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Hypoglycemic Activity of Endotoxin

II. Mechanism of the Phenomenon in BCG-infected Mice

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The mechanism of the hypoglycemic activity of endotoxin in hyperreactive BCG mice was investigated. The mechanism was found to be an inhibition of the synthesis of glucose from noncarbohydrate sources. The possibilities of an induced hypermetabolic state and an induced release of insulin in response to endotoxin as causes for the hypoglycemic response were essentially ruled out. In addition, no clear-cut evidence of an insulin-like action by endotoxin was found in the in vivo setting.

In another paper, we described the hypoglycemic effect of endotoxin when administered to mice rendered hyperreactive to endotoxin by *Mycobacterium bovis* BCG infection (hereafter called BCG mice) by zymosan injections, or by adrenalectomy (J. W. Shands, Jr., et al., Proc. Soc. Exp. Biol. Med., *in press*). Unlike normal mice which die after 18 to 20 hr and which have relatively low blood glucose concentrations at death, zymosan-treated mice and particularly BCG mice tend to die in less than 4 hr, frequently with convulsions, and to manifest severe hypoglycemia. The intravenous administration of 1.0 μg of endotoxin to BCG mice reduced the blood sugar concentrations from a mean of 99 mg/100 ml to a mean of 10 mg/100 ml in 4 hr time, and 0.025 μg of endotoxin produced a significant depression of blood glucose concentration.

Basically, the endotoxin-induced hypoglycemia that we have observed in BCG mice could result from two general metabolic derangements, either an increase in glucose consumption or a decrease in glucose production, or perhaps from a combination of these factors. Observations of other investigators suggested that the former possibility was the correct one. Endotoxin has been found to cause an uncoupling of oxidative phosphorylation (3, 12), and conceivably BCG mice might be much more susceptible than normal mice to this phenomenon. Therefore, they might exhaust their glucose rapidly because of a general hypermetabolic state. Alternatively, because endotoxin has been reported to have an insulin-like action (17), the endotoxin-induced hypoglycemia might result from a selective stimulation of glucose utilization. This latter possibility was particularly attractive since in BCG mice there is a marked hyperplasia of the reticuloendothelial system, and some of the cells of this system not only remove endotoxin from the circulation (7, 13) but also increase their glycolytic rate in its presence (18).

In this paper, we report the results of experiments which were designed to clarify the mechanism or mechanisms involved in the production of hypoglycemia. Since the responses of BCG mice and normal mice to lethal doses of endotoxin are so strikingly different, we attempted to compare the responses of these two groups in the expectation that the differences would be reflected in one or more of the pathways of intermediary metabolism.

MATERIALS AND METHODS

Animals. CD-1, pathogen-free, female mice, weighing 20 to 25 g and obtained from Charles River Breeding Laboratories, North Wilmington, Mass., were used in this study. Before use in experiments, the mice were fasted overnight, unless otherwise indicated.

Endotoxin. The endotoxins were prepared in this laboratory from a smooth strain of *Salmonella typhimurium*. Cell walls of this bacterium were obtained with the combined procedures of pressure cell disruption, trypsin digestion, and repetitive washing. The clean cell walls were then extracted by the phenol-water procedure described by Westphal, Lüderitz, and Bister (16).

BCG infection. Animals were made hyperreactive to endotoxin by intravenous (iv) infection with *M. bovis* strain BCG as described by Suter and Kirsanow (15) and were used in experiments 10 to 14 days later.

Endotoxin challenge was by the iv route in 0.2 ml of saline. At various intervals thereafter, groups of mice were bled from the retroorbital plexus or were killed with chloroform and bled via cardiac puncture. Blood lactates and pyruvates were determined by the methods described in Sigma Technical Bulletins no. 825-UV and 725, respectively. Blood glucose was determined in duplicate by the *o*-toluidine method described by

Dubowski (8) or with Glucostat (Worthington Biochemical Corp.).

Temperatures. Visceral temperatures of mice were taken via rectal probe attached to an electric universal thermometer (type TE 3; Elektrolaboratoreit A/S, Copenhagen Denmark). Mice were adapted to the laboratory temperature (25 + 2 C) in separate cages for 1 day before the experiments.

