content was extremely low but still measurable. At the end of 24 hours no norepinephrine could be determined in the heart. The alterations in cardiac phosphorylase *a* activity reported here show some correlation with the changes in norepinephrine concentration described by Paasonen and Krayer(11). During the period immediately following reserpine administration, when the heart is being depleted of norepinephrine, a stimulation of phosphorylase *a* occurs. This is followed by a return of enzyme activity to control values after one or 3 hours, and after 24 hours, when the heart is depleted of norepinephrine, there is a significant decrease in phosphorylase *a* activity. These results suggest that the norepinephrine stored in the heart plays a role in the regulation of the activity of cardiac phosphorylase *a*.

Summary. Total phosphorylase was higher in rat and rabbit hearts than in frog hearts and greater in ventricles than in atria of all 3 species. Reserpine administration caused an initial increase in cardiac phosphorylase *a* followed by a marked diminution.

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Inhibition of Erythropoietin Formation in Hypoxic Rats by Hexose Analogues.* (30400)

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Hypoxemia or anemia are known to stimulate erythropoietin formation whereas hyperbaric oxygen(1) and hypertransfusion polycythemia(2) depress erythropoiesis, probably through a depression of erythropoietin formation. The latter thus appears to be inversely proportional to the oxygen tension in erythropoietin producing tissue. Metabolic pathways and the mechanism which accelerates synthesis of the active hormone in response to low pO_2 have remained obscure. The present study examines the role of glycolysis in erythropoietin formation. It was prompted by reports on erythropoietin formation in several types of tumors(3). Moreover, the kidney, which produces most of the body's erythropoietin, exhibits in its medulla a high glycolytic metabolism. The exact site of erythropoietin formation in the kidney is unknown, but it seemed worthwhile to explore whether glycolysis perhaps represents the common link in erythropoietin formation in these diverse tissues. Erythropoietin serum levels were measured in hypoxic rats with or without administration of D-glucosamine (GA) or 2-desoxy-D-glucose (2-DG). These hexose analogues depress the rate of glycolysis through competitive inhibition of hexokinase(4). They reportedly do not affect cellular respiration, and their administration should thus curtail glycolysis without inducing alterations in the pO_2 of erythropoietin producing tissue.

Methods. Female Sprague-Dawley rats (200 to 225 g) in groups of 7 were fasted for 16 hours before experiments. Group (1) re-

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ceived nothing, Group (2) 500 mg glucose, Group (3) 200 mg 2-desoxy-D-glucose (Nutritional Bioch. Corp.), Group (4) 500 mg D-glucosamine hydrochloride (Sigma) plus 1 mM sodium bicarbonate, Group (5) the same dose of GA plus 100 mg sodium pyruvate, all per 100 g of body weight. The compounds were given in a fluid volume of 8 ml per rat in 3 divided doses: one-half by stomach tube one hour before hypoxia, one-fourth i.p. at start of hypoxia, and one-fourth i.p. 3 hours later. All groups were exposed for 6 hours at an ambient pressure of 320 mm Hg (pO_2 = 67 mm Hg). The rats were then quickly exsanguinated, and erythropoietin concentrations in serum were measured by bio-assay in polycythemic mice as described(5). Each assay mouse received 2 ml of serum subcutaneously, and 6 mice were used for the pooled serum of each group of rats. Erythropoietin concentrations are expressed in units of Erythropoietin Standard B (Nat. Inst. Medical Research, London).

Metabolic measurements were made in parallel experiments by exposing rats of the 5 groups to 9% O_2 in N_2 (pO_2 = 67 mm Hg). Their O_2 consumption was measured at 21°C environmental temperature by a modification of Benedict's closed system(6), and gas volumes were corrected to S.A.T. Blood samples were drawn by transcutaneous puncture of the left heart within 2 minutes after removal of the rats from the gas mixture. Blood pH was measured by Beckman blood electrode, GA concentration according to Elson(7), lactic and pyruvic acid by the enzymatic methods of Olson and of Segal(8). Blood glucose was determined by Benedict's method and corrections were made for the measured reducing power of GA present.

