EFFECTS OF BACTERIAL ENDOTOXIN ON METABOLISM

I. Carbohydrate Depletion and the Protective Role of Cortisone*

By L. JOE BERRY, Ph.D., DOROTHY S. SMYTHE, and LEONA G. YOUNG

(From the Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania)

(Received for publication, April 8, 1959)

The ability of cortisone and adrenocorticotropic hormone (ACTH) to protect experimental animals against the toxic and/or febrile effects of bacterial endotoxins has been reported by a number of investigators (1–8) and denied by others (9, 10). The underlying mechanism(s) responsible for this action of adrenal cortical hormones remains obscure. It frequently is suggested, however, that protection against endotoxins is related to the anti-inflammatory effect of this group of steroids. If further study proves this to be the case, it would represent only one level of understanding. There would remain the need for a biochemical explanation of how the hormone protects and, ideally, why there may be exceptions to the protection. The present report, while not pretending to provide any final answers to this complex problem, does offer some insight into possible metabolic bases for the actions of endotoxin and cortisone.

For several decades it has been known that endotoxins from Gram-negative bacteria injected into experimental animals produce an initial hyperglycemia (11, 12). This is followed by a prompt fall in blood sugar to hypoglycemic levels with a concomitant decrease in liver glycogen (13). Cortisone, on the other hand, promotes a glyconeogenesis and a negative nitrogen balance (14). It is on the basis of these established facts that the experiments described below were undertaken.

Methods

Preparation of Endotoxin.—Salmonella typhimurium, strain SR-11, was grown overnight in brain-heart infusion broth (Difco), or, in some instances, on brain-heart infusion agar. Either the broth culture itself or isotonic sodium chloride washings from the agar surface were sedimented in a refrigerated centrifuge at 5°C. The cells were extracted with acetone by mixing them in an omnimixer, the cup of which was immersed in an ice water bath. Following centrifugation, the supernatant was discarded and fresh acetone was added. The extraction procedure was repeated six to eight times, or until the acetone was colorless. Ether was then substituted for acetone and the bacteria were further extracted until a colorless

* Supported by Contract AF 41(657)-196 between Bryn Mawr College and the School of Aviation Medicine, United States Air Force, and by a National Science Foundation grant.

solvent remained (usually 3 to 4 washings). The dried material was stored over drierite at 5°C. in a desiccator. All endotoxin injections were administered intraperitoneally with the desired quantity suspended in 0.5 ml. of isotonic salt solution. Further purification of the crude endotoxin was not undertaken in this laboratory but a few tests were conducted with purified *Serratia marcescens* lipopolysaccharide (Difco, control 109753) and *Salmonella typhosa* endotoxin kindly supplied by Dr. Maurice Landy.

Glycogen and Blood Sugar Determinations.—Blood sugar was determined by the method of Nelson (15) on samples collected in a paraffined watch glass following decapitation of the mouse with sharp scissors. Liver and muscle (abdominal body wall) glycogens were measured according to the procedure of Kemp and Kits van Heijningen (16). To estimate the total carbohydrate in a mouse, the skin, feet, tail, stomach, and intestinal tract were removed and the carcass was blended for 3 minutes in 5 per cent trichloroacetic acid in a chilled omnimixer. A suitable aliquot was analyzed by the method of Mendel, Kemp, and Myers (17). Both glycogen procedures depend upon the development of 5-hydroxymethyl furfural from glucose in the presence of hot sulfuric acid. Sufficient color is developed to reliably measure 10 micrograms of glucose equivalents in the model 14 Coleman spectrophotometer.

Cortisone and Dibenzyline.—Cortisone was administered subcutaneously as a suspension of cortisone acetate (Nutritional Biochemicals, Cleveland) in isotonic saline solution. The suspension, stabilized with a drop of tween 80, was prepared in a glass homogenizer with teflon pestle and used immediately thereafter. Each injection was given in a total volume of 0.5 ml. Dibenzyline[1] (*N*-phenoxyisopropyl-*N*-benzyl-B-chloroethylamine hydrochloride), a sympatholytic drug, was administered intraperitoneally in mice with the desired dose, 10 mg./kg. body weight, contained in 0.5 ml. of isotonic saline and dilute ethanol. The latter was used to dissolve the dibenzyline before diluting it in saline. The final alcohol concentration was 10 per cent and this alone produced no detectable changes in body carbohydrates.

Rectal Temperatures of Mice.—Rectal temperatures of mice were determined by inserting a thermistor probe into the rectum of mice and reading the temperature from a telethermometer (Yellow Springs Instrument Company, Yellow Springs, Ohio) to which the probe was connected. The instrument was calibrated against a sensitive mercury thermometer.

Detection of Typhoid Carrier Mice.—Individual mice were color-coded on the pelt and a fecal pellet from each was macerated in 1 ml. of saline. One-half of a Petri dish of SS agar (Difco) was inoculated with a wire loopful of the fecal material. Following 48 hours of incubation at 37°C. the presence of colonies typical of members of the *Salmonella-Shigella* genera was taken as evidence of typhoid carriers. Fecal discharge of typhoid organisms is not always observed in carrier animals, hence a negative culture is no guarantee of freedom from salmonellosis. A positive culture, on the other hand, is proof that the animal is a carrier even though it subsequently may become free of the pathogen. A number of spleens taken from animals at the time of sacrifice were cultured on SS agar and with only rare exceptions the fecal diagnosis was confirmed. For the sake of brevity, animals with positive fecal cultures will be referred to as "carriers" and those with negative fecal cultures will be called "negatives."

Studies with Mouse Liver Mitochondria.[2]—Mouse liver mitochondria were prepared according to the procedure recommended by Hogeboom and Schneider (18) for rat liver mitochondria. Adenosinetriphosphatase (ATPase) activity of the mitochondria was determined in a final volume of 3.0 ml. contained in 15 ml. conical centrifuge tubes. This volume of 3.0 ml. consisted of 1.5 ml. of isotonic sucrose, KCl and $MgCl_2$ solution at pH 7.4; 0.5 ml. of the compound to be tested, *i.e.* either 1 mg. *S. typhimurium* endotoxin suspended in double dis-

[1] The dibenzyline was generously supplied by Smith, Kline, and French, Philadelphia.

