EFFECTS OF BACTERIAL ENDOTOXIN ON METABOLISM

II. Protein-Carbohydrate Balance Following Cortisone. Inhibition of Intestinal Absorption and Adrenal Response to ACTH*

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(Received for publication, April 8, 1959)

The role of the adrenal cortex in an animal's response to bacterial infections and to bacterial endotoxins has been the subject of much research and speculation.

Just recently, Brooke, Kass, and Hechter (1) reported that adrenalectomized rats are killed by one one-thousandth the dose of endotoxin required to kill normal animals. This dramatic observation is far superior to any other in the literature in emphasizing the protection afforded by adrenal hormones against the toxic manifestations of an endotoxin. It conveys no information, however, as to why this should be true. In the preceding paper (2), it was indicated that the therapeutic action of cortisone in endotoxin-poisoned mice was linked, perhaps, to the higher levels of carbohydrate it produced. Since Long *et al.* (3) found that the glyconeogenesis initiated by adrenal cortical hormones was accompanied by protein degradation and a negative nitrogen balance, it is to be assumed that the carbohydrate formed in mice given both endotoxin and cortisone is synthesized, at least in part, from body protein. This premise is subject to experimental verification.

The classical relationship which permits urinary nitrogen excretion to be used in calculating protein catabolized can now be combined with a simple procedure (4) for measuring total body carbohydrate to establish a balance between the two quantities. Results of such experiments comprise part of this report.

Other facets of the problem have also been evaluated. It must be assumed that the adrenal cortical hormones produced endogenously in response to adrenocorticotropic hormone (ACTH) actively protect experimental animals against injections of endotoxins. The importance of studies with ACTH instead of with exogenous cortisone is, therefore, apparent. Under selected conditions, the inability of ACTH to substitute, metabolically, for cortisone in animals poisoned with endotoxin is described.

Comparison is made also between the effect of an endotoxin derived from

* 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.

Salmonella typhimurium and that of a known number of the same bacteria killed by pasteurization. Earlier studies (5–7) established that the number of viable *S. typhimurium* present at the instant of death in mice experimentally infected with mouse typhoid was constant within 1 logarithmic unit, regardless of survival time or the treatment used to alter the animal's resistance to infection. Death seemed to be attributable, therefore, to the lethal effect of a given population of viable pathogenic organisms. To supplement these studies, selected changes induced in mice with known numbers of killed bacterial cells are compared with the same changes as they occur during the active infection. By knowing the number of viable bacteria at the time the alteration appears, it is possible to draw inferences as to the extent to which the toxic manifestations of an infection are due to living bacteria or to their endotoxins released *in vivo*.

Methods

Endotoxin.—A comparatively crude endotoxin, prepared from *Salmonella typhimurium* strain SR-11, as previously described (2), was used in all experiments. A suspension of heat-killed bacteria was obtained by sedimenting an overnight brain-heart infusion broth (Difco) culture in the cold, washing with isotonic NaCl solution, making a dilution count, and then pasteurizing at 60°C. for 30 minutes. The final material was stored in the cold in vaccine bottles.

Determination of Carbohydrates.—Liver and muscle (abdominal body wall) glycogens were determined colorimetrically by the method of Kemp and Kits van Heijningen (8). Total carbohydrate in the mouse was analyzed according to the procedure of Mendel, Kemp, and Myers (9) following the technique of Berry, Smythe, and Young (2).

Urinary Nitrogen Determination.—Stainless steel wire cloth, 16 mesh, was used to fabricate small cages that were placed over 100 mm. glass funnels. Urine from two mice was collected overnight (about 17 hours) in a test tube containing toluene, into which the stem of the funnel was inserted. The volume of urine was measured and nitrogen was determined by microKjeldahl analysis. Total nitrogen excreted per mouse per 17 hours was calculated. Mice were either fasted during the collection period or each pair was provided with 15 ml. of a solution which contained 500 mg. tryptone (Bacto) and 1000 mg. sucrose. All the fluid was consumed during the 17 hour period. In one experimental series mice were fed by stomach tube with a fluid mixture containing 200 mg. tryptone (Bacto) in 0.5 ml. In another series a slurry prepared from Purina dog chow biscuit was administered by stomach tube.

