SYMPOSIUM ON BACTERIAL ENDOTOXINS[1]

III. Metabolic Effects of Endotoxins on Mammalian Cells

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The endotoxins of gram-negative bacteria in minute amounts elicit an array of characteristic physiological alterations in mammals including such diverse phenomena as production of fever (1, 3), stimulation of resistance to infection (12, 21, 29), and protection against radiation injury (26). Higher dosage induces a state of stress, acute shock, or eventual death. In tumor-bearing animals, extensive intratumoral hemorrhage is associated with necrotization of the malignant tissue (25). In the present paper the occurrence of a possible metabolic common denominator in this wide range of phenomena will be considered.

Previous work (10, 31, 32) has demonstrated an insulin:anti-insulin hormonal mechanism that regulates the rate of glycolysis in certain tumors and normal tissues. Three years ago, as the result of metabolic studies in vitro in a series of experimental tumors, we observed that endotoxins exerted a stimulatory effect on cell glycolysis remarkably similar to the action of insulin (33). Endotoxin in concentrations of 0.003 to 0.3 μg per ml stimulated the formation of acid aerobically from glucose as much as severalfold; at the same time, oxygen uptake remained essentially unaffected. Anaerobic glycolysis was stimulated to a lesser extent.

In certain tumors, glycolysis and growth are inhibited by a state of stress in the host (10, 31). This glycolytic inhibition, which apparently involves steroid action, is reversed in vitro by insulin. In such tumors, endotoxin was found to exert an effect remarkably similar to that of insulin (33, 34). Since insulin stimulation often masked glycolytic stimulation by endotoxin, a common locus of action was suggested. Moreover, glycolysis in tumors not sensitive to stress, or to insulin, remained unaffected by endotoxin.

We were particularly impressed by the fact that the degree of stimulation of tumor glycolysis observed in vitro with a series of endotoxin preparations paralleled their potencies in eliciting endotoxic phenomena in vivo (33). Furthermore, reduction in the host-reactive properties of endotoxin by an enzyme system present in normal serum (13), or in liver homogenate (28), was paralleled by a similar decrease in its effect on glycolysis (33). These, and other findings, led us to postulate that the properties required for elicitation of reactions in the host by such macromolecular materials were the same as those involved in influencing glycolysis in vitro. It appeared likely, as we had previously shown for insulin, that these glycolytic effects of endotoxin resulted from a specific counteraction of the endogenous, stress-modifiable inhibitor mechanism that controls glucose metabolism.

The tumors employed in these studies have proved to be unusually favorable tools for studying the metabolic actions of endotoxin and insulin. The phenomena observed, however, are by no means unique for malignant tissues. Cohn and Morse (4–6) reported glycolytic stimulation, without respiratory impairment, with rabbit polymorphonuclear leukocytes. Of great importance was the fact that they correlated this glycolytic stimulation with an enhancement of phagocytic capacity, a phenomenon already shown by Sbarra and Karnovsky (19, 20) and Karnovsky and Sbarra (11) to be dependent on glycolysis. Cohn and Morse (5, 6) pointed out that it was not possible to decide from their experiments whether the endotoxin-induced stimulation of leukocyte glycolysis was an expression of increased membrane permeability or a direct enzymatic interaction. Yunis and Harrington (35) reported that pyrogenic polysaccharides stimulated glycolysis in normal bone

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marrow and in circulating leukemic leukocytes. With a high concentration of endotoxin these workers also obtained some evidence for an increase in adenine incorporation into ribonucleic acid of leukocytes. Martin (14) reported acceleration of glycolysis in leukocytes following exposure to bacterial pyrogens. However, he attributed this effect to a diminution of oxygen consumption and suggested that the observed alteration in respiratory function occurred in the cyclophorase system. Later, Martin et al. (15, 16) noted that insulin in vitro increased lactate production in leukocytes of diabetic subjects and that glycolysis of leukocytes was increased by low concentrations (0.03 μg per ml) of Pyromen (endotoxin from *Pseudomonas aeruginosa*).

Hochstein (8, 9) has shown in mitochondrial preparations of mouse melanoma that the inhibitory effects of sex steroids, or the stimulatory effects of insulin on glycolysis, are eliminated by supplying glucose 6-phosphate instead of glucose as substrate, thereby demonstrating a localization of the effects of these hormones at the hexokinase reaction. Recently, Giger (7) has obtained similar stimulation of glycolysis in mitochondrial preparations of melanoma and of brain with endotoxin and insulin.

There are many reports dealing with the stimulatory effects of bacterial lipopolysaccharides on the chemotactic or phagocytic capacities of leukocytes (2, 22, 27). Schär and Meier (24) have recently shown that endotoxin-induced stimulation of migration in vitro of leukocytes is glucose dependent, and is counteracted by cortisone (23). Snell (27) has shown that cortisone in sufficient dosage blocks chromium phosphate removal by Kupffer cells in mice in vivo, and that lipopolysaccharides can overcome this effect, yielding a net stimulation.

Space does not permit a more comprehensive review of reported stimulatory effects of endotoxin on the metabolic and functional activities of cells. It suffices to say that it is well established that endotoxin produces glycolytic stimulation, and that this stimulation can be associated with an increase in functional capacities of the cells.

