, interest in heterotransplantation waned. New exploration of this field now seems warranted because of success with immunosuppressive measures in homografts (*2*) and because of difficulties in obtaining suitable human organs. In two patients in terminal uremia, renal heterografts from nonhuman primates were used when no homografts were available.

*Case 1.* A 32-year-old female with a documented history of hypertension and pyelonephritis during her seventh pregnancy in 1958 was admitted to Charity Hospital in March 1963 with symptoms of weakness, vomiting, weight loss, and abdominal pain. Examination showed a lethargic, chronically ill patient with blood pressure of 150/100. Laboratory studies included a hematocrit of 23 percent; urinalysis showed pyuria, albuminuria, and specific gravity of 1.007. Other studies included the following: blood urea nitrogen, 120 mg per 100 ml of blood; creatinine, 6 mg per 100 ml of serum; creatinine clearance, 14 ml/min; and urine culture, *aerobacter aerogenes*.

In May 1963, the patient was readmitted to Charity Hospital in uremic coma. Tracheostomy was performed. She improved with medical management but was readmitted in August 1963 because of congestive heart failure and progression of uremia. On admission she showed blood pressure of 180/100, ascites, and marked edema. Hematocrit upon admission was 21 percent; hemoglobin, 6.2; blood urea nitrogen, 160 mg per 100 ml; and creatinine, 17.2 mg per 100 ml of serum; urinalysis showed specific gravity of 1.008, albuminuria and pyuria.

Peritoneal dialysis was begun, and a search was made for a human kidney from either a volunteer or cadaver. Because of progressive deterioration and the lack of a homograft, a heterograft was used. On 8 October 1963, the donor, a rhesus monkey, was prepared with hypothermia and anticoagulation. Both kidneys, the aorta, and the vena cava were removed *en bloc* and transplanted into the right iliac fossa of the patient. The aorta and vena cava of the graft were anastomosed to the external iliac artery and vein, respectively, of the recipient. The ureters were implanted into the bladder through submucosal tunnels. The time of ischemia was 46 minutes. Immunosuppressive agents included azathioprine (Imuran), steroids, actinomycin C, and azaserine. Mannitol was given during and immediately after the operation.

Urine appeared in the ureteral orifices 10 minutes after completion of the anastomoses. Urinary output reached a maximum of 3500 ml on the 1st day and creatinine clearance rose to 24 ml/min. Additional laboratory data are given in Table 1.

On 13 October an episode of acute abdominal pain and tenderness prompt-