Oxygen consumption. Oxygen consumption rates of mice were determined in a respirometer consisting of a flask to hold the mouse and calibrated siliconized glass tubes. The flask containing the mouse was immersed in a water bath at 30 C. To absorb CO_2, 15 g of soda lime was added and the apparatus was gassed with 100% oxygen. A drop of water was placed at the end of the glass tube, and the amount of oxygen consumed was calculated from the distance the drop moved down the tube.

Glucose clearance. Groups of fasted normal and BCG mice and similar groups of mice given endotoxin iv 1 hr previously (400 and 1 μg, respectively) were injected iv with 0.2 μc of ^{14}C-glucose (U) with 1 mg of glucose carrier. At 4-min intervals, 0.02 ml of blood was obtained from the retroorbital plexus and deproteinized in 0.25 ml of 3% trichloroacetic acid. The samples were centrifuged, and 0.1 ml of the supernatant fluid was added to 10 ml of Bray's solution (4) and was counted in a Packard scintillation spectrometer (model 3002).

$^{14}CO_2$ evolution. Mice were adapted to a gas train consisting of 50-ml syringes through which air was pulled and bubbled through 5 ml of NCS^{tm} (Nuclear-Chicago Corp.). Groups of normal and BCG mice and similar groups challenged with endotoxin 1 hr previously were given 0.1 to 0.2 μc ^{14}C-labeled substrate, and the evolved $^{14}CO_2$ was collected for four consecutive 15-min periods. A 0.5-ml sample of the NCS was added to 20 ml of PPO Spectrofluor (Nuclear-Chicago Corp.; 10 g/liter) and counted in a Packard scintillation spectrometer (model 3002).

Gluconeogenesis. Gluconeogenesis was measured in normal and in BCG mice and in similar groups of mice poisoned with endotoxin (400 μg for normal and 1.0 μg for BCG mice). One hour after the endotoxin poisoning, blood samples were taken for glucose determinations. The mice were then injected intraperitoneally with 20 or 100 μM sodium pyruvate containing 3 μc of sodium pyruvate-*2*-^{14}*C*. Paired blood samples were subsequently obtained from each mouse. One of the paired samples was used to determine glucose concentration. The other (0.05 ml) was placed on a mixed-bed resin column (0.3 by 8 cm) consisting of Dowex-50-X8 (100 to 200 mesh) in the H^+ form and Dowex-1-X8 (100 to 200 mesh) in the HCO_3^- form, a procedure similar to that reported by Corredor et al. (6). A 0.1-ml sample of the eluate was added to 10 ml of Bray's solution and was counted in a Packard scintillation spectrometer model 3002. The remaining eluates of several samples were spotted on paper and chromatographed in *n*-butyl alcohol-acetic acid-water beside a known glucose sample to ascertain that the counts obtained were in glucose. The columns were also tested for their ability to retain pyruvate. When 0.1 μc of sodium pyruvate-*2*-^{14}*C* was placed on a column, no counts above background were found in the eluate.

Radiochemicals. We obtained the following radiochemicals from Nuclear-Chicago Corp: sodium *n*-butyrate-*1*-^{14}*C*, 15.0 mc/mmole; sodium pyruvate-*1*-^{14}*C*, 11.8 mc/mmole; sodium pyruvate-*2*-^{14}*C*, 31 mc/mmole; and ^{14}C-D-glucose (U), 307 mc/mmole.

RESULTS

Several experiments were performed to assay the possibilities of an endotoxin-induced hypermetabolism in BCG mice or a stimulation of glucose utilization. First, both oxygen consumption and visceral temperatures were measured in normal and in BCG mice before and after endotoxin administration. The basal oxygen consumption for normal mice at 30 C and at a barometric pressure of 760 mm of Hg was 2.56 $\pm$ 0.45 ml of O_2 per g per hr and that for BCG mice was 2.79 $\pm$ 0.3 ml of O_2 per g per hr. Neither value changed significantly after the administration of endotoxin. In Fig. 1, the effect of endotoxin on the visceral temperatures of normal and BCG mice is presented. Normal mice given approximately three LD_{50} of endotoxin sustained a drop in temperature as Berry et al. previously reported (3). The mean basal temperature of the BCG mice was similar to that of the normal mice, and this value dropped considerably after endotoxin administration, some mice attaining temperatures below 28 C before death. Therefore, BCG mice appeared to have an augmented hypothermic response to endotoxin.