Comparative Activities of Different Endotoxins

Table 1 shows the correspondence observed between biological potencies in vivo and glycolytic activity in vitro in a series of endotoxic products. The preparations of high potency in eliciting fever and tumor damage likewise display the highest glycolytic activity in vitro. Lipid A was active only at very high dosage. One of the most active preparations, from *Salmonella abortus equi*, gave maximal glycolytic stimulation (44%) at 0.03 μg per ml, and approximately two-thirds maximal stimulation (29%) at 0.003 μg per ml. On the other hand, a less active preparation from *Serratia marcescens* yielded 41% stimulation at 0.1 μg per ml and only 29% stimulation at 0.03 μg per ml. In this range, Pyromen was without significant effect. The greater activity of *S. abortus equi* endotoxin is consistent with the difference in fever indices of the two preparations, 0.71 μg versus 5.0 μg, respectively (fever index, 40 cm²; *see* Table 1).

TABLE 1. *Comparison of glycolysis-stimulating activities of various endotoxins with their activities in vivo*

Endotoxin source	Assay in vivo		Concn producing maximal glycolytic stimulation
	Fever index 40 cm²	Tumor damage ED_{50}	
	μg	μg	μg/ml
Salmonella enteritidis	0.14	0.3	0.03–0.3
Salmonella abortus equi	0.71	1–3	
Pseudomonas pseudotuberculosis			
"Rough"	0.27	3–10	
"Smooth"	2.50	10–30	
Salmonella typhosa	0.63	1–3	0.3–3.0
Serratia marcescens	5.00	3	
Pyromen (*Pseudomonas aeruginosa*)	>80	16–24	25
Lipid A (*Escherichia coli*)	200	875–1,000	160

Specific Inactivation of Endotoxic Activities Paralleled by Loss of Glycolytic Effects

The relationship between biological and glycolytic potencies of endotoxin was also examined in another way. When endotoxin was submitted to reagents that degraded this macromolecule and eliminated biological activity (18), it was found that similar losses in glycolysis-stimulating potency occurred. Figure 1 shows that as endotoxin was progressively hydrolyzed by treatment with 0.1 N acetic acid at 100 C, the

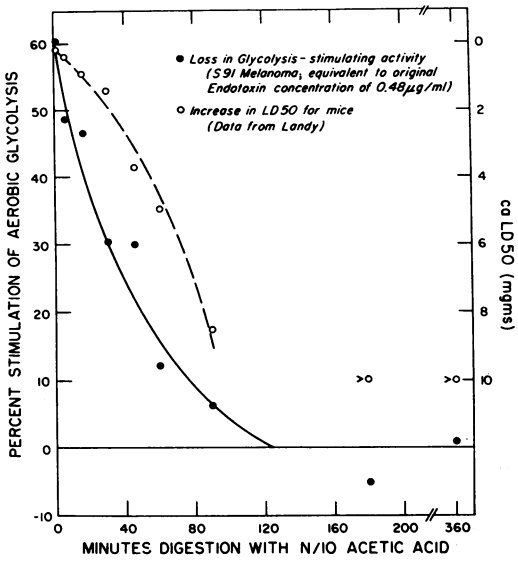

FIG. 1. *Inactivation of Salmonella enteriditis endotoxin by digestion with 0.1 N acetic acid at 100 C (see reference 33).*

loss in glycolytic potency generally paralleled the loss in biological activity as shown by the increase in the amount required for a 50% lethal effect in mice. Table 2 shows that when endotoxin was acted upon by host enzymes in human plasma (13), or in rabbit liver homogenate (28), the glycolysis-stimulating capacity of the polysaccharide was destroyed. This coincided with a loss of biological potency in vivo.

These and other results made it clear that there is a close correlation between glycolysis-stimulating activity in vitro and biological properties in vivo of products that ranged from the most highly purified endotoxins to weakly active materials such as lipid A.

Tumor Spectrum Showing Graded Glycolytic Responses to Endotoxin

The qualitative similarities between the action of endotoxin and insulin on glycolysis were shown in still other ways. Tumors could be arranged in a "spectrum" according to their sensitivity to glycolytic inhibition by certain steroids, or, conversely, to stimulation by insulin (Table 3). When endotoxin and insulin were compared in such a spectrum of tumors, it was evident that the largest responses to endotoxin, or to insulin, occurred in tissues most sensitive to

TABLE 2. *Inactivation of the glycolysis-stimulating property of endotoxin from Serratia marcescens by incubation with host enzymes*

Addition in vitro	Aerobic acid formation* by melanoma cells (125 mg slices/vessel)	
	Microliters CO_2/hr	Per cent effect
Control	42.3	
Endotoxin reacted with citrated human plasma at 37 C for 1 hr†	44.5	5
Endotoxin reacted with rabbit liver homogenate at 37 C for 1 hr‡	39.0	−8
Untreated endotoxin (0.36 μg/ml final concn.)	65.5	54

* Incubated at 37 C in Krebs-Ringer-bicarbonate medium with 0.3% glucose, 0.3% sodium bicarbonate, and 10% mouse ascitic serum. Gas phase 95% O_2 plus 5% CO_2.

† One milliliter of physiological saline containing 200 μg of endotoxin was incubated with 0.8 ml of citrated plasma at pH 9.5. Vessels contained the equivalent of 0.36 μg of original endotoxin per ml.

‡ One milliliter of physiological saline containing 10 μg of endotoxin was incubated with 0.05 ml of rabbit liver homogenate at pH 9.5. Vessels contained the equivalent of 0.36 μg of original endotoxin per ml.

inhibiton by steroid, in this instance testosterone.[3] Under ordinary conditions, glycolysis in K-2 carcinoma was practically insensitive to the effects of host stress in vivo, or to steroids in vitro (10, 31, 32). Consequently, as expected, this tumor showed little or no direct response to insulin or endotoxin.