[2] The assistance of Miss Nancy Coyne in carrying out this experiment is gratefully acknowledged.

tilled water, or recrystallized 2,4-dinitrophenol, DNP, (Nutritional Biochemicals) dissolved in double distilled water at 7×10^{-4} M, or double distilled water, alone, to serve as the control; 0.5 ml. of the freshly prepared mitochondria, and 0.5 ml. adenosinetriphosphate (ATP) (Sigma) at a concentration of 6.07 mg./ml., pH 7.4. The ATP was added to the tubes at intervals of 15 seconds so that the reaction could be precisely timed. All tubes were incubated at 31°C. for 10 or 20 minutes. The reaction was stopped by the addition of 0.5 ml. of a 50 per cent solution of trichloroacetic acid. The tubes were centrifuged and aliquots from each were analyzed in duplicate for inorganic phosphate by the method of Fiske and Subbarow (19).

TABLE I

Mortality Data (Survivors/Total Number) for Different Strains of Mice Injected Intraperitoneally with S. typhimurium Endotoxin and Protected with Subcutaneous Injections of Cortisone Acetate

The per cent survival is shown in parentheses. Animals designated as "negatives" failed to yield colonies on SS agar typical of members of the *Salmonella-Shigella* genera following inoculation of the medium with a saline suspension of fecal pellets.

Mouse strain and endotoxin dosage	No. of mice surviving/total animals (per cent surviving)		
	Endotoxin alone	Endotoxin + 5 mg. cortisone	Endotoxin + 1 mg. cortisone
CF-1-negatives (2 mg.)	3/35 (9%)	26/35 (74%)	4/25 (16%)
SW-negatives (0.75 mg.)	1/26 (4%)	22/26 (85%)	
HF-negatives (0.75 mg.)	4/30 (13%)	23/30 (77%)	

Mice.—Three strains of female mice were used in the experiments: CF-1 mice (Carworth Farms), HF mice (Huntingdon Farms), and SW mice (Swiss Webster mice, Rockland Farms). They were housed in small metal cages, about ten mice to the cage. White pine shavings were used as bedding and they were fed Purina dog chow *ad libitum*. Water was available at all times.

RESULTS

Protection by Cortisone of Mice Given Lethal Doses of Endotoxin.—Table I summarizes the mortality data of mice of different strains given intraperitoneal injections of *S. typhimurium* endotoxin alone and endotoxin immediately after a subcutaneous injection of cortisone acetate. CF-1-negative mice, *i.e.* mice with fecal cultures negative on SS agar, were readily protected with 5 mg. of cortisone but not with 1 mg. of cortisone against an LD_{90} dose of endotoxin. Five mg. of cortisone also protected SW-negative mice and HF-negative mice from an LD_{90-95} dose of endotoxin. The endotoxin used with SW and HF mice was prepared at a different time and was more toxic than

that given CF-1 animals, as judged by the dosage required to kill approximately 90 per cent of mice. There are suggestions of strain differences of these mice in response to endotoxins, yet the specific differences found in Table I are small and not statistically significant under the conditions of the experiments.

HF carrier mice, on the other hand, showed 90 per cent survival against a quantity of endotoxin that killed 87 per cent of non-carriers, as seen in Table II. When similar mice were injected with a lipopolysaccharide derived from *S. marcescens*, both negative and carrier mice were equally susceptible, Table II. This suggests that the carrier mice are either immune to the *Salmonella* endotoxin, possibly because of specific circulating antibodies, or else resist its

TABLE II

Mortality Data (Survivors/Total Number) for HF-Negative and HF-Carrier Mice Injected Intraperitoneally with Either S. typhimurium Endotoxin or S. marcescens Lipopolysaccharide

The per cent survival is shown in parentheses. Animals designated as "carriers" yielded colonies typical of members of the *Salmonella-Shigella* genera on SS agar inoculated with a saline suspension of fecal pellets.

Mouse strain	No. of mice surviving/total animals (per cent surviving)	
	S. typhimurium endotoxin (0.75 mg.)	*S. marcescens* lipopolysaccharide (0.5 mg.)
HF-negatives	4/30 (13%)	7/20 (35%)
HF-carriers	18/20 (90%)	9/20 (45%)

toxicity for other reasons. There is no experimental basis for a choice between the two but the high degree of immunity against salmonellosis reported by Hobson (20) for typhoid carrier mice may be related.

The effectiveness of cortisone in protecting mice against a lethal dose of endotoxin is significantly diminished by a large dose of endotoxin. It may be observed in Table III that 5 mg. cortisone protected only 15 per cent of HF-negative mice against 2.0 mg. endotoxin (instead of 0.75 mg. used for the data of Tables I and II). On the other hand, 1 of 24 carrier mice survived the same dose of endotoxin alone while, of those also given cortisone, only 50 per cent survived. Whether these observations can account for the failure of cortical steroids to protect against endotoxins as reported in the literature (9, 10) is not known.

Carbohydrate Levels in Mice Following Injections of (a) Endotoxin, (b) Cortisone Acetate, and (c) Both.—Profound changes in liver and muscle glycogen and

in blood sugar occurred 17 hours after the intraperitoneal injection of an approximately LD_{90} dose of endotoxin. This interval of time was chosen for the analyses because, under the conditions of the experiments, the first deaths from endotoxin poisoning usually occurred after 18 hours and were completed by 48 hours. Results of carbohydrate determinations with CF-1 mice are given in Table IV A. The top line gives control values and the second line the results after injecting 2 mg. of the same *S. typhimurium* endotoxin used for the data with CF-1 mice in Table I.

