The Hypoglycemic Activity of Endotoxin
I. Occurrence in Animals Hyperreactive to Endotoxin
(33568)

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When given to an intact normal animal large doses of endotoxin may produce sequential hyperglycemia, glycogen depletion, and hypoglycemia (1–4). The reactivity of animals to endotoxin can be altered by several manipulations which may decrease the LD_{50} dose of endotoxin as much as 100–1000-fold. Among these manipulations are: (a) systemin infection with *Mycobacterium bovis* BCG[1] (5), (b) zymosan injections (6), (c) adrenalectomy (7), and (d) poisoning by carbon tetrachloride (8). In addition, during a brief period (11–15 days of age) the chick embryo has a marked susceptibility to endotoxin which is not found either before or after these dates (9). Data indicate that in some of these systems very small doses of endotoxin cause even greater depletion of carbohydrate reserves. Rapid glycogen depletion has been found in BCG mice given endotoxin (10), and when given to 11-day chick embryos or to guinea pigs poisoned with CCl_4, endotoxin in very small doses was found to produce marked hypoglycemia (8, 11).

Although these data suggest that altered carbohydrate metabolism is involved in endotoxin lethality, a direct cause–effect relationship has not been established. However, the experiments with carbon tetrachloride-poisoned guinea pigs and those with chick embryos have shown that the endotoxin induced hypoglycemia is itself of sufficient magnitude to explain many deaths.

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[1] Hereafter called BCG mice.

The observations above led us to investigate the exdotoxin induced hypoglycemic response of various animals made hyperrreactive to endotoxin to ascertain whether (a) profound hypoglycemia is a regular occurrence in endointoxicated hyperreactive animals, and (b) whether or not the phenomenon contributes to the overall mortality.

Materials and Methods. Animals. CD-1, pathogen-free, female mice obtained from Charles River Breeding Laboratories, North Wilmington, Mass., were used. Prior to experiments the mice were fasted overnight, unless otherwise indicated. New Zealand rabbits were obtained from a local breeder.

Endotoxin. The endotoxins were prepared in this laboratory using a smooth strain of *Salmonella typhimurium*. Cell walls of this bacterium were obtained using the combined procedures of pressure cell disruption, trypsin digestion, and repetitive washing. The clean cell walls were then extracted by the phenol–water procedure as described by Westphal *et al.* (12).

BCG infection. Animals were made hyperreactive to endotoxin by intravenous infection with *M. bovis* strain BCG as described by Suter and Kirsanow (5). The animals were challenged intravenously with endotoxin in 0.2 ml of saline, and at desired intervals groups were bled from the retroorbital plexus or were killed with chloroform and bled via cardiac puncture.

Blood glucose was determined in duplicate by the *o*-toluidine method described by Dubowski (13) or by Glucostat (Worthington Biochemical Corp.).

Zymosan treatment. Zymosan (Nutritional Biochemicals Corp.) was suspended in saline by boiling; and mice were injected ac-

TABLE I. Blood Glucose Concentration in Normal Mice after Endotoxin Challenge.[a]

Time after challenge (hr)	Blood glucose (mg/100 ml)
Control	136 ± 13.0[b] (4)[c]
2	130 ± 14.0 (4)
4	92 ± 12.0 (4)
6	88 ± 8.0 (4)
10	88 ± 8.0 (4)
At death	78 ± 9.4 (14)

[a] Mice were challenged i.v. with about 3 LD_{50}'s (800 μg) of endotoxin.
[b] Mean ± SE.
[c] Number of mice.

cording to the schedule described by Benacerraf *et al.* (6).

Adrenalectomy. Mice were adrenalectomized through dorsolateral incisions after ether anesthesia. They were subsequently maintained on isotonic saline and were used in experiments 3 days after surgery.