It is probable that the same insulin:anti-insulin mechanism regulates glucose metabolism in most mammalian tissues. However, the degree of glycolytic sensitivity to inhibition of this mechanism varies considerably in different tissues. In melanoma, saturating levels of insulin were somewhat more effective than saturating levels

[3] The effect, observed in the present study, of antiglycolytic steroids counteracting endotoxin stimulation of glycolysis may be related to the well known effects of steroids in counteracting endotoxin lethality, or other actions, in vivo (*see* e.g., M. Brooke, E. H. Kass, and O. Hechter, "Protective effect of steroids against bacterial endotoxin." Federation Proc. **18:** 560, 1959).

of endotoxin in stimulating aerobic glycolysis. Under anaerobic conditions this differential was magnified. Table 4 illustrates such relationships. It is noteworthy that the stimulating effect of endotoxin was not removed from the cells by repeated washing. Aerobic glycolysis was 34% higher in the slices preincubated with endotoxin than in the controls incubated in Krebs-Ringer solution only. The addition of endotoxin in vitro (column 3, Table 4) raised the rates to the same level (68 μl per hr) in both preincubated samples. Insulin in vitro (columns 4 and 5, Table 4) raised glycolysis to levels above the rate with endotoxin alone. When the tissue was gassed anaerobically (second horizontal column, Table 4), glycolysis was greatly increased, and rates were higher with endotoxin in vitro, even in the presence of insulin.

TABLE 3. *Comparative anaerobic glycolytic responses to testosterone, endotoxin, and insulin in a "spectrum" of tumors*

Tumor	$Q_{CO_2}^{N_2}$ * of controls	Per cent change in glycolytic rate			
		Testosterone, 100 μg/ml	Endotoxin, 4 μg/ml	Insulin, 14 μg/ml	Endotoxin plus insulin
"Stressed" S91 melanoma	5.1	−66	87	174	168
"Normal" S91 melanoma	7.1	−59	30	70	65
S91A amelanotic melanoma	12.3	−30	12	15	17
K-2 carcinoma (ascites)	29.0	7	0	0	0

* Microliters of CO_2 released per mg dry weight per hr.

Endotoxin Action on Leukocytes

In tissues where myeloid elements predominate, such as rabbit bone marrow, rabbit peritoneal exudates, and human myelogenous leukemias, the stimulating action of endotoxin usually exceeded that of insulin (Fig. 2). In lymphatic leukemia, however, insulin generally stimulated glycolysis to a greater extent than did endotoxin (Fig. 3). Aerobic glycolysis in lymphatic leukemia was accentuated by the use of Krebs-Ringer medium (Fig. 3). In serum there was little aerobic glycolysis in lymphocytic cells. Considerable variation in absolute metabolic rates and effects was encountered depending on the particular conditions. These differences were probably quantitative rather than qualitative. They may, however, have an important bearing on responses in vivo to endotoxin in such tissues

TABLE 4. *Persistence of endotoxin effect on aerobic and anaerobic glycolysis after washing of tissue slices of S91 melanoma*

Slices were preincubated for 83 min aerobically (95% O_2 plus 5% CO_2) in Krebs-Ringer-bicarbonate plus 0.3% glucose and 10% mouse ascitic serum with, or without, endotoxin at 37 C. A definite endotoxin-induced glycolytic stimulation was evident. The endotoxin concentration during preincubation was 4 μg per ml. After preincubation, the tissue was washed three times with fresh aliquots of Krebs-Ringer medium, and glycolytic measurements made as indicated in the table.

Measurement	Addition in vitro after preincubation							
	None		Endotoxin 4 μg/ml		Insulin 14 μg/ml		Endotoxin plus insulin	
	Control*	Endotoxin†	Control	Endotoxin	Control	Endotoxin	Control	Endotoxin
Aerobic glycolysis (μl CO_2:hr:130-mg slices)	46	62	68	68	94	92	98	94
Anaerobic glycolysis (μl CO_2:hr:130-mg slices)	326	382	356	372	436	484	484	492

* Controls preincubated in Krebs-Ringer medium.

† Preincubated in Krebs-Ringer medium with endotoxin.

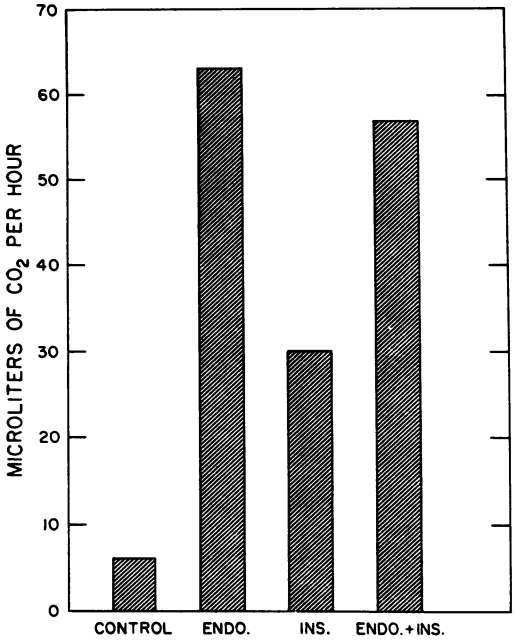

FIG. 2. *Effects of endotoxin, and of insulin, on aerobic glycolysis in human chronic myelogenous leukemia (0.2% glucose) 40 mm³ cells per vessel.*

