The results provide direct evidence that certain parenchymal marrow lymphocytes can produce GVH lymph node enlargement. In current experiments highly labelled large blast-like cells have been seen in radioautographs of F_1 hybrid popliteal lymph nodes 2–3 days after the footpad injection of H^3-uridine labelled cells from lymphocyte-rich marrow fractions of parental strain rats. Some of the marrow lymphocytes therefore appear to be capable of blastogenic transformation in response to histocompatibility antigens in vivo as well as in vitro.

The great majority of small lymphocytes in the bone marrow of rats and guinea-pigs are newly-formed, locally-produced cells, which have a short intramyeloid life span and migrate continuously via the blood stream to the spleen and lymph nodes[1,8–10]. The marrow is therefore a major site of small lymphocyte production. If the marrow cells which produce GVH reactions are among this population of newly-formed small lymphocytes the marrow may provide a continous source of small lymphocytes capable of reacting against foreign cells in the blood and other peripheral lymphoid tissues. On the other hand, recent radioautographic studies in this laboratory as well as those of other investigators[11] have indicated the presence of a small number of long-lived small lymphocytes in rat marrow. Further radioautographic studies are therefore required to determine whether the cells possessing GVH activity might be contained within this latter subpopulation of small lymphocytes in the marrow[12].

Résumé. Des suspensions de cellules provenant de la moëlle osseuse, ou des fractions cellulaires d'une moëlle osseuse d'une souche parentale ont été injectées dans la plante des pieds de rats hybrides (F_1). Le poids des nodules lymphatiques poplités constitue un test sensible de la réaction greffe-receveur qui résulte de l'injection des cellules et montre qu'une telle réaction peut être provoquée par certains lymphocytes provenant du parenchyme de la moëlle.

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Studies on Glucose-6-Phosphate Dehydrogenase: Variability in ATP Inhibition[1]

Glucose-6-phosphate dehydrogenase (G-6-PD) activity is tremendously important to erythrocyte integrity. Where activity is decreased, the erythrocyte is unusually susceptible to 'oxidant' drugs, which may markedly shorten its in vivo life[2]. The enzyme is inhibited by adenosine-triphosphate (ATP)[3], various steroids[4,5], and palmityl-CoA[6,7].

The effect of ATP on G-6-PD activity is particularly important because the inhibition constant (K_i) is within fluctuating levels of intracellular ATP concentration and because ATP helps regulate glycolysis by inhibiting fructose-6-phosphate kinase[8].

A discrepancy between G-6-PD activity of sheep hemolysates (measured under optimum spectrophotometric conditions) and the ability of erythrocytes to reduce glutathione[9], suggested studying effects of ATP on sheep erythrocyte G-6-PD.

Materials and methods. All blood samples, except one human sample (an aliquot of citrated blood 4 weeks in storage), were collected using heparin as an anticoagulant.

Glucose-6-phosphate-dehydrogenase activity was measured by following the reduction of nicotinamide-adenine-dinucleotide phosphate (NADP) at 340 nm recorded on a Gilford automatic recording spectrophotometer. The reaction mixture for hemolysates (1.0 ml) contained 0.2 m*M* NADP, 0.1 *M* *Tris*-buffer (pH 8.0), $MgCl_2$ and 0.6 m*M* glucose-6-phosphate at 25 °C[10].

Glucose-6-phosphate dehydrogenase was partially purified, using previously described techniques[10]; all preparations were dialyzed overnight against *Tris* 105 m*M*, pH 8.0; 2.7 m*M* EDTA; 7 m*M* β-mercapto-ethanol and 10 μ*M* NADP prior to the experimentation. For kinetic studies the reaction mixture was lowered to pH 7.35, the temperature raised to 30 °C, $MgCl_2$ omitted, and variable amounts of ATP (0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 m*M*) and glucose-6-phosphate (0.0125, 0.025, 0.05, 0.01, 0.3, 0.4, and 0.6 m*M*) were used[3]. The reaction was initiated by adding the enzyme as the last component. The Michaelis-Menten constant (*Km*) for glucose-6-phosphate, the inhibition constant, and their respective standard errors were calculated using a least square fit to the Michaelis-Menten equation[11], assuming the inhibition competitive and observed velocities equal in variances.

Results. Competitive inhibition was not observed using the sheep enzyme (Table). The reaction rates with ATP were in the expected range for samples without ATP.

Observed *Km* values for both human and sheep G-6-PD are higher than previously reported[9,10,12] – probably because we lowered the pH of the assay mixture and used a higher temperature. The *Ki* value for 1 sample (human, 1) was approximately half that observed in the other 2 samples; hemolysate activity of that one was approximately 20% of normal (1.6 μm NADP/min/g

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hemoglobin). This subject has no known clinical or hematological abnormalities.

Discussion. Variability in ATP inhibition of human G-6-PD and failure to observe inhibition with sheep G-6-PD is another variant characteristic of this enzyme. *Ki* for ATP likely should be considered one of the parameters required to characterize new G-6-PD variants; *Ki* may also belong in the list of desirable studies that the WHO scientific group outlined[13].