Cortisone and ACTH.—Cortisone and ACTH were each administered subcutaneously. Cortisone acetate (Nutritional Biochemicals, Cleveland) was suspended in isotonic NaCl solution with a drop of tween 80 as stabilizer. ACTH (George Breon Company, New York, or Armour Laboratories, Chicago) was injected as a gelatin suspension in two, four, or eight unit quantities. Within the limits of our determinations, the response did not vary with dose.

Mice.—Three different 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). Under the conditions of the experiments, each strain was indistinguishable from the others. They were housed in small cages, about 10 mice to the cage, in an animal room maintained at 25° ± 1°C. White pine shavings were used as bedding and they were fed Purina dog chow *ad libitum*. Water was available at all times.

RESULTS

Effect of Endotoxin and Cortisone on Urinary Nitrogen Excretion.—Table I summarizes the results of urinary nitrogen analyses on fasted animals with and without the subcutaneous injection of 5 mg. cortisone acetate and on fasted endotoxin-poisoned mice with and without the cortisone. An LD_{90} dose, approximately, of *S. typhimurium* endotoxin was administered intraperitoneally. Both HF and SW mice, all weighing 20 ± 1 gm., were employed. It is important to use animals of the same weight (age) for these experiments since nitrogen loss is related to size and maturity.

TABLE I

Urinary Nitrogen Excretion Data for Two Strains of Mice with and without Endotoxin and with and without Cortisone

Each value is the mean ± the standard deviation. The number of separate determinations is given in parentheses.

Experimental treatment	Urinary N excretion in mg. N per mouse per 17 hr. for	
	HF mice	SW mice
Control—without food and water 17 hrs.	13.3 ± 4.7 (10)	13.6 ± 1.9 (7)
17 hrs. without food and water + 5 mg. cortisone	21.6 ± 3.4 (8)	22.2 ± 3.2 (8)
17 hrs. without food and water + 0.75 mg. endotoxin	11.2 ± 1.8 (8)	12.2 ± 2.3 (13)
17 hrs. without food and water + 5 mg. cortisone + 0.75 mg. endotoxin	20.3 ± 2.3 (7)	20.8 ± 3.7 (15)

The injection of cortisone led, in all cases, to an increase in urinary nitrogen excretion. Calculating the quantities involved shows that the minimum increase due to cortisone, 8.3 mg., occurred in HF control mice (21.6 minus 13.3) while the maximum, 9.1 mg., was obtained with the same strain given endotoxin (20.3 minus 11.2). SW mice under these conditions excreted, in both cases, an excess of 8.6 mg. nitrogen. The quantity of protein catabolized in order to yield the extra urinary nitrogen was, therefore, nearly constant. Using 16 per cent as the average nitrogen content of animal protein, the injection of 5 mg. cortisone acetate in mice resulted in the breakdown of between 52 and 57 mg. protein (8.3 or 9.1 × 6.25). A corresponding weight of carbohydrate could either be spared or synthesized as a result (see below).

The data of Table I show quite conclusively that endotoxin injections do not alter significantly (*a*) the basal rate of protein degradation over a period

of 17 hours of fasting (compare lines 1 and 3 of the table) and (*b*) the protein catabolized in response to cortisone administration in fasted mice.

Quantitative Estimation of Protein Breakdown and Carbohydrate Synthesis.— As indicated in the introduction, it should be possible to calculate the total protein degraded and the total carbohydrate synthesized over a period of about 17 hours in mice injected with cortisone. In order to do this with fed animals for comparison with the more easily handled fasted mice, food was offered in solution. The animals readily accepted this material and, in all instances, the 15 ml. proffered them disappeared during the overnight period.

