14. Geschwind, I. I., Li, C. H., in: Hypophyseal Growth Hormone, Nature and Actions, R. W. Smith, O. H. Gaebler, C. N. H. Long, eds., McGraw-Hill, New York, 1955, p28.

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Anomeric Specificity of Human Erythrocyte Glucose-6-Phosphate Dehydrogenase.* (31222)

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In the earlier literature the substrate specificity of glucose-6-phosphate dehydrogenase (G-6-PD) was considered to be absolute for glucose-6-phosphate (G-6-P)(1). Subsequent investigators reported that the enzyme isolated from human erythrocytes has activity with the 6-phosphate derivative of 2-deoxyglucose, galactose, and glucosamine but not with mannose-6-phosphate, ribose-5-phosphate, and unphosphorylated glucose(2,3). However, yeast G-6-PD has been shown to use unphosphorylated glucose as a substrate when it is present in high concentrations, and to be specific for the β anomer(4). Recently Salas *et al*(5) have shown that this specificity for the β anomer also exists for the phosphorylated sugar, *i.e.*, G-6-P.

The anomeric specificity of red blood cell G-6-PD may not be of great physiologic importance in considering the metabolism of glucose, since both the α and β anomer of G-6-P are formed by hexokinase. In considering the metabolism of α glucose-1-phosphate, as derived from either glycogen or galactose, however, the anomeric specificity of G-6-PD is critical, since the product of phosphoglucomutase is only the α anomer of glucose-6-phosphate.

The purpose of this communication is to show that G-6-PD from human erythrocytes can use glucose as a substrate when it is present in sufficient concentration and that this activity is specific for the β anomer. The enzyme is also shown to be specific for the β anomer of G-6-P.

Materials and methods. G-6-PD was prepared from human erythrocytes by the method of Kirkman (1962) through the first ammonium sulfate fraction. Yeast hexokinase, adenosine triphosphate, triphosphopyridine nucleotide, glucose-6-phosphate, and α and β anomers of glucose were obtained from Sigma Chemical Co. All reactions were measured by following the appearance of TPNH at 340 mμ, using a Gilford Model 2000 Absorbance Recorder.

Results. It is apparent that the human red cell enzyme can use free glucose to reduce TPN and that this activity is specific for the β anomer (Fig. 1). No separation of enzyme activity was found during the purification procedure. The reaction rate with .1 M glucose is approximately 1% of that found with .6 mM G-6-P. The failure of Kirkman(2), and Greiling and Kisters(3) to demonstrate this reaction may be related to a low substrate concentration.

When glucose is used as a substrate for G-6-PD, the reaction is stimulated by $NaHCO_3$, is inhibited by Mg^{++} and phosphate ions, and has a pH optimum of approximately 9.0. In this respect it is similar to G-6-PD activity with DPN and G-6-P(6).

Fig. 2 shows the anomeric specificity of G-6-PD with respect to G-6-P. Yeast hexokinase, which acts on both anomers at approximately the same rate(5), was used to generate α and β G-6-P. When α glucose is used there is an appreciable lag which is not present with the β anomer. Since both anomers are formed by the hexokinase reaction and since spontaneous anomerization of the phosphorylated sugar is very rapid, it appears unlikely that anomerization of G-6-P would be limiting under physiological conditions.

Discussion. Red cell G-6-PD has been

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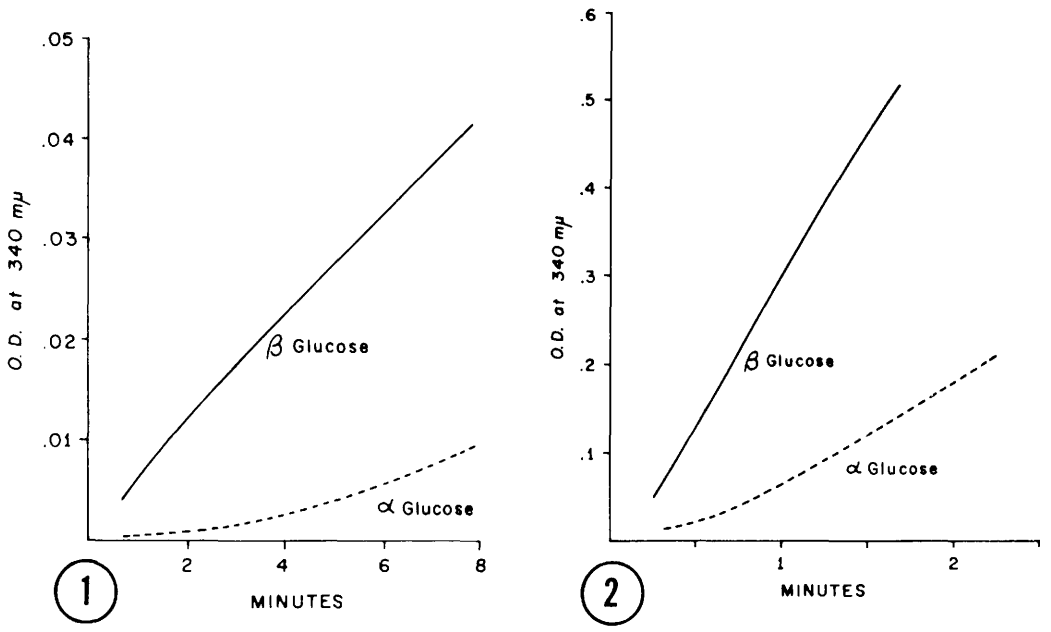

FIG. 1. Comparison of α and β glucose as substrate for erythrocyte glucose-6-phosphate dehydrogenase. The reaction mixture contained: 63 μMoles Tris, pH 8.0; .75 μMoles TPN; and .96 unit glucose-6-phosphate dehydrogenase in a final volume of 3 ml. The reaction was started by addition of .3 mMole of solid α or β glucose. T = 25°C.