Results. Table I shows the effect of endotoxin on the blood glucose concentrations of normal mice and substantially confirms what has been found by others (1–4); namely, after endotoxin, blood glucose fell below the usual fasting level. In spite of this, the data show that, by and large, these animals were able to maintain an adequate blood sugar concentration and only a few became hypoglycemic (blood sugar <40 mg/100 ml) even at the time of death. In this experimental system, therefore, one cannot attribute death directly to hypoglycemia.

The response of the hyperreactive BCG mice was quite different. Table II illustrates the effect of endotoxin at three dose levels: 0.025, 0.1, and 1.0 μg. Even at the lowest dose given, there was a substantial decline in the mean blood glucose concentration over a 4-hr period, and with the higher doses hypoglycemia was produced. Since the profound hypoglycemia 4 hr after endotoxin was often accompanied by convulsions and death, it appeared that the hypoglycemic activity and the lethality of endotoxin in these animals might be causally related.

To illustrate this relationship better, the hypoglycemic activity of endotoxin and its lethality were studied during the development of hyperreactivity in BCG mice. It has been found that the susceptibility of mice to endotoxin increases exponentially after BCG infection, reaching a maximum after 10 days (14). The hypoglycemic effect of endotoxin was therefore assayed in mice on days 4, 7, and 10 after BCG infection. Groups of mice were also set aside to determine the mortality, which was tabulated as deaths occurring in 4 hr or less and mortality at 24 hr. The data in Table III show that with time after BCG infection, the increase in susceptibility to the lethal activity was accompanied by an increasing hypoglycemic response and a striking decrease in time to death.

This particular response to endotoxin is not a peculiarity of BCG mice. The BCG-infected rabbits also responded in a similar manner, as shown in Fig. 1. In this experiment three rabbits were given 2 ml of a 10-day broth culture of BCG intravenously. Ten days later these animals and three controls were challenged with 10 μg of endotoxin intravenously. The BCG-infected rabbits convulsed and died in 2–4 hr with hypoglycemia, whereas the control rabbits remained normoglycemic and survived.

Zymosan treatment and adrenalectomy, two other manipulations which render mice hypersusceptible to endotoxin, were studied

TABLE II. Blood Glucose Concentrations in BCG Mice after Endotoxin Challenge.

Time after challenge	Blood glucose (mg/100 ml) LD_0 0.025 μg	LD_{30} 0.1 μg	LD_{85} 1.0 μg
Control	114 ± 8.8[a] (7)[b]	126 ± 12.3 (7)	99 ± 5.0 (7)
30 min	99 ±12.7 (7)	85 ± 6.5 (7)	86 ±13.8 (7)
1 hr	91 ± 3.4 (7)	113 ± 8.8 (7)	69 ± 6.9 (7)
2 hr	68 ± 9.2 (7)	41 ± 9.2 (7)	30 ± 4.2 (7)
4 hr	69 ± 9.6 (7)	35 ± 15.0 (10)	10 ± 5.0 (7)

[a] Mean ± SE.
[b] Number of mice.

TABLE III. Blood Glucose Concentrations after Endotoxin Challenge during Development of Hyperreactivity in BCG Mice.

Time after BCG (days)	Fasting blood glucose (mg/100 ml)	Blood glucose (mg/100 ml) 3–4 hr after 1.0 μg of endotoxin	Deaths/total	
			<4 hr	24 hr
0	155 ± 6.7[a] (5)[b]	89 ± 8.5 (5)	0/10	0/10
4	135 ± 7.1 (5)	100 ± 7.6 (5)	1/10	3/10
7	142 ±10.7 (5)	27 ± 9.4 (5)	4/10	7/10
10	106 ± 7.1 (5)	7 ± 1.3 (5)	9/10	9/10

[a] Mean ± SE.
[b] Number of mice.