Second, the rate of utilization of glucose was quantitated in two ways. First, by measuring the rate of removal of ^{14}C-glucose from the peripheral circulation of mice before and after a lethal dose of endotoxin and, secondly, by measuring the rate of oxidation of ^{14}C-glucose to $^{14}CO_2$. Figure 2, a log plot of glucose clearance with time, shows no

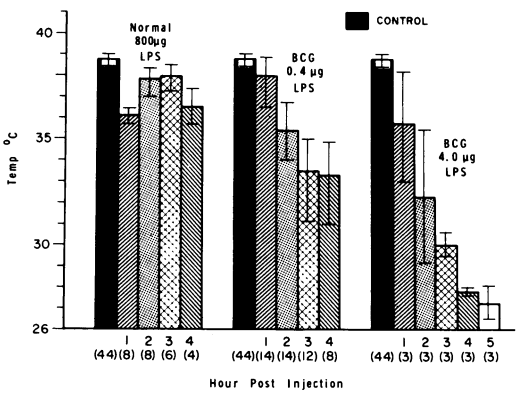

FIG. 1. *Temperature response of normal and BCG mice to endotoxin.*

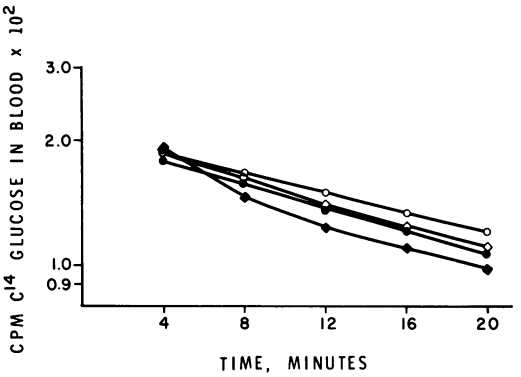

FIG. 2. *Clearance of ^{14}C-glucose (U) from the blood of normal mice (closed circles), normal mice given 400 μg of endotoxin iv 1 hr previously (open circles), BCG mice (open squares), and BCG mice given 1.0 μg of endotoxin IV 1 hr previously (closed squares). Each point represents the mean counts per min per 0.3 μliter of blood from six mice.*

appreciable differences in the clearance rates of normal mice, BCG mice, or similar groups of mice given lethal doses of endotoxin. Figure 3 shows the cumulative evolution of $^{14}CO_2$ from normal and BCG mice fasted for 18 hr and the $^{14}CO_2$ evolved from similar groups of mice given a lethal dose of endotoxin (400 and 1.0 μg) 1 hr previously. The rates of glucose oxidation in the normal and BCG control mice were essentially the same, and it is apparent that endotoxin did not stimulate glucose oxidation but tended to depress it, particularly in normal mice. Figures 4 and 5 show the effect of lethal doses of endotoxin on the oxidation of pyruvate-*1*-^{14}C and butyrate-*1*-^{14}C. There was no effect on the oxidation of these substrates in normal mice, and a 15 to 20% reduction was effected in BCG mice (*P* values: butyrate, < 0.01; pyruvate, < 0.05).

Third, Table 1 presents data comparing the blood glucose, lactate, and pyruvate levels of fasted BCG mice given endotoxin. There was no difference in the lactate or fasting glucose concentrations of BCG versus normal mice, and there was an insignificant difference in pyruvate concentrations. In BCG mice given endotoxin, blood lactate was unchanged, whereas pyruvate rose about twofold and glucose declined.

Although the data on glucose utilization did not support the concept, it was conceivable that the endotoxin-induced hypoglycemia of BCG mice could be the result of an endotoxin-induced release of insulin or of an abnormal sensitivity to insulin. The first possibility was investigated by use of diabetic mice. Both normal and BCG mice were made diabetic by the iv administration of 130 mg of alloxan hydrate (Mann Research Laboratories, New York, N.Y.) per kg. After 5 days, mice with blood sugars greater than 250 mg/100 ml (determined by Dextrostix, Ames Co., with a drop of tail blood) were selected. After fasting for 3 hr, "normal" diabetic and BCG diabetic mice were challenged with 800 and 4.0 μg of endotoxin, respectively. Controls and challenged animals were randomly selected. All of the animals were killed 4 to 5 hr after challenge, and blood glucose concentrations were determined. No significant difference in the blood glucose concentrations of the controls and challenged animals in the "normal" diabetic group was found. Figure 6 shows the results from the BCG diabetic mice. In the challenged group, most of the mice had glucose levels below the lowest of the controls and some had levels as low as 5 mg/100 ml. With the Mann-Whitney approximation, the difference between the control and challenged groups was significant at the 0.5% level. Therefore, it appears that the hypoglycemic action of endotoxin does not depend on an endogenous supply of insulin.