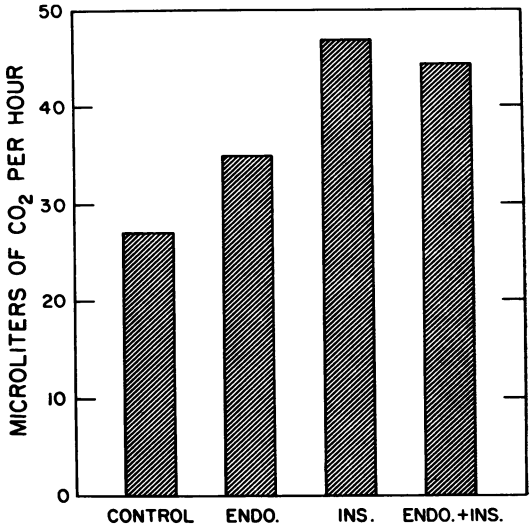

FIG. 3. *Effects of endotoxin, and of insulin, on aerobic glycolysis in human chronic lymphocytic leukemia (0.2% glucose) 40 mm³ cells per vessel.*

Dissociation of Glycolytic Stimulation and Cell Injury

Direct exposure in vitro of certain cells to high concentrations (10 to 50 μg per ml) of even the most potent endotoxins produced no evident injury. For example, melanoma cells, after incubation at 37 C for 3 hr with 30 μg of *S. marcescens* endotoxin per ml, in spite of glycolytic stimulation, showed no loss of viability when implanted into an appropriate host. Melanoma slices were incubated in Krebs-Ringer medium at 37 C with either 0.3 or 30 μg of *Salmonella enteritidis* endotoxin per ml. Respiration was not inhibited by either concentration of endotoxin, and the level of glycolytic stimulation was the same in both cases.

Measurements of intracellular levels of high energy phosphate (adenosine diphosphate plus adenosine triphosphate) by Joseph Seitz in our laboratory, showed that such levels were increased in melanoma slices incubated aerobically with either endotoxin or insulin. These results indicate that in this tumor endotoxin does not directly produce uncoupling or other damage to the energy metabolism of the cell. This view is further supported by the data in Fig. 4 where it can be seen that the stimulation of glycolysis in melanoma was not masked by the presence of the uncoupling agent 2,4-dinitrophenol (2,4-DNP). An endotoxin-insensitive tumor, the K-2 carcinoma, responded to 2,4-DNP, but not to endotoxin. Other experiments showed that insulin-treated melanoma tissue reacted to 2,4-DNP in the same way, although the stimulation with insulin was somewhat greater than with endotoxin. These results are consistent with the concept that in the "uncoupled" melanoma cells the insulin- and endotoxin-sensitive hexokinase reaction is still a rate-limiting step for glycolysis. As would be expected, no such effect occurs in the K-2 carcinoma where, under the conditions employed, the hexokinase reaction is not under notable hormonal restraint, and therefore not notably subject to stimulation by insulin or endotoxin. Data obtained with melanoma by Hochstein (8, 9), and by Giger with K-2 ascites and melanoma, using insulin or endotoxin at the mitochondrial level [(7) and *unpublished data*], support this interpretation.

Secondary Effects on Glycolysis in Vivo: Biphasic Responses

A. Melanoma

Although S91 melanoma cells do not appear to be affected adversely by endotoxin in vitro,

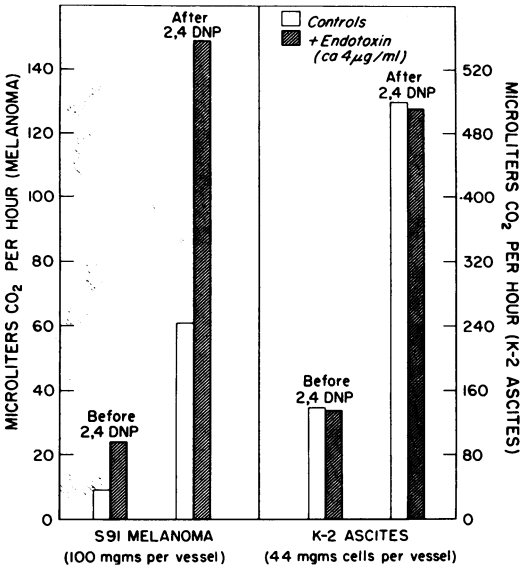

FIG. 4. *Effect of endotoxin (from Serratia marcescens) on aerobic glycolysis in S91 melanoma and K-2 ascites carcinoma in the presence and absence of 2,4-dinitrophenol (DNP). Krebs-Ringer-bicarbonate plus 0.3% glucose and 10% mouse ascitic serum. Endotoxin concentration about 4 μg per ml; 2,4-DNP, 40 μg per ml.*

this is not always the case in vivo. When endotoxin was administered intravenously to mice bearing melanoma, an intitial glycolytic stimulation (as measured in vitro) took place (Fig. 5). In this case, glycolysis of the melanoma was above control levels within 10 min after intravenous injection of 20 μg of *Serratia marcescens* endotoxin. This was observed under both aerobic and anaerobic conditions. Respiration was not affected significantly. Further addition of endotoxin in vitro produced additional glycolytic stimulation. However, in other S91 tumors both respiration and glycolysis were below normal levels 26 hr after intravenous injection of endotoxin. Further addition of endotoxin stimulated glycolysis but not respiration. There was considerable variation in response of melanoma to endotoxin in vivo. However, in the absence of visible hemorrhage and necrosis a biphasic stimulation of glycolysis occurred. Thus, for example, 43 hr after injection of endotoxin, glycolytic rates were found to be slightly above control levels.