TABLE II

Urinary Nitrogen Excretion, Calculated Protein Degradation (Urinary N × 6.25), and Total Carbohydrate in HF Mice Fed, Fasted, and Fasted plus Given Endotoxin

Determinations were made on animals of each group injected subcutaneously with 5 mg. cortisone acetate as well as on controls. Each value in the table is the mean of a minimum of six separate determinations.

Experimental treatment	Urinary nitrogen	Protein breakdown	Carbohydrate	Total carbohydrate
	mg.	*mg.*	*mg./gm.*	*mg.*
(*a*) Fed	26.7	166.9	7.1	83.8
(*b*) Fed + cortisone	44.9	280.6	17.7	194.7
(*b*) − (*a*)		113.7		110.9
(*c*) Fasted	13.3	83.1	2.0	22
(*d*) Fasted + cortisone	21.6	135.0	5.8	69.6
(*d*) − (*c*)		51.9		47.6
(*e*) Endotoxin	12.2	76.3	0.9	10.8
(*f*) Endotoxin + cortisone	20.8	130.0	2.7	32.4
(*f*) − (*e*)		53.7		21.6

By assuming that each mouse drank half the total volume, the nutritive intake per animal was known.

Under these experimental conditions, mice excreted 26.7 mg. urinary nitrogen as indicated in line (*a*), Table II. The total degradation of protein, obtained by multiplying 26.7 × 6.25, is 166.9 mg. The same mice were utilized also for the analyses of total carbohydrate. The values 7.1 mg./gm. and 83.8 mg. per mouse were found. Similarly, mice injected with 5 mg. cortisone at the beginning of the 17 hour period of urine collection and permitted to drink the nutritive solution yielded the data of line (*b*), Table II. The difference due to cortisone in quantity of protein breakdown and carbohydrate stored agree within less than 3 per cent (113.7 *vs.* 110.9) as shown by the values in line (*b*) − (*a*).

The same procedure was carried out for fasted mice and the results are listed in lines (*c*), (*d*), and (*d*) − (*c*), Table II. Here, also, the protein breakdown

attributable to the effects of the 5 mg. cortisone acetate (51.9 mg.) agrees within about 8 per cent of the excess carbohydrate (47.6 mg.). From the results with fed and with fasted mice, one is able to conclude that cortisone contributes to protein degradation and not, significantly, to carbohydrate utilization. A balance would not result otherwise.

By the same technique, the role of endotoxin was assessed. Lines (*e*), (*f*), and (*f*) − (*e*), Table II, contain results that prove endotoxin upsets the balance between protein catabolism and carbohydrate synthesis. There is a deficiency in carbohydrate (21.6 mg. instead of 53.7) due either to an increase in its utilization or to an impairment in synthesis, possibly from non-carbohydrate precursors. There is no evidence for the former but in the preceding paper (2) endotoxin was reported to interfere with the conversion of injected glucose into liver glycogen, not, however, into muscle carbohydrate. Since mice given endotoxin refused for the entire collection period of 17 hours to drink the nutritive fluid, it was not possible to extend the observations to cover this situation.

Additional facts can be derived from the data of Table II. Fed mice excrete 13.4 mg. more urinary nitrogen than fasted mice (26.7 minus 13.3). The solution consumed per mouse had 250 mg. tryptone with 13 per cent nitrogen or about 36 mg. total nitrogen. Hence the 22+ mg. of tryptone nitrogen unaccounted for was, presumably, available for growth and repair at the rate of approximately 1 gm. per week, a reasonable weight increment for 20 gm. mice. Fed cortisone-injected mice, on the other hand, excreted 31.6 mg. (44.9 minus 13.3) of the 36 mg. consumed and hence were almost in nitrogen balance. A total carbohydrate of 7.1 mg./gm. for fed mice compares favorably with that for control mice permitted free access to chow biscuits (see Table V). Since each animal used for the data of line (*a*), Table II, consumed 500 mg. sucrose and 250 mg. tryptone over a 17 hour period, this amounted to 44 mg. of food per hour. (Note: the caloric value of protein and carbohydrate per unit weight is about equivalent.) From the rate of oxygen consumption by mice (10), it can be calculated that 40 to 45 mg. of glucose, or its equivalent, is required per hour. The fed mice, on this basis, were in caloric balance.