BCG mice were not abnormally reactive to

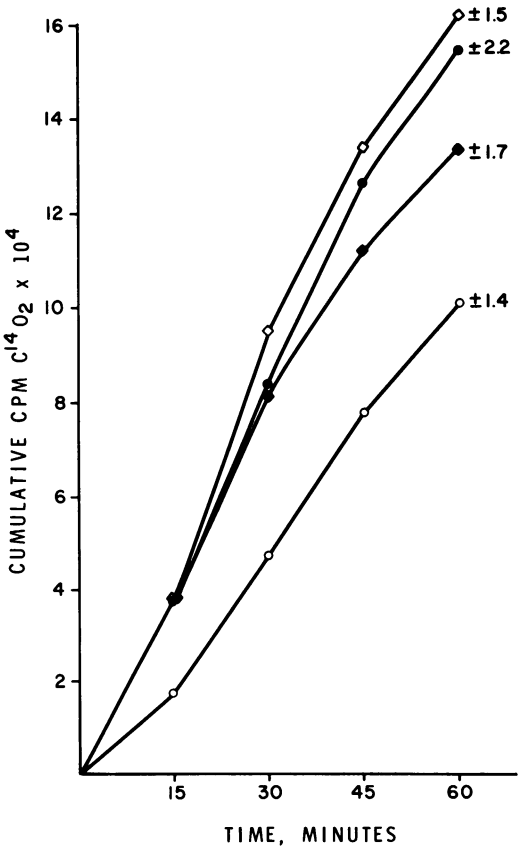

FIG. 3. *Evolution of $^{14}CO_2$ from fasted mice given 0.2 μc of ^{14}C-glucose (U) iv. Key same as that in Fig. 2.*

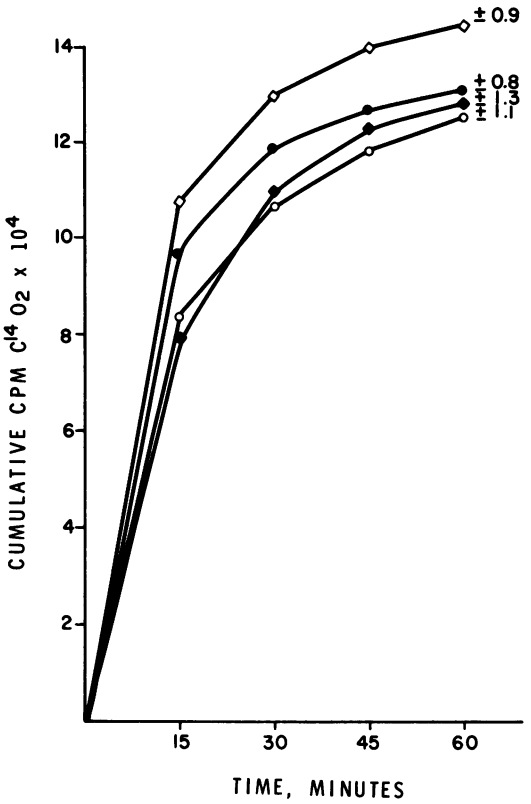

FIG. 4. *Evolution of $^{14}CO_2$ from fasted mice given 0.1 μc of sodium pyruvate-1-^{14}C iv. Key same as that in Fig. 2.*

insulin (Table 2). After graded doses of insulin (Iletin, regular; Eli Lilly & Co.), the depression of blood glucose at 30 min was similar for normal and BCG mice. This experiment also points out the interesting fact that in BCG mice insulin has less hypoglycemic activity than an equivalent weight of endotoxin (assuming 22 μg of insulin/unit).

Insulin tolerance was also assayed by a standard insulin tolerance test to compare the responses of normal, BCG, and adrenalectomized mice (Table 3). When compared with normal mice, BCG mice had only a minor impairment in their ability to recover from a large dose of insulin. In contrast, adrenalectomized mice given the same dose of insulin became hypoglycemic in 20 min and many died with convulsions.