Particular attention is directed to the total carbohydrate listed for control mice in the last column of the first line of the table. Mice weighing about 20 gm. have approximately 1 gm. of liver, 10 gm. of muscle, and 2 ml. of blood.

TABLE III

Mortality Data (Survivors/Total Number) for HF-Negative and HF-Carrier Mice Injected Intraperitoneally with a Large Dose of the Same S. typhimurium Endotoxin Used for Data of Table II and with Subcutaneous Injections of Cortisone Acetate

The per cent survival is shown in parentheses.

Mouse strain and endotoxin dosage	No. of mice surviving/total animals (per cent surviving)	
	Endotoxin alone	Endotoxin + 5 mg. cortisone
HF-negatives (2 mg.)	0/20 (0%)	3/20 (15%)
HF-carriers (2 mg.)	1/24 (4%)	12/24 (50%)

Liver contains 55 mg. glycogen (5.5 per cent of 1000 mg.), muscle 33 mg., and blood 3 mg., or a total of 91 mg. of carbohydrate. The skin, feet, tail, and intestines weigh 6 to 7 gm., so the carcass weight on which the values in the last column are based was about 14 gm. The 6.5 mg./gm. agrees closely with 91 mg./14 gm. In similar manner, the carbohydrate values following endotoxin yielded approximately the same calculated total as that shown in the last column of row 2. These data prove that endotoxin resulted in a true reduction in body carbohydrate amounting, in this case, to 80 per cent of the total.

The next two lines in Table IV A show the increase in glycogen that accompanied cortisone injection. The smaller dose, 1 mg., nearly doubled the carbohydrate per unit weight of carcass while 5 mg. of cortisone yielded almost a fourfold increase. According to Long *et al.* (14), storage of carbohydrate under these conditions indicates a conversion of body protein into glycogen. One mg. cortisone acetate failed to compensate, however, for the carbohydrate loss associated with an injection of 2 mg. endotoxin (Table IV A). It has been

seen that 1 mg. cortisone also offered little protection against the lethal effects of the endotoxin (Table I). A higher level of carbohydrate was to be found

TABLE IV

Carbohydrate Data for Different Strains of Mice Given Intraperitoneal Injections of S. typhimurium Endotoxin, Cortisone, or Both

Each value is the mean ± the standard deviation. The numbers in parentheses are the number of separate determinations made for each value.

Experimental treatment	Blood sugar	Liver glycogen	Muscle glycogen	Total body carbohydrate
	mg. per cent	*per cent by wt.*	*per cent by wt.*	*mg./gm.*
A. CF-1 mice				
Controls	149 ± 6 (24)	5.5 ± 0.6 (27)	0.33 ± 0.06 (15)	6.5 ± 1.2 (15)
2 mg. endotoxin	37 ± 13 (17)	0.21 ± 0.07 (16)	0.10 ± 0.04 (17)	1.3 ± 0.5 (17)
1 mg. cortisone	111 ± 22 (13)	8.1 ± 1.1 (11)	0.45 ± 0.05 (7)	12.7 ± 3.8 (11)
5 mg. cortisone	144 ± 13 (17)	14.6 ± 2.1 (17)	0.93 ± 0.05 (17)	25.3 ± 5.14 (17)
2 mg. endotoxin + 1 mg. cortisone	58 ± 17 (15)	0.30 ± 0.14 (18)	0.11 ± 0.02 (13)	1.6 ± 0.7 (13)
2 mg. endotoxin + 5 mg. cortisone	52 ± 10 (6)	0.85 ± 0.13 (6)	0.24 ± 0.05 (6)	3.55 ± 0.8 (6)
At death				0.9 ± 0.1 (5)
B. SW mice				
17 hrs. after 0.75 mg. endotoxin	44 ± 10 (8)	0.15 ± 0.04 (8)	0.06 ± 0.03 (8)	0.9 ± 0.2 (8)
17 hrs. after 0.75 mg. endotoxin and 5 mg. cortisone	99 ± 10 (8)	1.21 ± 0.47 (8)	0.14 ± 0.04 (8)	2.7 ± 0.5 (8)

in mice injected with 5 mg. cortisone and 2 mg. endotoxin (line 6, Table IV A) than with the endotoxin alone. Associated with the greater percentage survival afforded by 5 mg. of hormone (Table I) was a "protection" of total carbohydrate. It is not known whether the greater amount of glycogen was due to greater synthesis, to diminished loss, or to both. Presumably, however, the

glyconeogenesis initiated by cortisone compensated in part for the carbohydrate loss resulting from the action of endotoxin.

The final value listed in the last column of Table IV A was obtained with mice at the moment of death from a lethal dose of endotoxin. It is impossible to know the specific or biochemical lesion causing death under these conditions or whether the quantity of glycogen found was actually incompatible with continued survival. It was slightly lower, nevertheless, than values obtained with surviving but gravely ill animals.

TABLE V

Carbohydrate Data for HF-Negative Mice Given Intraperitoneal Injections of S. typhimurium Endotoxin, Cortisone, or Both

All mice were fasted for the 5 hour period preceding the analyses. Each value is the mean ± the standard deviation. The numbers in parentheses are the number of separate determinations made for each value.