to see whether these would also lead to a hypoglycemic response to endotoxin. Groups of mice were given five doses of 1 mg of zymosan intravenously on alternate days and 1 day after the last dose were challenged with 20 μg of endotoxin. As shown in Table IV, almost half the challenged mice died within 4 hr with convulsions, and at death had a mean blood glucose of 10 mg/100 ml. Adrenalectomized mice were also unable to maintain an adequate blood glucose concentration and were quite hypoglycemic at the time of death (Table V). The hypoglycemia in these animals, however, was neither as severe nor as rapid in development as that of BCG-infected mice, in spite of the fact that

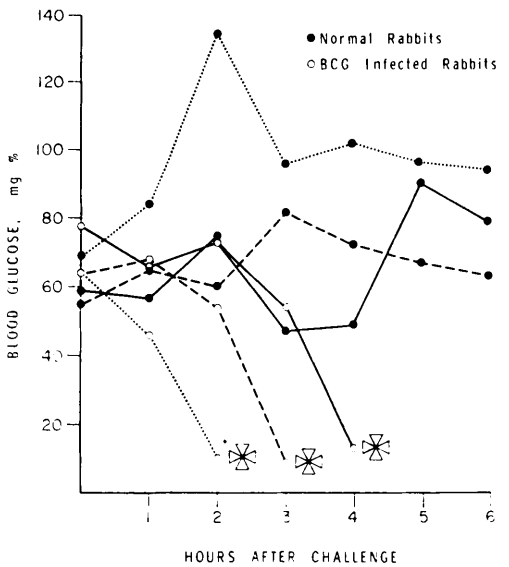

FIG. 1. Blood glucose concentrations in normal and BCG-infected rabbits. All rabbits were given 10 μg of endotoxin i.v. at 0 time.

TABLE IV. Blood Glucose Concentrations in Zymosan-Treated Mice after Endotoxin Challenge.[a]

Time after challenge (hr)	Blood glucose (mg/100 ml)
Control	132 ± 9.1[b] (14)[c]
4	56 ± 8.8 (15)
At death[d]	10 ± 1.4 (12)

[a] Mice were challenged i.v. with 20 μg of endotoxin.
[b] Mean ± SE.
[c] Number of mice.
[d] All deaths occurred prior to 4 hr, frequently with convulsions.

the LD_{50} was approximately the same. This was also reflected in the clinical observations that the adrenalectomized mice tended to die later (>6 hr) and only occasionally had seizures. This suggests that the seizures and early deaths in BCG-infected mice resulted from the rapidity with which the blood glucose fell.

If death in hyperreactive mice is directly related to endotoxin induced hypoglycemia, it

TABLE V. Blood Glucose Concentrations in Adrenalectomized Mice after Endotoxin Challenge.[a]

Time after challenge (hr)	Blood glucose (mg/100 ml)
Control	98 ± 4.9[b] (7)[c]
3	59 ± 10.6 (7)
6	37 ± 5.7 (7)
At death	22 ± 3.4 (9)

[a] Mice were challenged i.v. with 1.0 μg endotoxin (LD_{80}).
[b] Mean ± SE.
[c] Number of mice.

would be logical to assume that survival would be improved by the administration of glucose. Efforts to show this have met with variable success, in part because of the very large and frequent doses of glucose which must be given. In our hands, the administration of 100 mg of glucose intravenously every hour to challenged BCG mice was of questionable benefit. One of 10 mice given glucose died prior to 7 hr, whereas 5 of 10 controls convulsed and died during the same period. Rough quantitation of blood glucose prior to each dose was performed with Dextrostix (Ames Co.) and a drop of blood. None of the animals was hyperglycemic at these times, and some were severely hypoglycemic. Treatment was stopped at 8 hr and by 24 hr all the animals in both groups were dead.