Results and discussion. GA is rapidly cleared from the blood stream(9) and frequent injections are necessary to maintain competitive levels over prolonged periods. Experiments were therefore limited to a hypoxic exposure of 6 hours duration, which was found sufficient to raise plasma erythropoietin in untreated rats to readily measurable levels. Free GA is unstable and the administration of its hydrochloride caused severe acidosis. In subsequent experiments, therefore, sodium bicarbonate or pyruvate were given in addition. Erythropoietin concentrations, measured in 4 separate experiments, were significantly lower in GA and 2-DG rats than in controls (Table I). The serum of the former contained between 20 and 50 mg% of GA. In control assays, therefore, 1 and 10 mg of GA was added *in vitro* to a known quantity of erythropoietin. Table II shows that the added GA did not affect the erythropoietic response of the assay mice. Erythropoietin is not stored to any significant degree at the site of its renal formation(10), and the observed lower blood levels in GA and 2-DG treated rats are therefore attributed to a diminished formation.

The rate of erythropoietin formation is in first order dependent on the degree of hypoxia, and the lower erythropoietin values in analogue-treated rats could have been the result of their being less hypoxic than the controls, either due to increases in their arterial O_2 supply or to decreases in their rate of O_2 consumption. Hematocrits did not differ significantly in various groups. The volume of fluid administered was the same and it seems unlikely that the injections would have increased cardiac output in the analogue-treated group and not in the glucose con-

TABLE I. Effect of Glucosamine (GA) and 2-Desoxy-Glucose (2-DG) Administration on Erythropoietin in Serum of Rats After 6 Hours Exposure to 320 mm Hg Ambient Pressure.

Treatment	No. of groups	Units/ml serum
None	4	.24 ± .02
GA	1	.07
GA + pyruvate	3	.08 ± .02*
GA + bicarbonate	3	.07 ± .01*
2-DG	3	.09 ± .01*
Glucose + pyruvate	3	.28 ± .07

* Difference is significant ($p < .01$) in comparison to untreated groups.

TABLE II. Effect of *in vitro* Addition of Glucosamine to Erythropoietin (E) on Fe^{59} Incorporation in Assay-Mice. (Mean and S.D. in 6 mice per group.)

	% Fe^{59} incorporated
.3 U E	18 ± 2.5
.3 U E + 1 mg GA	20 ± 4.8
.3 U E + 10 mg GA	17 ± 5.3

TABLE III. Metabolic Data on Hypoxic Rats With or Without Administration of Glucosamine (GA) or 2-Desoxy Glucose (2-DG). Mean and SEM, 6 rats in each group.

Treatment	O_2 consumption, ml/hr/100 g	Blood pH	Glucose, mg %	GA, mg %	Lactate, mg %	Pyruvate, mg %	L/P
None	102 ± 5	7.39 ± .02	105 ± 4	0	21.4 ± 3.9	2.0 ± .3	10.6 ± 1.0
GA + pyruvate	99 ± 4	7.46 ± .06	163 ± 11	24.1 ± 2.2	18.4 ± 2.6	1.9 ± .2	10.2 ± 2.2
GA + bicarbonate	101 ± 3	7.41 ± .05	158 ± 9	20.9 ± 1.7	18.2 ± 2.3	2.0 ± .3	9.2 ± .5
2-DG	95 ± 3	7.45 ± .06	172 ± 14	0	14.3 ± 1.8	1.9 ± .2	8.0 ± .8
Glucose + pyruvate	95 ± 4	7.51 ± .02	176 ± 6	0	36.9 ± 6.2	3.6 ± .3	10.2 ± .8