Several investigators have reported discrepancies between G-6-PD activity (as measured spectrophotometrically) and clinical manifestations. Hemolysates from nonanemic individuals with the Mediterranean-type G-6-PD deficiency have less activity (0.9% of normal) than some Caucasians with chronic nonspherocytic hemolytic disease, like the Chicago variant (9–26% of normal activity)[14]. G-6-PD activity in hemolysates does not correlate with severity of primaquine sensitivity in vivo[15]. Some of the discrepancies may be accounted for by differences in vivo enzyme stability, pH optimum, and altered kinetics. The influence of other substrates on proteins in the intact erythrocytes may be equally important.

Km-G6P and *Ki*-ATP of erythrocyte glucose-6-phosphate dehydrogenase

	Km-G6P (μM)	*Ki*-ATP (μM)
Human		
1	78.0 ± 7.4[a]	1110.0 ± 302.0
2	108.0 ± 4.3	2280.0 ± 408.0
3	112.0 ± 6.4	2190.0 ± 536.0
Sheep		
1	70.6 ± 4.6	[b]
2	68.3 ± 4.5	[b]
3	179.2 ± 11.5	[b]

[a] ± Standard error. [b] Not observed.

Effects of changes in *Km*-G-6-P and *Ki*-ATP can be estimated using relative velocity equations of DIXON and WEBB[16]. Assuming G-6-P concentration of 27 μM[17] a 75% decrease in the *Km*-G-6-P increases the inhibited relative reaction rate 37% (from 0.197 to 0.257). Decreasing the *Ki*-ATP one-half (assuming an ATP concentration of 1.35 mM) decreases the reaction 25% (from 0.197 to 0.131) when the *Km*-G-6-P is 110 μM and the G-6-P is 27 μM. Because both the *Km*-G-6-P and the *Ki*-ATP are lowered in this particular mutant, the relative reaction rate does not differ significantly (0.131 for normal verus, 0.133 for the mutant). However, the calculations are based on the reaction rate relative to the maximum velocity and do not consider actual enzyme activity per gram hemoglobin.

Zusammenfassung. Es wird eine Glukose-6-Phosphat-Dehydrogenase in Erythrozyten des Menschen beschrieben, bei der die Michaelis-Konstante für Glukose-6-Phosphat und die Inhibitor-Konstante für Adenosintriphosphat erniedrigt sind. Erythrozyten des Schafes zeigen keine kompetitive Hemmung mit ATP.

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Effect of bacterial Antigens, Propranolol, and Insulin on the Susceptibility of Mice to Hypothermic Stress

Accumulated evidence suggests that animals can be rendered more susceptible to certain noxious stimuli by decreasing their blood sugar levels or by preventing the usual hyperglycemic response to stress[1–8]. 4 agents possessing the above stress-enhancing and glucose-lowering properties have been extensively studied in our own and other laboratories. These include the histamine-sensitizing factor (HSF) of *Bordetella pertussis*, bacterial endotoxin, insulin, and β-adrenergic blocking drugs such as propranolol. In most instances, these agents have been found capable of dramatically increasing the sensitivity of experimental animals to a wide variety of pharmacological, immunological and physical stresses[1–14].

In 1957 MUNOZ and SCHUCHARDT reported that several days after inoculation with pertussis vaccine, mice were rendered significantly more susceptible to cold exposure than uninoculated mice. The component of *B. pertussis* responsible for this effect was not determined. More recently, BERRY[16] has shown that bacterial endotoxin possesses a similar enhancing effect when injected into mice immediately prior to their being placed in a cold environment.

B. pertussis contains at least 2 components, HSF and endotoxin, capable of inducing hypoglycemia, and sensitizing mice to diverse stresses[1,9,12,13]. We considered it of interest to determine the relative contributions of HSF and endotoxin in increasing the sensitivity of mice to cold. This paper will also report the effects of 2 other hypoglycemic agents, insulin and propranolol, on the susceptibility of mice to low temperatures.

Materials and methods. In preliminary experiments we determined that uninoculated, or saline-injected female CFW mice, housed in groups of 8 to 10 in copper pans, could survive for more than 24 h when placed in a refrigerator at a temperature of 3–6 °C. Any increase in susceptibility to cold among experimentally treated mice was indicated by a high mortality within 24 h of cold treatment. A pertussis extract (PE) was prepared as previously described[17] by lysing *B. pertussis* organisms in a blender and precipitating the lysate with ammonium sulphate. This preparation has been shown to contain the bulk of the HSF activity of whole vaccine[17,18]. Endotoxin is also present in this extract[19]. Groups of 8–10 CFW female mice weighing 14–18 g, were injected i.p. with the following preparations: 1. 45 µg N of PE, 2. the same but heated at 100 °C for 30 min, 3. 100 µg *Salmonella typhosa* endotoxin (Difco), 4. 1 mg of propranolol (Ayerst Labs), 5. 0.5 unit of regular insulin (Iletin, Lilly), and 6. saline solution. Mice received a single injection of the first 3 preparations either 5 days before or im-