Protection of endointoxicated mice by glucose was approached in one other way. It was reasoned that the induction of a diabetic state with alloxan, thereby causing hyperglycemia, decreased glucose utilization and increased gluconeogenesis, might provide some protection against endotoxin in BCG mice. We were unable to document significant protection in alloxan-treated animals. However, experiments with alloxan diabetic BCG mice showed that lethality and hypoglycemia did not necessarily accompany one another. In one experiment BCG mice were made diabetic by the i.v. administration of 130 mg/kg of alloxan hydrate (Mann Lab). Three days later 5 mice were selected randomly as controls and the remainder were given 4.0 μg of endotoxin i.v. When the mice became moribund they were bled and the blood glucose concentrations were determined. The data showed that the mice which died in less than 5 hr died with hyperglycemia. Those which died in the ensuing hour had a pronounced fall in blood glucose concentration but were essentially normoglycemic at death.

Discussion. The data show that endotoxin induced severe hypoglycemia in BCG mice and rabbits. This effect was also evident, but to a lesser extent, in zymosan-treated and in adrenalectomized mice. In the BCG and in the zymosan-treated mice, the hypoglycemia produced was often of sufficient magnitude to be a direct cause of death. This was supported by the clinical behavior of these mice, i.e., convulsive seizures followed by death.

Our data and the data of others (8, 11) suggest that a hypoglycemic response is a common denominator in those experimental systems characterized by a marked susceptibility to endotoxin. The evidence indicates, however, that it is but one manifestation of hyperreactivity. Attempts to save endointoxicated mice by maintaining an adequate blood glucose level have certainly not achieved overwhelming success. Reports by Berry *et al.* (10) and by Farrar and Watson (8) indicate that the administration of exogenous glucose prolongs life but has little effect on the ultimate mortality. Our experience with glucose adminstration was quite similar, and in our observations it became abundantly clear that although seizures could be prevented, mice which were given glucose still appeared to be as sick as the controls. Indeed, in one experiment, nonfasted, alloxan diabetic, BCG mice were killed in 4 to 5 hr with 4 μg of endotoxin, and although there was a fall in blood glucose concentrations, they did not become hypoglycemic, nor did they have seizures. Therefore, the hyperreactivity must involve mechanisms, other than the hypoglycemic one, which are lethal but perhaps compatible with longer survival.

Is the hypoglycemic activity of endotoxin due to some direct intervention into intermediary metabolism or is the effect mediated via some other physiological disturbance? For instance, it is known that irreversible hemorrhagic shock will lead to hypoglycemia and glycogen depletion (15), and is it possible that the hypoglycemia of endointoxicated BCG mice is effected via a drastic reduction in blood pressure? At present we have no data on the blood pressure responses of mice to endotoxin. However, to our knowledge the consistent and profound hypoglycemia that we have observed has not been found after hemorrhagic shock. Strawitz *et al.* (15) found that the mean blood glucose of rats subjected to hemorrhagic shock was 82 mg/100 ml at death with a range from 28 to

171 mg/100 ml. This is quite similar to our observations in normal mice given endotoxin but is unlike the response of BCG mice. Moreover, our concern with irreversible hypotension leading to hypoglycemia may be irrelevant, since it has been postulated, with some evidence, that the irreversible phase of hemorrhagic shock is a result of absorbed endotoxin (16).

The mechanism by which endotoxin produces hypoglycemia in BCG mice has not been defined. The possibilities that (a) endotoxin induces a hypermetabolic state, (b) causes insulin release or hypersensitivity, or (c) causes a block in gluconeogenesis in BCG mice have been investigated and will be the subject of a separate publication.

Summary. Mice were made hyperreactive to endotoxin by BCG infection, zymosan injections, and by adrenalectomy, and the effect of endotoxin on the blood sugar concentration of these animals was determined. All of the mice developed hypoglycemia after small doses of endotoxin and in the BCG mice and in the zymosan-treated mice the hypoglycemia was often of sufficient magnitude to cause death. Efforts to save endointoxicated BCG mice by intermittent glucose administration were unsuccessful, and experiments with diabetic mice showed that the hypoglycemic and lethal effects of endotoxin were not necessarily associated with one another.

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