Experimental treatment	Blood sugar	Liver glycogen	Muscle glycogen	Total carbohydrate
	mg. per cent	*per cent by wt.*	*per cent by wt.*	*mg./gm.*
5 hrs. after 0.75 mg. endotoxin	70 ± 5.5 (8)	0.16 ± 0.02 (8)	0.05 ± 0.02 (8)	1.1 ± 0.1 (8)
5 hrs. after 0.75 mg. endotoxin + 5 mg. cortisone	85 ± 7.2 (8)	0.85 ± 0.3 (8)	0.10 ± 0.01 (8)	2.6 ± 0.24 (8)
5 hrs. of fasting alone	130 ± 7.5 (8)	3.23 ± 0.65 (8)	0.17 ± 0.03 (8)	4.4 ± 0.4 (8)

In Table IV B, carbohydrate data are summarized for SW mice following the injection of endotoxin alone and endotoxin plus 5 mg. cortisone. Endotoxin alone at a dosage level 96 per cent fatal (Table I) lowered blood sugar and liver and muscle glycogen to near depletion levels. The total carbohydrate is the same as that in CF-1 animals at death. This was not unexpected, since the mice employed for the determinations were near death when the assays were performed. When cortisone was given with the endotoxin, the carbohydrate levels of blood and muscle were more than doubled and liver glycogen was elevated about eightfold. These larger values were reflected in the threefold increase in total carbohydrate (2.7 mg./gm. *vs.* 0.9 mg./gm.). As seen in Table I, 85 per cent of SW mice given both endotoxin and cortisone survived.

Carbohydrate analyses made 17 hours after administration of endotoxin raise the question as to how much inanition contributes to the depletion of glycogen. The fact that carbohydrate loss was greater in mice given endotoxin and fasted for 5 hours than in mice simply fasted for the same period of time can be seen in the data of Table V. These results with HF-negative mice clearly

prove that endotoxin exaggerated the glycogen disappearance. It is particularly important to note that the values shown in the first two lines of Table V agree closely with comparable data for SW mice at 17 hours, Table IV B. Thus, with endotoxin alone the carbohydrate dropped promptly and continued at a new low steady-state level for at least a period of 12 hours. Animals given both endotoxin and cortisone had more carbohydrate than those given endotoxin alone, presumably because of glyconeogenesis, and their reserves appeared to be in a new steady-state level. For this to occur, the hormone is either absorbed slowly over an extended period of time or is not rate-limiting in its action. Since albino mice are reported (21) to have a basal metabolic

TABLE VI

Carbohydrate Data for HF-Negative Mice Given Intraperitoneal Injections of S. typhimurium Endotoxin and the More Highly Purified Lipopolysaccharide of S. marcescens and S. typhosa

Each value is the mean ± the standard deviation. The numbers in parentheses are the number of separate determinations made for each value.

Experimental treatment	Blood sugar	Liver glycogen	Muscle glycogen	Total carbohydrate
	mg. per cent	*per cent by wt.*	*per cent by wt.*	*mg./gm.*
17 hrs. after 0.75 mg. crude *S. typhimurium* endotoxin	62 ± 10.6 (9)	0.15 ± 0.05 (12)	0.06 ± 0.03 (11)	0.9 ± 0.34 (9)
17 hrs. after 0.5 mg. lipopolysaccharide (*S. marcescens*)	71 ± 7.8 (7)	0.22 ± 0.1 (7)	0.06 ± 0.02 (7)	1.3 ± 0.18 (7)
17 hrs. after 0.5 mg. purified *S. typhosa* endotoxin	67 ± 7 (7)	0.25 ± 0.06 (7)	0.06 ± 0.01 (7)	1.3 ± 0.19 (7)

rate such that 40 to 45 mg. glucose (or its equivalent) is consumed per hour, then normal animals have no more than about 2 hours reserve. The unabsorbed food in the digestive tract would still be available but once this were depleted, continued existence would depend primarily on utilization of body reserves of fat and protein. These relationships are treated in more detail in the paper that follows (22).

Comparison of Endotoxins from Different Sources and of Different Purity on Carbohydrate Levels of Mice.—That endotoxins of varying purity, as judged by the procedures used in their preparation and their relative nitrogen content, yielded almost identical carbohydrate levels in mice 17 hours postinjection can be seen in the data of Table VI. The "crude" *S. typhimurium* endotoxin (8 to 10 per cent N) at the dosage indicated (0.75 mg.) was 87 per cent lethal for HF mice (Table I) while 0.5 mg. of *S. marcescens* lipopolysaccharide (2.3 per cent N) was about 20 per cent lethal for similar animals. The *S. typhosa*

endotoxin (0.6 per cent N) (23), also in 0.5 mg. amounts, failed to kill any of seven mice. The supply did not permit additional tests. Perhaps the slightly lower liver glycogen and total carbohydrate seen with the *S. typhimurium* endotoxin is attributable to the more toxic dose administered.

Effect of Dibenzyline on Carbohydrate Changes Due to Endotoxin.—While the preceding data show that cortisone "protects" the carbohydrate reserves of mice against depletion by endotoxin, the following group of experiments was designed to offer some insight into the causes of the rapid disappearance of glycogen in animals injected with endotoxin. The extent to which this phe-

TABLE VII

Effect of Intraperitoneal Injections of 10 Mg./Kg. Dibenzyline on Survival and on Body Carbohydrate of CF-1 Mice Injected Intraperitoneally with S. typhimurium Endotoxin

Dibenzyline was given 1 hour prior to the endotoxin. Each carbohydrate value is the mean ± the standard deviation. The numbers in parentheses are the number of separate determinations made for each value.

Experimental determination	Result with mice given	
	Endotoxin alone	Endotoxin and dibenzyline
Survivors / Total No.	11/20	9/20
Blood sugar, *mg. per cent*	80 ± 17 (19)	77 ± 14 (18)
Liver glycogen, *per cent*	1.5 ± 1.1 (17)	1.9 ± 1.2 (17)
Total carbohydrate, *mg./gm. carcass*	3.3 ± 1.0 (8)	2.7 ± 1.0 (8)

nomenon is mediated through epinephrine was investigated by pretreating mice with dibenzyline, a drug known to prevent the hyperglycemia and breakdown in liver glycogen produced by epinephrine (24, 25). The data of Table VII show, however, that dibenzyline neither protected against the lethal effects of endotoxin nor did it alter the related carbohydrate changes. Any interaction between the adrenal medulla and bacterial endotoxins, therefore, appears to be secondary rather than primary even though two recent reports (26, 27) offer convincing evidence for epinephrine depletion in endotoxin-poisoned animals.