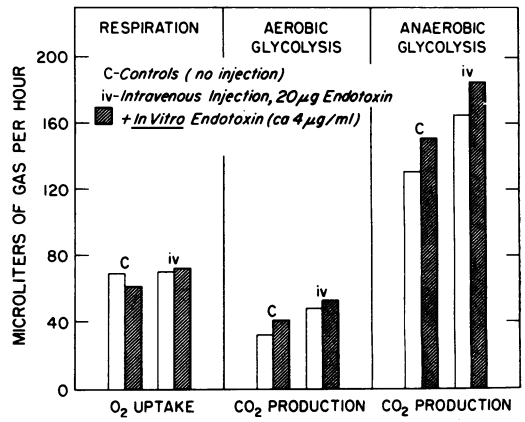

FIG. 5. *Effects of intravenous endotoxin on metabolism of S91 melanoma. All measurements made in Krebs-Ringer-bicarbonate plus 10% mouse ascitic serum. Respiration determined in the presence of 0.025% bicarbonate, and glycolysis with 0.3% bicarbonate. Gas phases 100% O_2 (4 N NaOH in center wells) for respiration, and 95% O_2, or 95% N_2, plus 5% CO_2 for glycolytic determinations. Temperature 37 C. (Ten minutes after injection of 20 μg endotoxin; 100 mg tissue per vessel.)*

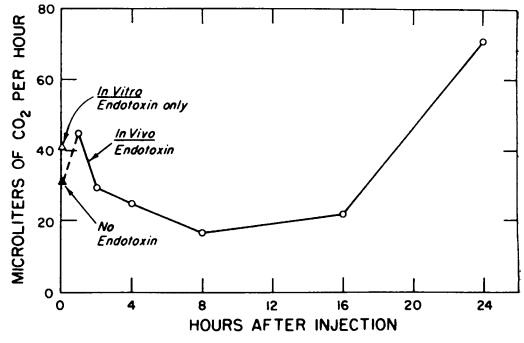

FIG. 6. *Effect of intravenous endotoxin (from Salmonella enteriditis) on glycolysis in mouse peritoneal macrophages. Krebs-Ringer-bicarbonate plus 0.3% glucose, gas phase 95% N_2 plus 5% CO_2 at 37 C. Endotoxin, 5 μg per mouse; 20 × 10^6 cells per vessel.*

B. Mouse Peritoneal Macrophages

In mice given endotoxin, biphasic glycolytic responses were clearly apparent in peritoneal macrophages. Whitby et al. (30) have described these effects in relation to changes in nonspecific resistance to infection. Figure 6 shows the effects obtained with 5 μg of endotoxin injected intravenously into CAF mice. The peritoneal

TABLE 5. *Effect of intravenously injected endotoxin (from Salmonella enteritidis) on anaerobic glycolysis of mouse peritoneal macrophages*

Endotoxin was given at the indicated dosages and glycolytic measurements in vitro were made after the indicated time periods. Krebs-Ringer solution plus 0.3% sodium bicarbonate and 0.3% glucose. Gas phase 95% N_2 plus 5% CO_2 at 37 C.

	Time after injection of endotoxin						
	Time = 0 (controls)	30 min		11 hr			
Dosage of endotoxin (μg per mouse)	0	1	50	0.05	0.5	5	50
Glycolytic rate (μl CO_2:20 × 10^6 cells:hr)	22	14	41	77	47	16	5

macrophages were washed out of successive groups of animals at the time periods indicated in the figure. Sixty minutes after injection, anaerobic glycolysis was slightly higher than in control cells where endotoxin was supplied in vitro only. Following the initial stimulation, glycolytic rates declined to levels below the control without endotoxin. Thereafter, a secondary rise occurred in cells from endotoxin-injected mice, and 24 hr after injection glycolytic rates were considerably above the initial level of stimulation.

In another experiment, 50 μg of endotoxin were injected intravenously; 9 hr after injection glycolysis was 57% below the control rate, at 16 hr after injection the inhibition was only 27%, at 72 hr the rate was 16% above normal, and at 96 hr it was 52% above the normal level. The glycolytic rate was still 52% above the control level at 6 and also 8 days postinjection, at which time the experiment was terminated.

It was demonstrated repeatedly that, with high doses of endotoxin, glycolytic stimulation was evident earlier, but secondary lowering of the rate of glycolysis also appeared earlier, was greater, and was more prolonged. Some of the observed glycolytic-endotoxin dose-time relationships are illustrated in Table 5. Within 30 min after 50 μg of endotoxin, glycolysis was 86% above the control level; however, 11 hr after injection the rate was 77% *below* the control. On the other hand, if the dose of endotoxin was reduced to 1 μg no significant effect was discernible 30 min after injection. However, by 11 hr, the smallest dose tested, 0.05 μg per mouse, produced maximal (250%) stimulation.

C. Mouse Spleen

Results similar to those obtained with macrophages were seen with spleen cells of normal mice. However, secondary inhibition of glycolysis in spleen was rapidly followed by a marked secondary rise that was maximal about 42 hr after injection, and by 72 hr the glycolytic rate had almost returned to control levels.