Since the above data failed to support the possibilities of induced hypermetabolism, insulin release, or insulin-like activity as mediators of endotoxin hypoglycemia in BCG mice, the possibility of defective gluconeogenesis was investigated. A strong suggestion that endotoxin did impair the synthesis of glucose from noncarbohydrate sources was found in two experiments with D-mannoheptulose. This compound produces a transient diabetic state by blocking insulin release and, therefore, stimulates gluconeogenesis

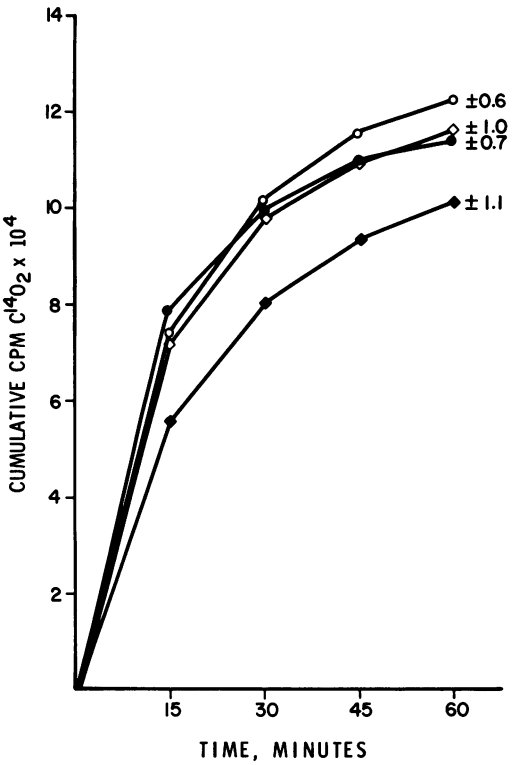

FIG. 5. *Evolution of $^{14}CO_2$ from fasted mice given 0.1 μc of sodium butyrate-1-^{14}C iv. Key same as that of Fig. 2.*

TABLE 1. *Blood lactate, pyruvate, and glucose concentrations in BCG mice after endotoxin challenge*[a]

Time after challenge (hr)	Lactate (mg/100 ml)	Pyruvate (mg/100 ml)	Glucose (mg/100 ml)
Control	22 ± 2.0[b] (18)[c]	1.05 ± 0.05 (18)	118 ± 3.6 (18)
1	22 ± 0.9 (24)	2.02 ± 0.05 (24)	83 ± 6.5 (24)
4	19 ± 0.04 (33)	1.88 ± 0.06 (33)	33 ± 3.5 (33)
Normal mice	26 ± 0.05 (15)	0.85 ± 0.04 (15)	118 ± 2.6 (15)

[a] Mice were challenged iv with 1.0 μg of endotoxin.
[b] Mean ± standard error.
[c] Number of mice.

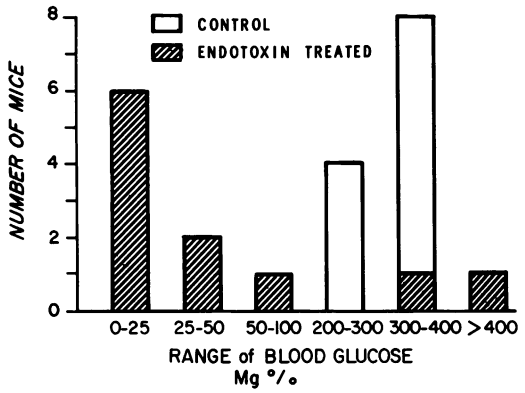

FIG. 6. *Blood glucose concentrations of fasted alloxan diabetic BCG mice and of diabetic BCG mice given 4.0 μg of endotoxin 4 to 5 hr previously.*

TABLE 2. *Insulin tolerance of normal and BCG mice*[a]

Amt of insulin (unit)	Blood glucose (mg/100 ml)	
	Normal	BCG
0.0	164 ± 7.0[b] (4)[c]	110 ± 4.5 (4)
0.1	89 ± 10.5 (4)	62 ± 1.5 (4)
0.2	79 ± 3.5 (4)	56 ± 3.5 (4)
0.4	59 ± 7.0	52 ± 3.5 (4)

[a] Blood glucose was measured 30 min after iv administration of insulin.
[b] Mean ± standard error.
[c] Number of mice.