Effect of Endotoxin on Intestinal Absorption and Motility.—Since endotoxin-poisoned mice refused to drink a nutritive solution made available to them, an intrastomachal injection containing 200 mg. tryptone was given instead. Intrastomachal injections were also given to control mice and to mice injected with both endotoxin and 5 mg. cortisone. In all instances, animals were kept in the urine collection cages for a total period of 17 hours and they were force-fed 6 hours from the beginning of this period. The results of urinary nitrogen determinations are shown in Table III. The control animals excreted the same amount of nitrogen, 26.1 mg., as those permitted to drink their food (line (*a*), Table II) while mice given endotoxin excreted no more nitrogen,

11.1 mg., than fasted poisoned mice (Table I). Urine nitrogen from cortisone-injected mice also given endotoxin was significantly less than that of the force-fed controls ($P = 0.05$) but it was almost precisely the same as that for fasted mice given cortisone (Table I), 21.1 *vs.* 20.3. As judged by these data, endotoxin appears to block completely either the absorption or utilization of ingested food.

At autopsy, the stomachs of force-fed poisoned mice were found to be distended with much of the tryptone suspension originally injected. In corresponding control animals, the stomachs were empty. For a period of 10 to 11 hours, therefore, the tryptone remained in the stomachs of endotoxin-

TABLE III

Urinary Nitrogen Excretion in HF Mice Given Intrastomachal Injections of 200 Mg. Tryptone 6 Hours after Being Placed in Metabolism Cages

Urine was collected for a total of 17 hours. Mice were injected with *S. typhimurium* endotoxin and/or cortisone at the beginning of the experiment. Each value is the mean ± the standard deviation with the number of separate determinations shown in parentheses.

Experimental group	Urinary N excretion in mg. N per mouse per 17 hrs.
Control	26.1 ± 2.6 (8)
Endotoxin	11.1 ± 2.0 (8)
Endotoxin + 5 mg. cortisone	21.1 ± 4.3 (7)

poisoned mice, including those given cortisone. This could occur only if the digestive tract were paralyzed.

Direct evidence that there was an impaired gastric motility was obtained in the following manner.

Mice were fasted for 17 hours. Half were injected intraperitoneally with *S. typhimurium* endotoxin and an hour later all were fed by stomach tube with 0.5 ml. of a slurry made from finely ground dog biscuit and sufficient bone charcoal to blacken it. After an additional 3 hours, the animals were sacrificed and the stomachs were analyzed for carbohydrate.

Mice given endotoxin retained 41.3 per cent of the carbohydrate content of the slurry injected while control mice had only 6 per cent, a quantity hardly measurable by the technique employed. These values are shown in Table IV. This experiment, covering a shorter period of time than the one in which urinary nitrogen was determined, clearly indicates that endotoxin inhibits gastric emptying.

Visually, it was striking to note, when the digestive tracts of these animals were stretched to full length, that, within 4 hours of forced feeding, practically all of the food in normal mice had passed into the large intestine and was visible as four to six carbon-black fecal pellets. Poisoned mice, however, had all the food in the stomach and upper third of the small intestine.

Effect of ACTH on Survival, Carbohydrate Levels, and Urinary Nitrogen Excretion in Mice Given Endotoxin.—The first line of Table V shows that a quantity of endotoxin survived by 75 per cent of control mice was survived by only 10 per cent of those given an injection of ACTH at the same time. This result was not anticipated since cortisone has been shown to protect animals against endotoxins (2, 11). The absence of a therapeutic value of ACTH under these conditions is partially explained by a study of the remaining

TABLE IV

Per Cent of Carbohydrate Retained in Stomachs of HF Mice 3 Hours after an Intrastomachal Injection of 18 Mg. Carbohydrate Contained in 0.5 Ml. of a Slurry Prepared from Purina Dog Chow Biscuit

Prior to the injection, all mice had been fasted for about 17 hours. Endotoxin was given 1 hour prior to the slurry. Each value is the mean ± the standard deviation. The number of separate determinations is given in parentheses.