Rectal Temperatures of Mice Given Endotoxin.—The pyrogenic effect of endotoxin, reviewed by Bennett and Beeson (28), might reasonably be expected to result in an elevated metabolism and, consequently, an accelerated loss of

carbohydrates. Under the specific conditions of the present experiments, however, a hypothermia was observed, as shown in Fig. 1. Mice given either the crude *S. typhimurium* endotoxin or the purified *S. marcescens* lipopolysaccharide had rectal temperatures that were never hyperthermic even in the early minutes postinjection. They were several degrees below normal after an hour and continued low for the succeeding 4 or 5 hours. Carbohydrate values at the end of this period were nearly as low as they were 17 hours after endotoxin administration (compare Tables IV and V). The absence of a febrile response in the mice used in these studies might be related to the large dose of endotoxin employed; otherwise, the effect is unexplained.

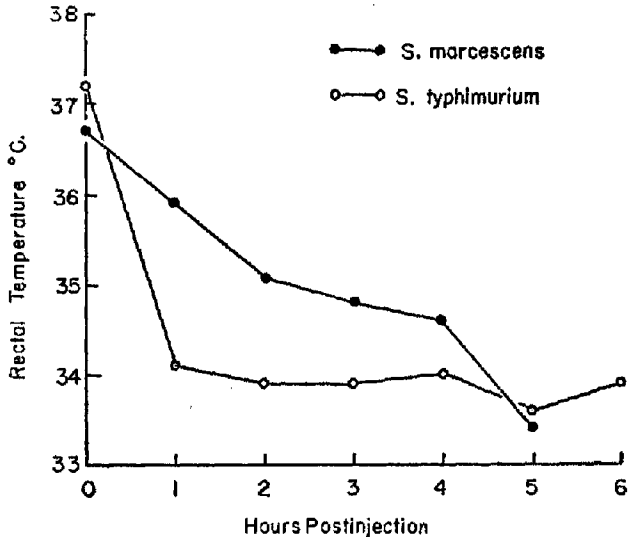

FIG. 1. Rectal temperatures of mice after intraperitoneal injection of 0.75 mg. *S. typhimurium* endotoxin or 0.5 mg. of *S. marcescens* endotoxin. Each point is the average of 10 separate determinations.

Glycogen Synthesis from Glucose in Fasted Mice and in Mice Given Endotoxin.— From the literature (see Discussion) there is reason to believe that bacterial endotoxins exert their effect on body carbohydrate by impairing glycogen synthesis. A comparison was made, therefore, of the manner in which the normal and the endotoxin-poisoned mouse responds to intraperitoneal injections of 50 mg. glucose. Normal mice were fasted until their carbohydrate levels approximated the values obtained for mice given endotoxin (compare line 1, Table VIII with line 2 Table IV). One hour after glucose, fasted animals showed a decided hyperglycemia and a threefold increase in liver glycogen. No significant change occurred in the blood sugar or liver glycogen of the endotoxin-treated mouse under the same conditions. On the other hand, muscle glycogen

appeared to have been synthesized in the mouse previously given endotoxin (see line 3, Table VIII) but there was no change in muscle glycogen of fasted mice given glucose. A marked contrast exists, therefore, between the glycogene-

TABLE VIII

Effect of Intraperitoneal Injection of Glucose on Body Carbohydrate of CF-1 Mice

Each value is the mean ± the standard deviation. The numbers in parentheses are the number of separate determinations made for each value.

Experimental treatment	Blood sugar	Muscle glycogen	Liver glycogen
	mg. per cent	*per cent*	*per cent*
Fasting for 17 hrs.	93 ± 12 (6)	0.08 ± 0.05 (7)	0.72 ± 0.4 (8)
Fasting for 17 hrs. and 1 hr. after 50 mg. glucose intraperitoneally	231 ± 22 (8)	0.09 ± 0.01 (8)	2.03 ± 0.33 (8)
17 hrs. after fasting and endotoxin and 1 hr. after 50 mg. glucose intraperitoneally	72 ± 12 (14)	0.23 ± 0.05 (13)	0.26 ± 0.11 (14)

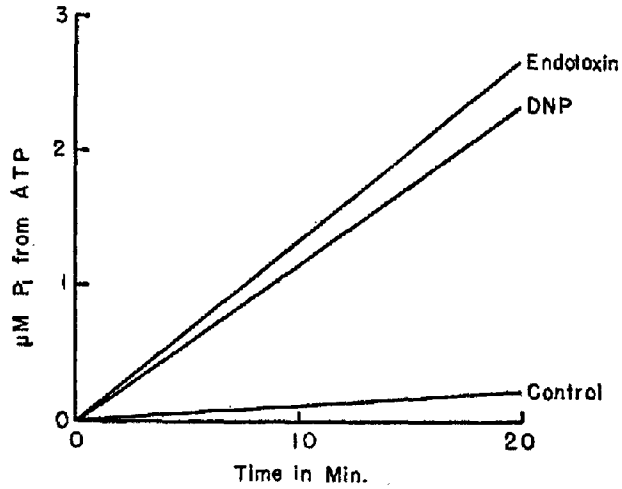

FIG. 2. Micromoles of inorganic phosphate released from ATP by mouse liver mitochondria in the presence of 1 mg. of *S. typhimurium* endotoxin or 10^{-4} M 2,4-dinitrophenol compared to that of control preparations.

sis resulting from glucose injection in fasted mice *versus* mice given endotoxin. In each case, the total amount of glucose accounted for in the assays was about the same; *i.e.*, 13 to 14 mg. The remainder of the 50 mg., presumably, was metabolized (*cf.* above for hourly metabolic rate of mice).