trols. Differences in blood pH undoubtedly resulted in some shifts of the blood oxygen dissociation curves and were probably also associated with differences in ventilation. There was, however, no correlation between blood pH and erythropoietin formation. Differences in arterial blood supply are therefore dismissed as a significant factor. Oxygen consumption is known to be influenced by type and concentration of substrate(11). The higher blood glucose concentrations (Table III) in analogue-treated groups reflect their diminished glucose utilization. The latter, however, did not result in appreciable lower pyruvate levels and injection of sodium pyruvate did not raise oxygen consumption in GA rats. Pyruvate concentration can thus be disregarded as a rate-limiting factor in the oxidative metabolism of the analogue-treated rats. The hourly O_2 consumption fluctuated somewhat but showed no temporal relation to the administration of the analogues. In the GA plus pyruvate group for example, the hourly values were 107, 108, 88, 98, 96, 103 ml/h/100 g *versus* 110, 110, 109, 88, 88, 109 in the untreated group. Total O_2 consumption during the 6-hour period of hypoxia did not significantly differ in various groups (Table III). The absence of major differences in oxidative metabolism is further supported by the nearly identical lactate/pyruvate ratios. The measurements thus gave no indication of a lesser degree of hypoxia in GA or 2-DG treated rats as the cause of their diminished erythropoietin formation, and the latter is attributed to a competitive inhibition of their glucose phosphorylization. The findings do not necessarily imply a rate regulating connection between glycolysis and erythropoietin formation. Erythropoietin is a glycoprotein (12), and the hexose analogues might have simply interfered with synthesis of the prosthetic group of the molecule. The significance of the demonstrated inhibition in regard to the mechanism which effects increased erythropoietin formation in response to hypoxia thus remains to be elucidated.

Summary. Administration of D-glucosamine or 2-desoxy-D-glucose suppressed erythropoietin formation in hypoxic rats. No evidence was found to account for this depression on the basis of oxygen supply or consumption, and it is therefore attributed to a competitive inhibition of glucose phosphorylization. The inhibition of erythropoietin formation by the hexose analogues could be the result of their interference with the synthesis of the carbohydrate moiety of the erythropoietin molecule, or it could imply a rate regulating role of glycolysis in erythropoietin formation.

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Demonstration of the Inherited Serum Group Specific Protein by Acrylamide Electrophoresis.* (30401)

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The inherited group specific protein (Gc) was first recognized by the technique of immunoelectrophoresis(1) and also demonstrated by the method of vertical starch gel electrophoresis(2) as a system of protein staining bands in the immediate post-albumin region(3). In a later modification the use of a discontinuous buffer system incorporating lithium hydroxide(4), improved the resolution and revealed more clearly the heterogeneous nature of the Gc system. This method suffers from the limitations imposed by variations in the behavior of different batches of hydrolyzed starch, a moderately long running time and the proximity of the leading Gc protein bands to the trailing border of the albumin. For routine typing of the common Gc phenotypes these difficulties are of no great importance, but it has been almost impossible to separate albumin from the genetic variants of Gc which possess a component with an electrophoretic mobility greater than that of the normal fast Gc 1-1 phenotype.

In these circumstances the method of acrylamide gel electrophoresis developed by Raymond(5) and applied by Peacock(6) offers some advantages. Surface effects, which are apparent in the starch gel system, are abolished and the geometry of the apparatus ensures a linear field within the cell. With the additional advantage of a stable uncharged gel matrix of uniform pore size, it seemed likely that with minor changes in the electrophoretic conditions, it would be possible to resolve the post-albumin region more clearly. This paper is concerned with the resolution of the Gc proteins in the post-albumin region of serum obtained by the method of vertical slab acrylamide gel electrophoresis.

Materials and methods. Apparatus: A vertical slab of acrylamide is prepared in a 3 mm thick mold as described by Raymond (7). The apparatus is constructed so that the gel mold, cooling plates electrodes and buffer reservoirs are in a single plexiglass unit. The apparatus employed was obtained from the E. C. Apparatus Corp., Philadelphia, Pa. The cooling bath circulator was obtained from Forma Scientific Inc. and the circulating buffer pump from Gorman-Rupp Industries, Inc. Variations of the standard Trishydroxymethylaminomethane (TRIS), disodium ethylenediaminetetraacetic acid (EDTA) and boric acid were used. (a) 10.09 g Tris, 1.3 g EDTA, 0.81 g boric acid in 1 liter pH 8.91, (b) 10.75 g Tris, 0.93 g EDTA, 5.04 boric acid in 1 liter pH 8.28. *Cooling:* In the initial experiments cooling was obtained by circulating tap water at approximately 12°C, but in the later experiments by circulating water at a temperature of 4°C. *Gel:* A 5% acrylamide gel was used throughout and made by dissolving 7.5 g of Cyanogum-41 (electrophoresis grade) in 150 ml of buffer. Gel formation was catalyzed by adding 0.3 ml of N,N,N′,N′,-tetramethylethylenediamine (TMED) and 300 mg of ammonium persulphate

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