Experimental group	Per cent carbohydrate in stomach after 3 hrs.
Endotoxin mice	41.3% ± 7.3% (8)
Control Mice	6.0% ± 1.7% (8)

data of Table V. ACTH stimulated glycogen synthesis in control mice, presumably by the release of glycocorticoids from the adrenal cortex, as a comparison of the first two columns of the table shows. A threefold increase in total carbohydrate was found in mice given the hormone. This change is about the same as that previously observed after an injection of 5 mg. cortisone acetate (2). The quantity of cortisone capable of protecting mice against endotoxin appears to be commensurate with the endogenous capabilities of the mouse, as far as carbohydrate synthesis is concerned. The glycogenetic effect of ACTH was almost completely blocked, however, in animals given endotoxin, as seen in the next two columns of Table V. Here, both groups of mice were severely depleted of carbohydrates, a characteristic consequence of endotoxin administration, and ACTH, in contrast to cortisone (2), was incapable of augmenting it. From the nitrogen excretion data, summarized in the last line of the table, the basis for these results becomes apparent. Mice with ACTH secreted more than twice as much urinary nitrogen overnight as con-

trol animals. Since the mice were fasted throughout the 17 hour period of urine collection, the larger nitrogen loss could only result from a breakdown of body protein. The non-nitrogenous residues remaining could serve, therefore, as a source of new carbohydrate. ACTH given to endotoxin-poisoned mice caused no increase in urinary nitrogen excretion.

These observations leave little doubt that endotoxin blocks the ability of the animal to respond to ACTH, at least to the extent of altering urinary nitrogen excretion and body carbohydrates. It is assumed that glycocorticoids

TABLE V

Carbohydrate and Urinary Nitrogen Excretion Data for CF-1 Mice Following Intraperitoneal Injections of S. typhimurium Endotoxin and/or Subcutaneous Injections of Adrenocorticotrophic Hormone

Each value is the mean ± the standard deviation. The number of separate determinations is given in parentheses.

Experimental determination	Mice given			
	No injection (controls)	8 units ACTH alone	8 units ACTH and endotoxin	Endotoxin alone
Survivors / Total No.			2/20 (10%)	15/20 (75%)
Liver glycogen, *per cent*	5.5 ± 0.6 (27)	8.8 ± 1.2 (10)	0.29 ± 0.10 (5)	0.24 ± 0.06 (8)
Muscle glycogen, *per cent*	0.33 ± 0.06 (15)	0.98 ± 0.1 (10)	0.10 ± 0.06 (5)	0.18 ± 0.01 (10)
Total carbohydrate, *mg./gm. carcass*	6.5 ± 1.2 (15)	20 ± 4.2 (10)	1.8 ± 0.27 (5)	1.4 ± 0.4 (15)
Urinary nitrogen, *mg./mouse/17 hrs.*	13.1 ± 4.5 (10)	29.9 ± 3.2 (8)	10.4 ± 1.6 (7)	11.2 ± 1.8 (8)

were not released from the adrenal cortex and, without the resulting glyconeogenesis, the glycogen stores of the mouse became depleted in the presence of endotoxin despite the injection of ACTH.