(14). When 40 mg of mannoheptulose was administered iv to fasted BCG mice, a low fasting glucose concentration was elevated to well over the usual fasting level by 1 hr (Fig. 7). In contrast, mice given a lethal dose of endotoxin (1 μg) 1 hr previously failed to respond. In a similar experiment with fewer mice and an LD_{60} of endotoxin (0.1 μg), more striking results were obtained (Fig. 8). Unchallenged BCG mice sustained marked elevations of blood glucose concentration after the administration of D-mannoheptulose. Three challenged BCG mice did not respond to mannoheptulose and died, whereas two responded normally and survived.

To confirm this postulated defect in gluconeogenesis, experiments were performed to quantitate the incorporation of pyruvate-2-^{14}C into blood glucose both in normal and in BCG mice and also to document in these mice the effect of a pyruvate load on blood glucose concentrations. Table 4 shows the results of three experiments comparing the responses of normal and BCG mice. The doses of pyruvate given were 100 μmoles in experiment 1 and 20 μmoles in experiments 2

TABLE 3. *Insulin tolerance of normal, BCG, and adrenalectomized mice*[a]

Time (min)	Blood glucose (mg/100 ml)		
	Normal	BCG-infected	Adrenalectomized[b]
Control	129 ± 4.8[c] (5)[d]	114 ± 6.3 (5)	131 ± 9.0 (5)
20	72 ± 9.8 (5)	55 ± 2.7 (5)	20 ± 4.0 (5)
30	61 ± 9.8 (5)	58 ± 3.1 (5)	31 ± 8.1 (5)
45	76 ± 5.4 (5)	49 ± 3.1 (5)	15 ± 2.2 (5)
60	53 ± 7.2 (5)	49 ± 5.8 (5)	
90	59 ± 13.9 (5)	49 ± 4.5 (5)	17 ± 4.5 (5)
180	113 ± 12.1 (5)	61 ± 9.8 (5)	

[a] Mice were given 0.2 unit of crystalline insulin vi in 0.2 ml of saline.
[b] About one-third of the challenged mice died prior to 90 min.
[c] Mean ± standard error.
[d] Number of mice.

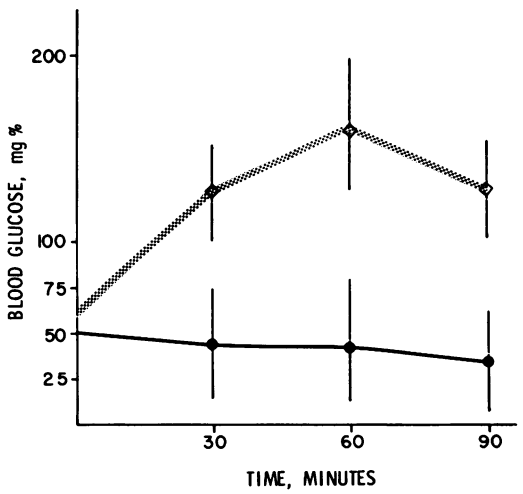

FIG. 7. *Effect of mannoheptulose on the blood glucose concentrations of fasted BCG mice. BCG mice given 40 mg of D-mannoheptulose iv at zero-time (dotted line); BCG mice given 40 mg of D-mannoheptulose iv 1 hr after a 1.0-μg dose of endotoxin (solid line). Each point represents the mean value from 10 mice.*

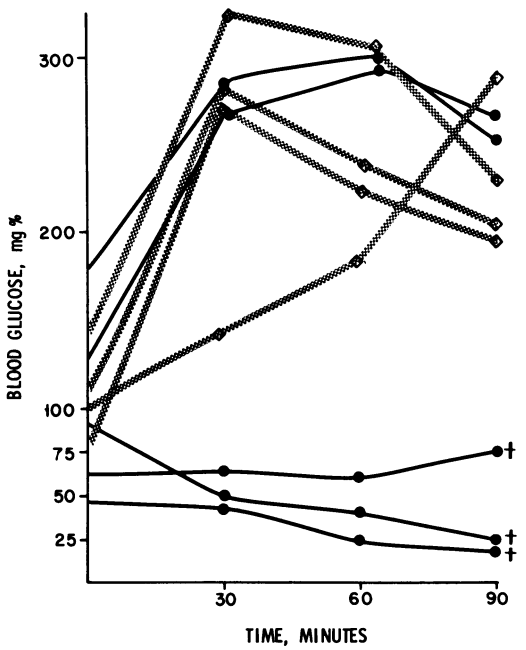

FIG. 8. *Effect of mannoheptulose on the blood glucose concentration of BCG mice. Key same as Fig. 7, except that the dose of endotoxin was 0.1 μg. Each line represents determinations from one mouse.*