In Vitro Effect of Endotoxin on Mouse Liver Mitochondria.—Mouse liver mitochondria in the presence of *S. typhimurium* endotoxin under *in vitro* conditions released inorganic phosphate from ATP at a rate slightly greater than that observed with DNP, Fig. 2. The mechanism involved in the splitting of ATP in the presence of endotoxin may be non-specific and dependent upon mitochondrial swelling (29), as indicated by a few preliminary experiments using the technique of Cleland (30). DNP is believed to act more specifically since it does not distort the fine structure of the mitochondria (31). The same end effect may be obtained, therefore, by entirely different processes and need not be considered comparable. The indication of an interference with high energy phosphate metabolism offers a possible explanation, however, for many of the metabolic changes observed with endotoxins.

DISCUSSION

The ability of cortisone acetate to protect mice against the lethal action of bacterial endotoxin has been confirmed. No detectable protection results, however, with inadequate cortisone or with large doses of endotoxin.

The survival of experimental animals given a lethal dose of endotoxin and a therapeutic injection of cortisone seems to be related to carbohydrate metabolism, either directly or indirectly. Surviving mice, without exception, have greater carbohydrate reserves than those that succumb and, as well as can be judged, the quantity that remains appears to be essential for life. This, admittedly, is speculative but if one accepts as valid the fundamental biological concept that the existence of a living organism in an active state is dependent upon a continuous expenditure of energy, then it would follow that the exhaustion of energy sources is fatal. The remaining requirement becomes, then, a matter of ascertaining the lowest level to which energy reserves may fall. On the basis of the experiments reported in this and another (25) paper, mice have consistently died when their total carbohydrate drops to about 1 mg. per gm. of carcass weight. There is no proof, of course, that the carbohydrate level is causally related, under these conditions, to survival but it at least has the merit of reproducibility. On the other hand, the interaction between endotoxin and cortisone offers an opportunity to establish a balance between protein degradation and carbohydrate synthesis. This is the subject of the paper that follows (22).

Metabolic and endocrine processes as complex as those regulating carbohydrate balance do not permit ready explanations for changes in muscle and liver glycogen in endotoxin poisoning. Various mechanisms have been suggested.

Evans and Zwecker (32) and later Boivin and Mesrobeanu (33) postulated a release of epinephrine or related compound in animals given endotoxin. Dennis (34) found that the adrenals were depleted of chromaffin material in rabbits given a lethal dose

of typhoid endotoxin. More recently, Thomas (35) and Zweifach, Nagler, and Thomas (36) proposed that endotoxin sensitized tissues to epinephrine but this was not confirmed by Meyer and Ballin (37). The use of dibenamine, a compound closely related to dibenzyline, by Nickerson and Goodman (38) and subsequently by Boquet and Izard (39) failed to prevent, however, the hyperglycemic effect of endotoxin. Thus, it would appear unlikely, on the basis of reports in the literature and the data of Table VII, that the carbohydrate changes due to endotoxin are mediated through epinephrine.

Kun and Miller (40), working with fasted rabbits, administered massive doses of meningococcus or *Salmonella aertrycke* (*typhimurium*) endotoxin intravenously that were lethal in 2 to 3 hours. Under these extreme conditions, blood sugar rose at 30 minutes and then declined. Lactic acid and inorganic phosphate increased steadily in blood. In liver and muscle, glycogen decreased while lactic acid increased. Kun also reported (41) that *Salmonella* endotoxin administered intraperitoneally to rats inhibited completely the *in vivo* conversion of glucose to liver glycogen. Under *in vitro* conditions, the uptake of glucose by rat diaphragm or muscle extracts (42) was prevented by endotoxin alone but insulin partially reversed it. These findings are in agreement with those presented in Table VIII.

This greater sensitivity of liver to bacterial endotoxins, compared to that of muscle, may be explained by the recent results of several groups of workers. Liver is clearly more active in the removal of foreign particulate matter and hence contains a higher concentration of endotoxin than muscle.

Rowley, Howard, and Jenkins (43), for example, injected, intravenously, P^{32}-labelled lipopolysaccharide from *Escherichia coli* into mice and found liver and spleen to contain half of the total radioactivity that disappeared from the blood. Mayne and Jones (44) with C^{14}-labelled polysaccharide complex from *Klebsiella pneumoniae* found that livers of injected mice contained a large fraction of the total radioactivity. This was also observed by Braude, Carey, Sutherland, and Zalesky (45) in livers of rabbits injected intravenously with Cr^{51}-labelled *E. coli* endotoxin. Muscle, by contrast, contained no more than approximately 1 per cent of the total radioactivity. These results suggest that muscle glycogen changes are more secondary than those of liver glycogen because of the *in vivo* distribution of the toxic material.

Kun and Abood (46) carried out *in vivo* and *in vitro* experiments which indicated an inhibition of pyruvate oxidation in animals injected with *Salmonella* or meningococcus endotoxins. The mechanism involved in this action was not explained but it was thought to be the primary cause for the toxicity of the bacterial product. Mager and Theodor (47) determined the influence of somatic antigen preparations derived from *Shigella paradysenteriae* type III on the oxygen consumption in rat or mouse liver mitochondria. They found oxygen consumption to be inhibited by a haptenic polysaccharide contaminating the antigens. Inhibition was most pronounced with those substrates the dehydrogenases of which were linked to pyridine nucleotides. The native hapten also uncoupled oxidative phosphorylation in rat liver mitochondria. A similar observation was reported by Fonnesu and Severi (48) in mitochondria isolated from rat livers showing cloudy swelling due to endotoxins. There is reason to

believe, therefore, that endotoxins interfere with the energetics of the organism. The carbohydrate changes with which this paper has been concerned would be a result of such disturbances and not a cause. This suggestion has the support of results published by Takeda *et al.* (49), who claimed that appropriate intravenous doses of ATP, approximately 1 mg. per 20 gm. mouse, completely protected against a lethal quantity of highly purified endotoxin from either *Salmonella paratyphi* B, or *Shigella flexneri* type Mita. They also reported that increased lactic and inorganic phosphate and decreased organic phosphorus and alpha ketoglutaric acid could all be prevented by injection of ATP but that hyperglycemia persisted.