Analysis of the experiments with tumor, macrophage, and spleen cells suggested that at least three separate, although interacting, processes are involved in producing these complex biphasic syndromes. An initial glycolytic stimulation (as measured in vitro) follows administration in vivo of endotoxin. However, when the level of injected endotoxin is sufficiently high, a state of general stress is produced in the host and the tissues are subjected to stress-accentuated glycolytic inhibition. Injection of endotoxin results in an increase in the level of ketosteroids in the circulation [Nadel et al. (17)]. Glycolytic inhibition is sufficient to overcome not only the initial endotoxin-induced stimulation, but also to lower the rate below endogenous control levels. Glycolytic stimulation is once again evident as the effects of the initial stress diminish. In a sense this is like a persistent insulin action that cannot be fully compensated for by the usual anti-insulin hormonal, or other control mechanisms.

D. Endotoxin-insensitive Tumors

Experiments with K-2 carcinoma made possible a clear-cut separation of the *reversible* glycolytic inhibitory effects of endotoxin-induced host stress from the irreversible injury resulting from endotoxin-induced damage to the blood vessels. Glycolysis in the K-2 carcinoma, in either solid or ascitic forms, is practically insensitive to inhibition by steroids, and host stress in vivo thus has no significant effect on its glycolysis (10, 31, 32). When 10 μg of endotoxin were given intravenously to mice with this tumor in the solid intramuscular form, extensive intratumoral hemorrhage occurrred. This was accompanied by a marked reduction in both respiration and

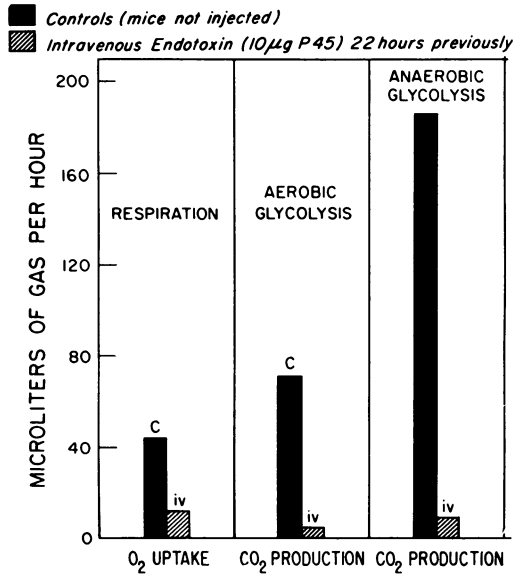

FIG. 7. *Effect of intravenous endotoxin (from Serratia marcescens) on metabolism of solid K-2 carcinoma. Conditions of measurement same as in Fig. 5.*

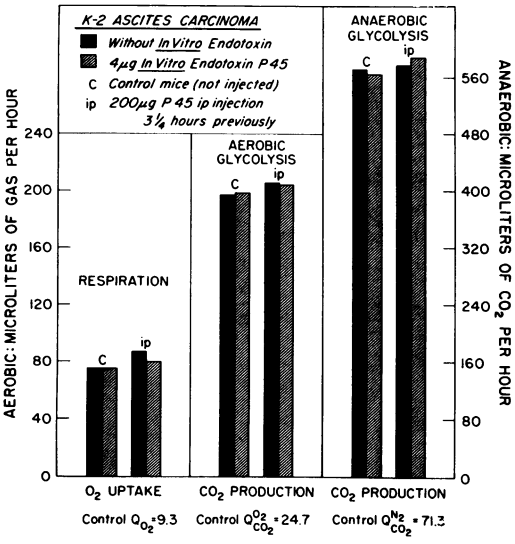

FIG. 8. *Effect of intraperitoneal endotoxin (from Serratia marcescens) on metabolism of K-2 ascites carcinoma. Conditions same as in Fig. 5.*

glycolysis (Fig. 7), indicative of extensive cell destruction. However, when the tumor was in the ascitic form, and thus relatively independent of the effects of host vascular impairment, a very different result was obtained. Injections of 200 μg of endotoxin directly into the peritoneal cavity did not significantly alter glycolytic or respiratory rates of the tumor cells (Fig. 8) even though the mice showed symptoms of acute stress. Subsequent further additions of endotoxin to the cells during incubation in vitro also had no significant effects on either respiration or glycolysis. These results make it clear that cells which are insensitive to endotoxin in vitro are likewise insensitive in vivo. However, such cells can be damaged *indirectly* by endotoxin when they are dependent upon endotoxin-sensitive vascular tissues of the host.

Scheme for in Vivo Metabolic Effects of Endotoxin

A scheme is proposed (Fig. 9) which seeks to relate some of the actions in vivo of endotoxin to a metabolic common denominator, that is, to stimulation of aerobic glycolysis. Thus certain cells, which may be referred to as "glycolysis-tolerant" cells (right side of figure), are apparently not directly injured by endotoxin. In fact, such cells can be stimulated to hypernormal activity. Persistence of an elevated glycolysis could lead to anabolic and functional increases as suggested in lines A, B, and C. On the other hand (left side of figure), there may also be cells which cannot tolerate a sustained elevation of aerobic glycolysis. Damage to such cells could initiate a sequence of events leading to various forms of injury and malfunction. Thus, primary vascular impairment would lead secondarily to irreversible damage even in endotoxin-tolerant cells (line B^1). Activation of a stress syndrome as shown in line C^1 would lead to temporary counteraction of the glycolytic stimulation initially produced by endotoxin in the tolerant cells. This latter inhibition would disappear as the effects of stress wear off and the stimulatory effects of endotoxin reappear.