and 3. It is evident that the rates of incorporation of pyruvate into blood glucose in normal and in BCG mice were similar. One hour after a lethal dose of endotoxin, normal mice were still able to incorporate pyruvate into glucose at a normal rate. However, in BCG mice the rate of incorporation fell significantly 1 hr after a lethal dose of endotoxin. The fluctuations in the blood glucose concentrations during these experiments were also of interest. The blood glucose concentrations of both the control and endotoxin-poisoned normal mice in experiment 1 rose precipitously and, 20 min after pyruvate administration, reached mean levels of 242 and 280 mg/100 ml, respectively. The BCG control mice of experiment 1 also sustained an elevation of mean blood glucose but only to 160 mg/100 ml. On the other hand, in each of the three experiments the endotoxin-poisoned BCG mice not only failed to elevate their blood glucose concentrations after pyruvate administration, but in each case actually sustained a net loss of blood glucose.

DISCUSSION

These studies show that the hypoglycemic response of BCG mice to endotoxin cannot be explained by the induction of a hypermetabolic state. The results of the experiments on oxygen consumption, visceral temperatures, and the rates of oxidation of various substrates after endotoxin administration essentially rule out this possibility. Therefore, the uncoupling of oxidative phosphorylation that endotoxin has been found to cause in vitro (3, 12) would appear to play a minor or insignificant role in the hypoglycemic response. Similarly, our data do not demonstrate that endotoxin possesses any clear-cut, insulin-like activity in the in vivo setting. The rate of glucose "clearance" from blood showed no appreciable differences between normal and BCG mice and similar mice poisoned with endotoxin. The validity of the clearance experiment might be questioned, since the procedure did not exclude products of intermediary metabolism which, although low in concentration in blood relative to glucose, would contain the ^{14}C label (other than CO_2 which was removed by acidification). The curves, therefore, really represent the equilibration of ^{14}C-labeled blood glucose with carbohydrate in tissue compartments and with pyruvate and lactate released into the circulation. Consequently, it is

TABLE 4. *Glucogenesis in BCG and normal mice*[a]

Expt	Time after pyruvate addition	Mean counts/min of ^{14}C-glucose				
		Normal mice (per ml of blood)		BCG mice (per ml of blood)		
		Control	With endotoxin	Control	With endotoxin	*P* values
	min	× 10^4	× 10^4	× 10^4	× 10^4	
1	10	6.1 ± 0.9[b]	5.5 ± 0.9	6.1 ± 0.4	4.7 ± 0.2	<.05
	20	6.6 ± 0.9	7.2 ± 0.8	6.9 ± 0.6	3.8 ± 0.5	<.01
2	15			5.6 ± 0.9	2.4 ± 0.9	<.001
	30			4.2 ± 1.0	2.1 ± 1.0	<.01
3	30			3.9 ± 1.0	1.2 ± 0.6	<.001

[a] Incorporation of ^{14}C label from pyruvate-2-^{14}C into glucose.
[b] Mean ± standard error.

more accurate to state that the rates of equilibration of blood glucose in the various groups of mice were essentially the same. This being so, it is difficult to conceive of a marked difference in utilization.

The data on lactates and pyruvates also fail to incriminate stimulated glucose utilization as a cause of hypoglycemia. In endotoxin-poisoned BCG mice, lactate concentrations failed to rise whereas glucose concentrations fell. The increase in serum pyruvate in BCG mice could be interpreted to signify enhanced glycolysis, but could also be explained by the rapid glycogen breakdown that occurs (2), by the slight decrease in pyruvate oxidation (Fig. 4), and perhaps by a block in one of the steps in gluconeogenesis, namely, the carboxylation of pyruvate to form oxalacetate. These data do not allow a categorical statement that enhanced glycolysis plays no role in the endotoxin hypoglycemia of BCG mice. However, they suggest that if enhanced glycolysis does play a role it is a minor one.