FIG. 2. Hexokinase-glucose-6-phosphate dehydrogenase coupled system. The reaction mixture (1 ml) contained Tris, pH 7.7, 10 μMoles; ATP, pH 7.0, 75 μMoles; TPN, .25 μMoles; $MgCl_2$ 5 μMoles; albumin, 50 μg%; hexokinase, .67 unit, and human erythrocyte glucose-6-phosphate dehydrogenase, 2.8 units at approximately 3°C. The reaction was started with 12 mMoles of freshly prepared α or β glucose.

shown to have a high degree of specificity of β-G-6-P. The inability of G-6-PD to metabolize the α anomer of G-6-P may well clarify some previously unexplained observations regarding galactose metabolism in erythrocytes. It has recently been reported that the methemoglobin reduction in nitrited, washed erythrocytes is not accelerated by methylene blue when galactose is used as substrate(7). This is in agreement with the lack of stimulation of oxygen consumption when methylene blue was added to red cells incubated with galactose(8). These findings were surprising in view of the known metabolism of galactose to G-6-P: galactose is phosphorylated to galactose-1-phosphate (Gal-1-P) by galactokinase; Gal-1-P reacts with uridine diphosphoglucose to form glucose-1-phosphate (Glu-1-P) in the presence of Gal-1-P uridyl transferase; and phosphoglucomutase catalyzes the conversion of Glu-1-P to G-6-P. It seemed, then, that the addition of methylene blue should divert the G-6-P formed from galactose through the hexose monophosphate pathway, accelerating the reduction of methemoglobin or stimulating the consumption of oxygen.

This failure of methylene blue to stimulate shunt activity may be explained by the specificity of G-6-PD for the α anomer.

Some Gal-1-P has been reported to be metabolized through the hexose monophosphate pathway in hemolysates(10,11). However, results from hemolysates cannot be compared with intact cell preparations unless precautions have been taken to maintain ATP levels.

Summary. Evidence is presented that human erythrocyte glucose-6-phosphate dehydrogenase is specific for the β anomer of glucose and glucose-6-phosphate. It is suggested that this specificity is responsible for the lack of stimulation of the hexose monophosphate pathway by methylene blue when galactose is

used as a substrate.

1. Glock, G. E., in Biochemists' Handbook, C. Long, ed., E. and F. Spon, Lond., 1961, p345.

2. Kirkman, H. N., J. Biol. Chem., 1962, v237, 2364.

3. Greiling, H., Kisters, R., Hoppe-Seylers Z. physiol. Chem., 1965, v341, 172.

4. Colowick, S. P., Goldberg, E. B., Bull. Res. Counc., Israel, 1963, v11A4, 373.

5. Salas, M., Vieula, E., Sols, A., J. Biol. Chem., 1965, v240, 561.

6. Levy, H. R., Biochem. Biophys. Res. Comm., 1961, v6, 49.

7. Beutler, E., Collins, Z., Scand. J. Haemat., 1965, v2, 343.

8. Betke, K., Baltz, A., Maas, U., Z. Kinderheilk, 1960, v84, 226.

9. Isselbacher, K. J., in Metabolic Basis of Inherited Disease, Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., eds., McGraw-Hill, N. Y., 1960, p208.

10. Beutler, E., Baluda, M. C., Donnell, G. N., J. Lab. Clin. Med., 1964, v64, 694.

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Susceptibility of Cultured Renal Cells from Different Species of Subhuman Primates to Simian Virus 40.* (31223)

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While many animal viruses are capable of infecting cultured renal cells derived from a broad spectrum of animals, limitations as to host specificity are well recognized. In an early *in vitro* study, Kaplan(1) found that poliovirus induced a cytopathic effect (CPE) in some subhuman primate cells but not in others. Cultured renal cells from rhesus *(Macaca mulatta)* and crab-eating *(M. cynomolgus)* monkeys were found to be susceptible while those from the capuchin monkey *(Cebus capucina)* were not. Similar observations have been made by Sweet and Hilleman(2) with Simian Virus 40 (SV40), a papovavirus, which was cytopathic to cultured renal cells from African green monkey *(Cercopithecus aethiops)* but not from rhesus except after a prolonged incubation period(3). Based on these differences, Hsiung *et al*(4) suggest that virus host range may be another criterion to aid in the grouping of primates. This article will contribute additional information to that previously reported(4) on those subhuman primates whose kidneys are either refractive or susceptible to CPE when infected *in vitro* with SV40.

Materials and methods. Trypsinized kidney cells from the different species of primates were grown in medium 199 (Hanks' base) with 0.05% yeastolate and 10% calf serum. All media used throughout the experiment contained 100 μg/ml of streptomycin and 100 U/ml of penicillin. The SV40, initially obtained from Dr. Fred Rapp, was prepared and titrated in primary African green monkey (AGM) kidney cultures. The infective volume for monolayer tube cultures was 0.2 ml of 10-fold serial dilution of stock virus.

Cells grown in Falcon disposable flasks (No. 3017) were exposed for 2 hr to $16^{6.7}$ $TCID_{50}$ of the virus and then washed 5 times with 4-ml volumes of maintenance medium before incubation. All infected and control cultured cells were maintained in medium 199 (Earle's base) supplemented with 3% calf serum. The cultures were retained for 35 days with medium changes made at 5- to 6-day intervals.

Results. As shown in Table I, cultured renal cells from different species of animals demonstrated varying degrees of susceptibil-

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