Finally, it is again emphasized that the capacity to stimulate glycolysis parallels to a remarkable degree the various biological activities of endotoxin preparations from various gram-negative bacteria by different isolation procedures. There are reasons for believing that immunological phenomena, and a state of hyperreactivity, are also involved in cellular reactions to endotoxin. Nevertheless, stimulation of aerobic glycolysis may well be a prime factor in

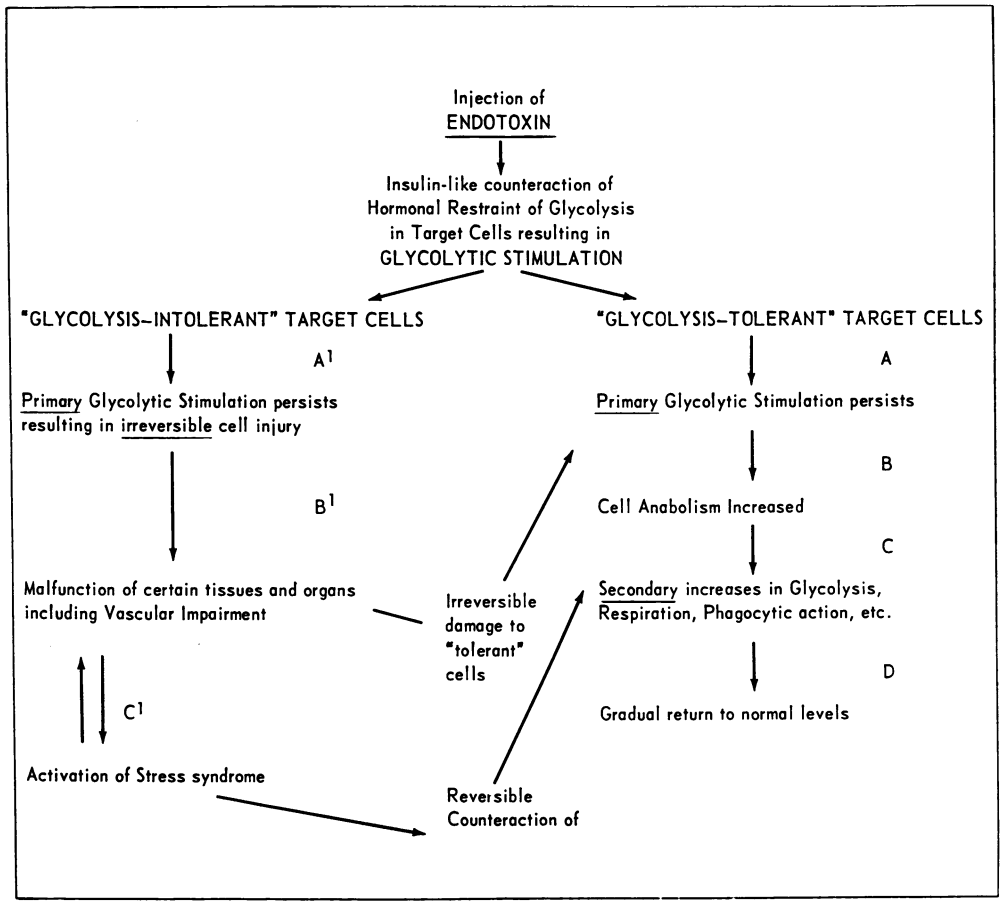

FIG. 9. *Proposed scheme for actions in vivo of endotoxin based on its metabolic effects*

initiating a chain of events that culminate in characteristic reactions evoked by endotoxin.

LITERATURE CITED

1. ATKINS, E. 1960. Pathogenesis of fever. Physiol. Rev. **40**:580–646, 1961.
2. BENACERRAF, B., AND M. M. SEBESTYEN. 1957. Effect of bacterial endotoxins on the reticuloendothelial system. Federation Proc. **16**:860–867.
3. BENNETT, I. L., JR., AND L. E. CLUFF. 1957. Bacterial pyrogens. Pharmacol. Rev. **9**:427–479.
4. COHN, Z. A. 1960. Relation of cell metabolism to infection with rickettsial and bacterial agents. Bacteriol. Rev. **24**:96–105.
5. COHN, Z. A., AND S. I. MORSE. 1960. Functional and metabolic properties of polymorphonuclear leucocytes. I. Observations on the requirements and consequences of particle ingestion. J. Exptl. Med. **111**:667–687.
6. COHN, Z. A., AND S. I. MORSE. 1960. Functional and metabolic properties of polymorphonuclear leucocytes. II. The influence of a lipopolysaccharide endotoxin. J. Exptl. Med. **111**:689–704.
7. GIGER, K. 1961. Endotoxin and insulin as stimulators of mitochondrial glycolysis in melanoma and brain. Proc. Am. Assoc. Cancer Research **3**:227.
8. HOCHSTEIN, P. E. 1957. Glycolysis by tumor mitochondria and the action of insulin. Science **125**:496–498.
9. HOCHSTEIN, P. E. 1959. Hormonal regulation of subcellular glycolysis in the S91 melanoma. *In* M. Gordon, [ed.], Pigment cell biology, p. 445–453. Academic Press, Inc., New York.
10. HUNTER, J. C. 1955. Differential effects of temperature on the growth of certain transplanted tumors in strain DBA mice. J. Natl. Cancer Inst. **16**:405–414.