Other possible causes for the hypoglycemic response have been ruled out. Glycosuria did not occur in BCG mice after endotoxin challenge, nor was there any histological evidence of adrenal damage. In addition, we found no evidence in BCG mice of a "shunting" of glucose into a storage compartment. Like Berry et al. (2), we found that liver glycogen stores were rapidly depleted and that there was negligible incorporation of ^{14}C label from glucose into liver fat.

The major defect leading to endotoxin hypoglycemia appears to be in glucose synthesis from noncarbohydrate sources, and evidence for such a defect was shown in three ways. First, endotoxin-poisoned BCG mice failed to raise their blood sugars after administration of D-mannoheptulose, which should stimulate gluconeogenesis. Second, the incorporation of radiolabeled pyruvate into blood glucose was decreased in endotoxin-poisoned BCG mice; third, these animals failed to raise their blood sugar after a pyruvate load.

The probable course of events in these mice is as follows. After endotoxin administration, the synthesis of new glucose is impaired. Blood glucose is maintained at normal levels for approximately 1 hr by the residual gluconeogenesis and by any reserves of liver glycogen present. After exhaustion of liver stores, the mice become hypoglycemic with the first significant drop in blood glucose, usually occurring 1.5 to 2 hr after challenge. In BCG mice, this series of events is aggravated by at least one additional factor, namely, their carbohydrate reserves are consistently lower than those of normal mice (2).

It is quite possible that impaired gluconeogenesis as a cause of hypoglycemia may be common to most, if not all, animals which are hyperreactive to endotoxin. This is true for adrenalectomized mice, BCG mice, and possibly for the 11-day chick embryo which in some respects behaves like an adrenalectomized animal. The same phenomenon may also pertain to CCl_4-poisoned animals which were found to have normal peripheral clearance of glucose after endotoxin challenge and decreased fatty acid oxidation (9, 10). A defect in fatty acid oxidation might well lead to a critical lack of energy and reduced nicotinamide adenine dinucleotide (NADH) necessary for new glucose synthesis.

Why BCG infection should so predispose animals to the hypoglycemic action of endotoxin is unknown, although there is a rather extensive literature on the biochemical alterations caused by tuberculous infection, i.e., a decrease in nicotinamide adenine dinucleotide-linked enzymes, etc. (1, 5). Our metabolic studies have revealed no striking differences between BCG and normal mice. However, after endotoxin challenge the oxidative metabolism of normal mice did differ from that of the BCG mice. Lethally poisoned normal mice continued to oxidize most substrates at a normal rate but manifested a pronounced decrease in glucose oxidation. Unlike the apparently selective abnormalities in normal mice, challenged BCG mice sustained a slight decline in oxidation of all substrates. We need additional data for the proper interpretation of these observations. However, the decline in substrate oxidation in challenged BCG mice is quite compatible with their drop in visceral temperature. The selective abnormalities in normal mice are more difficult to interpret and, since our data showed that glucose clearance was unchanged in these mice when given endotoxin, it is possible that glucose may be shunted into some alternate pathway.

One difference between BCG and normal mice reported by Berry et al. (2) and previously mentioned in this paper is that BCG mice have less carbohydrate reserve than normal mice. The reason for this difference is not clear. However, it is doubtful that a defect in gluconeogenesis is responsible, since our data show a normal rate of synthesis of glucose from pyruvate, and, in addition, BCG mice are not abnormally sensitive to insulin. It is possible that BCG mice have a defect in glycogen synthesis to account for their decreased stores. Such a defect, however, and the consequent reduction in glycogen stores could not by themselves account for the endotoxin hypoglycemia we have observed, since unchallenged BCG mice can be fasted beyond exhaustion of their glycogen stores without becoming hypoglycemic.

The failure to observe a defect in gluconeogenesis in lethally endotoxin-poisoned normal mice warrants some comment, particularly since a recent report by LaNoue et al. (11) described such a defect in liver slices from endotoxin-poisoned rats. In all probability, the discrepancy results from the timing of the metabolic studies. Our studies were performed 1 hr after a lethal dose of endotoxin, at which time the lethal effect had been set in motion and after which the mice could not be salvaged. However, during this period the mice were not severely ill. In contrast, the studies by LaNoue et al. (11) were performed on liver slices taken from rats at a time very close to the mean time until death.

At present, studies are underway to clarify the nature of the defect in gluconegenesis. Whether specific enzymes are at fault, whether long chain fatty acid oxidation is abnormal, or whether specific cofactors such as NADH are lacking are problems to be investigated.

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