Preliminary results from this laboratory with commercially available ATP and with *S. typhimurium* endotoxin failed to confirm the results of the Japanese workers but more careful tests are required before the basis for disagreement is understood.

SUMMARY

Mice of different strains were protected against the lethal effect of bacterial endotoxin by concurrent injections of cortisone. Either inadequate amounts of cortisone or excessive quantities of endotoxin voided the protection.

Analyses of blood sugar, liver glycogen, muscle glycogen, and total body carbohydrate in the skinned eviscerated carcass were carried out on different strains of mice given endotoxin and/or cortisone. Poisoned animals were virtually depleted of all carbohydrate while mice given cortisone alone had concentrations of carbohydrate from three to four times that of normal mice. Mice given a lethal amount of endotoxin and a protective dose of cortisone had two to three times as much carbohydrate as animals injected with the same amount of endotoxin alone but significantly less than that found in normal mice.

Dibenzyline failed to alter the lethal effect of endotoxin and to reduce the carbohydrate loss that accompanied endotoxin administration.

Endotoxin, at the dosage level employed, lowered the temperature of mice 2°–3°C. during the first hour or two postinjection and the temperature remained essentially unaltered during the next 4 to 5 hours. Loss in body carbohydrate in endotoxin-poisoned mice cannot be explained, therefore, as the result of an elevated metabolic rate accompanying hyperthermia.

Endotoxin prevented the conversion of injected glucose into liver glycogen but not into muscle glycogen.

Mouse liver mitochondria, in the presence of endotoxin, released from ATP approximately the same amount of inorganic phosphate as that released in the presence of dinitrophenol.

BIBLIOGRAPHY

1. Duffy, B. J., and Morgan, H. R., ACTH and cortisone aggravation or suppression of the febrile response of rabbits to bacterial endotoxin, *Proc. Soc. Exp. Biol. and Med.*, 1951, **78**, 687.

2. Germuth, F. P., Jr., Ottinger, B., and Oyama, J., The influence of cortisone on the evolution of acute infection and the development of immunity, *Bull. Johns Hopkins Hosp.*, 1952, **91,** 22.
3. Bennett, I. L., Jr., and Beeson, P. B., The effect of cortisone upon reactions of rabbits to bacterial endotoxins with particular reference to acquired resistance, *Bull. Johns Hopkins Hosp.*, 1953, **93,** 290.
4. Geller, P., Merrill, E. R., and Jawetz, E., Effects of cortisone and antibiotics on lethal action of endotoxins in mice, *Proc. Soc. Exp. Biol. and Med.*, 1954, **86,** 716.
5. Spink, W. W., and Anderson, D., Experimental studies on the significance of endotoxin in the pathogenesis of brucellosis, *J. Clin. Inv.*, 1954, **33,** 540.
6. Thomas, L., and Smith, R. T., Effect of cortisone on response to endotoxin in mature rabbits, *Proc. Soc. Exp. Biol. and Med.*, 1954, **86,** 810.
7. Chedid, L., Boyer, F., and Saviard, M., Nouveaux aspects de l'action antitoxique de la cortisone, *Ann. Inst. Pasteur*, 1954, **86,** 347.
8. Levitin, H., Kendrick, M. I., and Kass, E. H., Effect of route of administration on protective action of corticosterone and cortisol against endotoxin, *Proc. Soc. Exp. Biol. and Med.*, 1956, **93,** 306.
9. Jackson, E. B., and Smadel, J. E., The effects of cortisone and ACTH on toxins of *Rickettsiae* and *S. typhosa*, *J. Immunol.*, 1951, **66,** 621.
10. Ebert, R. V., Borden, C. W., Hall, W. H., and Gold, D., A study of hypotension (shock) produced by meningococcus toxin, *Circ. Rev.*, 1955, **3,** 378.
11. Menten, M. L., and Manning, H. M., Blood sugar studies on rabbits infected with organisms of the enteritidis-paratyphoid B group, *J. Med. Research*, 1924, **44,** 675.
12. Zeckwer, I. T., and Goodell, H., Blood sugar changes in fatal bacterial anaphylaxis in the rabbit, *J. Exp. Med.*, 1925, **42,** 43.
13. Delafield, M. E., A comparison of the changes in the blood sugar and blood phosphorus in rabbits following the injection of suspensions of different dead bacteria, *J. Path. and Bact.*, 1932, **35,** 53.
14. Long, C. N. H., Katzin, B., and Fry, E. C., The adrenal cortex and carbohydrate metabolism, *Endocrinology*, 1940, **26,** 309.
15. Nelson, N., A photometric adaptation of the Somogyi method for the determination of glucose, *J. Biol. Chem.*, 1944, **153,** 375.
16. Kemp, A., and Kits van Heijningen, A. J. M., A colorimetric micro-method for the determination of glycogen in tissues, *Biochem. J.*, 1954, **56,** 646.
17. Mendel, B., Kemp, A., and Myers, D. K., A colorimetric micro-method for the determination of glucose, *Biochem. J.*, 1954, **56,** 639.
18. Hogeboom, G. H., and Schneider, W. C., Intracellular distribution of enzymes. V. Further studies on the distribution of cytochrome c in rat liver homogenates, *J. Biol. Chem.*, 1950, **183,** 123.
19. Fiske, C. H., and Subbarow, Y., The colorimetric determination of phosphorus, *J. Biol. Chem.*, 1925, **66,** 375.
20. Hobson, D., Resistance to reinfection in experimental mouse typhoid, *J. Hyg.*, 1957, **55,** 334.
21. Morrison, P. R., Oxygen consumption of several mammals under basal conditions, *J. Cell. and Comp. Physiol.*, 1948, **31,** 281.