Urinary Nitrogen Excretion Following ACTH in Mice Given Known Numbers of Heat-Killed S. typhimurium.—First it was established that 10^9 heat-killed cells of *S. typhimurium* was an LD_{95+} for mice weighing about 20 gm. This is of interest since about 10^9 viable pathogens have been consistently cultured from mice at the moment of death with mouse typhoid (6, 7). Thus, numerically, there is an equivalent toxicity between living and dead organisms. Since *S. typhimurium* endotoxin was shown, in the preceding section, to block the increase in urinary nitrogen output elicited by ACTH, the number of cells

in a vaccine required for the same blockage was determined. The results are summarized in Table VI. No change was found with 10^5 cells and, while 10^7 bacteria produced a slight decrease, it was not statistically significant by rank test (12). With 5×10^7 cells, however, there was a significant drop in nitrogen output, to slightly more than half the control value, and with 10^8 cells urinary nitrogen excretion reached the same low value obtained after a lethal dose of endotoxin (*cf.* Table V). Greater variations in nitrogen values were found for mice within the groups given intermediate numbers of heat-killed bacteria than with those given either the largest or smallest numbers. It is believed that individual animals vary in their sensitivity to the vaccine and hence in the dose required to block the adrenocortical response to ACTH.

TABLE VI

The Influence of Known Numbers of Heat-Killed S. typhimurium on Urinary Nitrogen Excretion in CF-1 Mice Injected with ACTH

Each value is the mean ± the standard deviation for the number of separate determinations shown in parentheses.

Urinary nitrogen (in mg.) excreted per mouse per 17 hr. period following the simultaneous injection of the designated No. of heat-killed *S. typhimurium* and 2 units of ACTH				
Control (ACTH alone)	10^5 cells + ACTH	10^7 cells + ACTH	5×10^7 cells + ACTH	10^8 cells + ACTH
29.9 ± 3.2 (8)	29.5 ± 3.3 (8)	24.6 ± 4.8 (8)	17.1 ± 4.3 (7)	10.6 ± 1.4 (8)

Urinary Nitrogen Excretion Following ACTH in Mice Infected with S. typhimurium.—Mice were infected intraperitoneally with 10^5 *S. typhimurium*. Six hours later some of the animals were injected with four units of ACTH and placed in urine collection cages without food and water for a period of 17 hours. With another group of infected mice, ACTH was administered after 54 hours and urine was collected similarly for 17 hours. The average nitrogen content of the urine under the two sets of conditions is shown in Table VII. During the earlier period postinfection there was a decided decrease in nitrogen elimination, as compared with the later period, and also with the control value (Table VI). In fact, the infection between 6 and 23 hours resulted in almost the same urinary nitrogen output, in response to ACTH, as that found for mice injected with 5×10^7 heat-killed *S. typhimurium*. Between 54 and 71 hours, on the other hand, nitrogen excretion was almost the same as in control mice injected with ACTH. Whatever blocked the animal's ability to respond to the pituitary hormone in the early period postinfection was no longer effective after 54 hours. The question of whether the toxic manifestations of mouse typhoid are elicited by the living pathogens within the mouse or by those destroyed by its defenses assumes importance in this context. If it is dead bacteria, then about 5×10^7 had to be derived within 24 hours

from the infectious dose of 10^5 cells. (It is possible that the pathogens killed *in vivo* are more toxic than heat-killed cells.) By 54 to 71 hours, the killed cells are presumed to have disappeared or at least to have lost their ability to block the adrenal. Total viable counts of pathogens in mice at 24 and 72 hours post-infection were also determined. At 24 hours, there were from 5×10^5 to 5×10^6 cells, and at 72 hours there were from 2×10^7 to 2×10^8 cells in the majority of animals. If this accurately reflects the status of the infection, then the effects reported in Table VII cannot be directly related to numbers of living bacteria.

TABLE VII

Urinary Nitrogen Excretion Following ACTH Injection in CF-1 Mice at Different Times Postinfection

Mice were infected intraperitoneally at time zero with a saline dilution of an overnight brain-heart infusion broth culture containing about 10^5 cells in 0.5 ml. Each value is the mean ± the standard deviation for the number of separate determinations shown in parentheses.