11. KARNOVSKY, M. L., AND A. J. SBARRA. 1960. Metabolic changes accompanying the ingestion of particulate matter by cells. Am. J. Clin. Nutrition **8**:147–155.
12. LANDY, M., AND L. PILLEMER. 1956. Increased resistance to infection and accompanying alteration in properdin levels following administration of bacterial lipopolysaccharides. J. Exptl. Med. **104**:383.
13. LANDY, M., R. C. SKARNES, F. S. ROSEN, R-J. TRAPANI, AND M. J. SHEAR. 1957. Inactivation of biologically active ("endotoxic") polysaccharides by fresh human serum. Proc. Soc. Exptl. Biol. Med. **96**:744–747.
14. MARTIN, S. P. 1952. Metabolic effect of Piromen on human leukocytes. *In* Proceedings 5th Piromen Conference, p. 1–4. Baxter Labs., Inc., Morton Grove, Ill.
15. MARTIN, S. P., G. R. MCKINNEY, R. GREEN, AND C. BECKER. 1953. The influence of glucose, fructose, and insulin on the metabolism of healthy and diabetic subjects. J. Clin. Invest. **32**:1171–1174.
16. MARTIN, S. P., G. R. MCKINNEY, AND R. GREEN. 1955. The metabolism of human polymorphonuclear leukocytes. Ann. N. Y. Acad. Sci. **59**:996–1002.
17. NADEL, E. M., B. YOUNG, A. HILGAR, AND A. MANDELL. 1961. Effects of adrenocorticotropin and endotoxin on adrenal stimulation and resistance to infection. Am. J. Physiol. **201**: 551–553.
18. RIBI, E., W. T. HASKINS, M. LANDY, AND K. C. MILNER. 1961. Symposium on bacterial endotoxins. I. Relationship of chemical composition to biological activity. Bacteriol. Rev. **25**:427–436.
19. SBARRA, A. J., AND M. L. KARNOVSKY. 1959. The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. J. Biol. Chem. **234**:1355–1362.
20. SBARRA, A. J., AND M. L. KARNOVSKY. 1960. The biochemical basis of phagocytosis. II. Incorporation of C^{14}-labeled building blocks into lipid, protein and glycogen of leukocytes during phagocytosis. J. Biol. Chem. **235**:2224–2229.
21. ROWLEY, D. 1956. Rapidly induced changes in the level of non-specific immunity in laboratory animals. Brit. J. Exptl. Pathol. **37**:223–234.
22. SCHÄR, B. 1960. Zur Charakterisierung der Wirkung von Lipopolysacchariden auf Leukocyten *in vitro*. Helv. Physiol. et Pharmacol. Acta **18**:99–108.
23. SCHÄR, B., AND R. MEIER. 1960. Hemmung der durch Proteuslipopolysaccharide gesteigerten Leukozytenmigration in vitro durch antiinflammatorische Corticosteroide. Experientia **16**:315–316.
24. SCHÄR, B., AND R. MEIER. 1961. Abhängigkeit der Leukozyten Wanderung und ihrer Steigerung durch Proteuslipopolysaccharide von der Glucose-konzentration des Milieus. Experientia **17**:27–28.
25. SHEAR, M. J. 1955. Polysaccharides in biology. Trans. 1st Conference. Josiah Macy Jr. Foundation, New York.
26. SMITH, W. W., I. M. ALDERMAN, AND R. E. GILLESPIE. 1958. Hematopoietic recovery induced by bacterial endotoxin in irradiated mice. Am. J. Physiol. **192**:549–556.
27. SNELL, J. F. 1960. The reticuloendothelial system. I. Chemical methods of stimulation of the reticuloendothelial system. Ann. N. Y. Acad. Sci. **88**:56–57.
28. TRAPANI, R-J., V. S. WARAVDEKAR, M. LANDY, AND M. J. SHEAR. 1961. Inactivation *in vitro* of endotoxin by intracellular liver enzyme. Federation Proc. **20**:262.
29. WHITBY, J. L., AND D. ROWLEY. 1959. The role of macrophages in the elimination of bacteria from the mouse peritoneum. Brit. J. Exptl. Pathol. **40**:358–370.
30. WHITBY, J. L., J. G. MICHAEL, M. W. WOODS, AND M. LANDY. 1961. Symposium on bacterial endotoxins. II. Possible mechanisms whereby endotoxins evoke increased nonspecific resistance to infection. Bacteriol. Rev. **25**:437–446.
31. WOODS, M. W., J. C. HUNTER, AND D. BURK. 1955. Regulation of glucose utilization in tumors by a stress-modified insulin:anti-insulin system. J. Natl. Cancer Inst. **16**:351–404.
32. WOODS, M. W., AND J. C. HUNTER. 1959. Metabolic and growth responses of mouse melanomas, Krebs-2 carcinoma, and brain relative to stress, and insulin:anti-insulin hormones. *In* M. Gordon, [ed.], Pigment cell biology, p. 455–488. Academic Press, Inc., New York.
33. WOODS, M. W., M. LANDY, AND M. J. SHEAR. 1959. Effects of endotoxic polysaccharides on tumor glycolysis. Federation Proc. **18**:355.
34. WOODS, M. W., D. BURK, T. HOWARD, AND M. LANDY. 1961. Insulin-like action of endotoxins in normal and leukemic leukocytes and other tissues. Proc. Am. Assoc. Cancer Research **3**:279.
35. YUNIS, A., AND W. J. HARRINGTON. 1960. Metabolic effects of bacterial pyrogens upon human leukocytes. I. Studies on leukocyte respiration, glycolysis, and nucleic acid synthesis. J. Lab. Clin. Med. **55**:584–592.