22. Berry, L. J., and Smythe, D. S., Effects of bacterial endotoxin on metabolism. II. Protein-carbohydrate balance following cortisone. Inhibition of intestinal absorption and of adrenal response to ACTH, *J. Exp. Med.*, 1959, **110**, 407.
23. Landy, M., and Johnson, A. G., Studies on the O antigen of *Salmonella typhosa*. IV. Endotoxic properties of the purified antigen, *Proc. Soc. Exp. Biol. and Med.*, 1955, **90**, 57.
24. Harvey, S. C., Wang, C. Y., and Nickerson, M., Blockage of epinephrine-induced hyperglycemia, *J. Pharm. and Exp. Therap.*, 1952, **104**, 363.
25. Berry, L. J., and Smythe, D. S., Carbohydrate metabolism in normal and altitude exposed mice following arsenite poisoning, *Am. J. Physiol.*, 1959, in press.
26. Heiffer, M. H., Mundy, R. L., and Mehlman, B., Adrenal catechol amine concentrations following endotoxin administration, *Fed. Proc.*, 1959, **18**, 66.
27. Nykiel, F., Bass, N., Scherr, G., and Glaviano, V., Myocardial and adrenal medullary function in *E. coli* endotoxin shock, *Fed. Proc.*, 1959, **18**, 115.
28. Bennett, I. L., Jr., and Beeson, P., Properties and biologic effects of bacterial pyrogens, *Medicine*, 1950, **29**, 365.
29. Tapley, D. F., The effect of thyroxine and other substances on the swelling of isolated rat liver mitochondria, *J. Biol. Chem.*, 1956, **222**, 325.
30. Cleland, K. W., Permeability of isolated rat heart sarcosomes, *Nature*, 1952, **170**, 497.
31. Lehninger, A. L., Phosphorylation coupled to oxidation of dihydrodiphosphopyridine nucleotide, *J. Biol. Chem.*, 1951, **190**, 345.
32. Evans, C. L., and Zwecker, I. T., On the nature of the hyperglycemic response to injections of certain killed bacteria, *Brit. J. Exp. Path.*, 1927, **8**, 280.
33. Boivin, A., and Mesrobeanu, L., Sur l'action hyperglycemiante du complexe toxique, specifique et antigenique isole a partir du bacille d'Aertrycke, *Compt. rend. Soc. biol.*, 1934, **117**, 273.
34. Dennis, E. W., Toxicity of acid-soluble typhoid toxin for laboratory animals, *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 553.
35. Thomas, L., The role of epinephrine in the reactions produced by the endotoxins of Gram-negative bacteria. I. Hemorrhagic necrosis produced by epinephrine in the skin of endotoxin-treated rabbits, *J. Exp. Med.*, 1956, **104**, 865.
36. Zweifach, B. W., Nagler, A. L., and Thomas, L., The role of epinephrine in the reactions produced by the endotoxins of Gram-negative bacteria. II. Changes produced by endotoxin in the vascular reactivity to epinephrine in the rat mesoappendix and the isolated, perfused rabbit ear, *J. Exp. Med.*, 1956, **104**, 881.
37. Meyer, M. W., and Ballin, H. M., Failure to observe alteration of epinephrine activity after endotoxin, *Proc. Soc. Exp. Biol. and Med.*, 1959, **100**, 288.
38. Nickerson, M., and Goodman, L. S., Pharmacological properties of a new adrenergic blocking agent: N,N-dibenzyl-B-chloroethyl amine (Dibenamine), *J. Pharmacol. and Exp. Therap.*, 1947, **89**, 167.
39. Boquet, P., and Izard, Y., Recherches sur l'hyperglycemie experimentale par l'endotoxine typhique, *Comp. rend. Acad. Sc.*, 1951, **145**, 351.
40. Kun, E., and Miller, C. P., Effect of bacterial endotoxins on carbohydrate metabolism of rabbits, *Proc. Soc. Exp. Biol. and Med.*, 1948, **67**, 221.
41. Kun, E., Effect of bacterial endotoxin on glycogen synthesis, *Proc. Soc. Exp. Biol. and Med.*, 1948, **68**, 496.

42. Kun, E., Inhibition of the phosphorylation of glucose by meningococcal endotoxin, *J. Biol. Chem.*, 1948, **174**, 761.
43. Rowley, D., Howard, J. G., and Jenkins, C. R., The fate of P^{32}-labelled bacterial lipopolysaccharide in laboratory animals, *Lancet*, 1956, 336.
44. Mayne, Y. C., and Jones, R. S., Tissue distribution of C^{14}-labelled bacterial polysaccharide in guinea pig, rat, mouse and rabbit, *Proc. Soc. Exp. Biol. and Med.*, 1958, **98**, 734.
45. Braude, A. I., Carey, F. J., Sutherland, D., and Zalesky, M., Studies with radioactive endotoxin. I. The use of Cr^{51} to label endotoxin of *E. coli*, *J. Clin. Inv.*, 1955, **34**, 850.
46. Kun, E., and Abood, L. G., Mechanism of inhibition of glycogen synthesis by endotoxins of *Salmonella aertrycke* and type I meningococcus, *Proc. Soc. Exp. Biol. and Med.*, 1949, **71**, 362.
47. Mager, J., and Theodor, E., Inhibition of mitochondrial respiration and uncoupling of oxidative phosphorylation by fractions of the *Shigella paradysenteriae* type III somatic antigen, *Arch. Biochem. and Biophysics*, 157, **67**, 169.
48. Fonnesu, A., and Severi, C., Oxidative phosphorylation in mitochondria from livers showing cloudy swelling, *J. Biophysic and Biochem. Cytol.*, 1956, **2**, 293.
49. Takeda, Y., Muira, Y., Sazaki, H., and Kasai, N., The elimination of metabolic disturbances produced by the injection of *S. typhi*, *S. paratyphi* B, *S. flexneri* and the prevention of animals from death through the application of adenosine triphosphate, *Japan J. Exp. Med.*, 1955, **25**, 133.