Urinary nitrogen (in mg.) excreted per mouse per 17 hr. period at designated times postinfection following injection of 4 units of ACTH	
6 to 23 hrs. postinfection	54 to 71 hrs. postinfection
16.8 ± 1.7 (8)	28.5 ± 1.6 (7)

DISCUSSION

The imbalance, following cortisone injection, between protein catabolism and carbohydrate synthesis in endotoxin-poisoned mice appears to be due to a deficiency of carbohydrate. It should be possible to discriminate, experimentally, between a lack of synthesis and an accelerated breakdown of carbohydrate under these conditions. If so, the point at which endotoxin alters metabolism would be narrowed significantly.

Quite unanticipated was the discovery that endotoxin effectively blocks absorption from the digestive tract and diminishes gastro-intestinal motility. This followed from the fact that urniary nitrogen excretion in force-fed mice was the same as that of fasted mice. The extent to which lack of absorption from the digestive tract relates to inhibited peristalsis is unknown. Nevertheless, if the paralysis of smooth muscle in stomach and intestines also occurred in the musculature of the vascular bed, then many of the circulatory manifestations of endotoxin poisoning would be brought into clearer perspective (13–15). A recent report by Broitman *et al.* (16) presented evidence that succinoxidase activity of the intestinal mucosa of rats was reduced more than half by injections of lethal doses of endotoxin. This may or may not be relevant to impaired absorption and motility.

The concept that the adrenal cortex is involved in the survival of experi-

mental animals subjected to stressful situations (17) is widely accepted. The greatly increased sensitivity of adrenalectomized rats to endotoxins (1) would serve to strengthen that concept and place injections of endotoxin in the category of "stressful situation." Completely contradictory to this notion is the total loss in the ability of the adrenals of mice given endotoxin to respond to exogenous ACTH, as clearly indicated by the data of Tables V and VI. These results might be explained by a destruction or inactivation of ACTH *in vivo*. One is still at a loss, however, to understand why adrenalectomized animals should be more susceptible to endotoxin poisoning unless it is assumed that adrenal hormones other than glycocorticoids act beneficially against endotoxin. Such hormones would have escaped detection by the techniques employed. Much work remains before these phenomena become clear.

The use of known numbers of heat-killed *S. typhimurium* to block the adrenal response to ACTH, as evaluated by urinary nitrogen excretion, offers the interesting possibility of estimating the number of killed pathogens in an infected mouse. There could never be any assurance of a one-to-one equivalence between the toxicity of a heat-killed cell injected into a mouse and that of a bacterium destroyed by the body defenses of the host, but the net effect would be the same. A careful utilization of this approach to the problem of infectious disease could, conceivably, place within reach a conclusion as to whether an infected animal dies from living or dead cells.

SUMMARY

Urinary nitrogen excretion over a 17 hour period without food and water was determined for mice poisoned with endotoxin and for control mice with and without the subcutaneous injection of 5 mg. cortisone acetate. Endotoxin did not alter the quantity of nitrogen excreted, compared with that of control mice, but in both groups of animals cortisone increased urinary nitrogen by the same amount.

A balance between the quantity of protein catabolized (as estimated by increased urinary nitrogen excretion) and the total carbohydrate stored as a result of cortisone administration was found in fasted and in fed mice but not in endotoxin-poisoned mice.

Endotoxin inhibited the motility of the gastrointestinal tract and blocked the absorption of food.

Injections of ACTH in normal mice increased protein degradation and carbohydrate synthesis. In endotoxin-poisoned animals, ACTH failed to alter urinary nitrogen excretion and carbohydrate reserves. ACTH increased susceptibility to endotoxin.

The number of heat-killed cells of *S. typhimurium* required to block the increase in urinary nitrogen caused by an injection of ACTH was found to be 10^8. A partial block was found with 5×10^7 cells but not with 10^6 cells.

Mice infected with *S. typhimurium* excreted less than the normal amount of

urinary nitrogen in response to ACTH 6 to 23 hours postinfection while normal amounts of nitrogen were eliminated 54 to 71 hours postinfection. The number of viable cells in the mice during the earlier period was less than the number of dead cells required to alter the nitrogen output, while the reverse was true during the later peroid.

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