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-

mediately prior to cold treatment. Preparations 4 to 6 were injected 10 min before cold exposure. Previous studies had shown that this time interval was sufficient for both propranolol and insulin to sensitize mice to other stressful stimuli[2,3,12,14]. All of the inoculations were well tolerated by mice at room temperature and in no case did more than one mouse of any group die after injection when held at ambient temperature (25 °C).

Results. The Table indicates that, whereas all saline-injected mice survived in the cold for more than 1 day, each of the test preparations was capable of eliciting a high mortality within 24 h of cold exposure provided the appropriate time interval was used. Heated PE behaved similarly to endotoxin in inducing 100% mortality when injected immediately prior to cold exposure and no mortality when the interval was 5 days. Unheated PE gave high mortality whether exposure was immediate or delayed following injection. The Table further shows that insulin and propranolol can also increase the susceptibility of mice to cold stress. All of the above tests were repeated with similar results.

Discussion. These results indicate that the HSF of *B. pertussis*, and not its heat-stable endotoxin, is responsible for the enhanced lethality observed when *B. pertussis* is given about 5 days before the application of cold. This is shown by the fact that only an unheated extract of *B. pertussis*, high in HSF activity[17], could mimic the sensitizing effect of whole cell vaccine[15] when administered 5 days before hypothermic stress. On the other hand, exposure of the *B. pertussis* fraction to 100 °C for 30 min to destroy the HSF activity[18] rendered it incapable of increasing mortality when injected 5 days before cold treatment. Similarly *S. typhosa* endotoxin was ineffective at this 5 day interval.

In confirmation of the previously cited work of BERRY[16], however, when *S. typhosa* endotoxin, and heated or unheated PE, which has been shown to contain endotoxin[19], were administered to mice immediately prior to cold exposure, significant sensitization to cold did occur.

The HSF of *B. pertussis*, like propranolol, is capable of blocking β-adrenergic receptors of the autonomic nervous system[8,12,13]. As a consequence, these agents are able to attenuate the normal hyperglycemic response to epinephrine[8] which has been shown to play an extremely important role in combating cold stress[20–23].

The induction of hypoglycemia is known to predispose to or actually cause hypothermia[24]. This effect has been explained by a decreased caloric production in the absence of normal levels of blood glucose[24]. This mechanism may well account for the increased vulnerability to cold exhibited by mice treated with insulin or with endotoxin. The dose of insulin used in the present experiments, 0.5 unit, induced a marked hypoglycemia (24.4 mg % ± 8.4 S.E.). Similarly, the blood sugar levels and glycogen reserves of mice poisoned with endotoxin are known to be markedly reduced and the thermoregulatory ability of such mice is greatly impaired[16,25]. All of these findings emphasize the important role played by carbohydrate metabolism in influencing the susceptibility of an organism to hypothermic stress. They also add further support for the general thesis that there is an inverse relationship between the glycemic state of a host and its susceptibility to a wide variety of stressful stimuli[1–14,20].

Effect of bacterial antigens, propranolol, and insulin on the susceptibility of CFW mice to cold exposure (3–6 °C)

Sensitizing agent	Dose	Interval[a]	Dead/ total[b]
PE[c]	45 μg N	5 days	9/10
PE[c]	45 μg N	none	10/10
PE, 100 °C, 30 min[c]	45 μg N	5 days	0/10
PE, 100 °C, 30 min[c]	45 μg N	none	10/10
Endotoxin[d]	100 μg	5 days	0/10
Endotoxin[d]	100 μg	none	10/10
Propranolol	1 mg	10 min	8/8
Insulin	0.5 unit	10 min	8/10
Saline	0.5 ml	10 min	0/10

[a] Between i.p. injection and exposure to cold. [b] Deaths tabulated 24 h after exposure to cold. At room temperature none of the preparations killed more than 1 mouse during a similar observation period. [c] Pertussis extract (containing both HSF and endotoxin). [d] From *Salmonella typhosa*.

Zusammenfassung. *Bordetella pertussis* hat zwei Komponenten, eine mit derselben Wirkung wie Endotoxin von *Salmonella typhosa* mit sofortiger kältesensibilisierender Wirkung, die andere mit einer um 5 Tage verspäteten Sensibilisierung. Beide Komponenten können hypoglykämisch wirken.

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