THE INFLUENCE OF ENDOTOXIN ADMINISTRATION ON THE NUTRITIONAL REQUIREMENTS OF MICE*

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While the influence of the nutritional state on the course of infectious processes has been repeatedly emphasized, less attention has been paid to the influence of infection and other biological stresses on nutritional requirements. Observations recently made in our laboratory have uncovered a number of distinct phenomena related to this problem. They have revealed, for example, that the need for certain essential amino acids is greatly increased by infectious processes and physiological disturbances.

It will be shown in the present paper that albino mice maintained on a semisynthetic gluten diet low in lysine and threonine recover from the weight loss caused by administration of bacterial endotoxins much more slowly than do mice fed a better balanced diet.

Materials and Methods

Mice.—The NCS colony of mice maintained in our laboratories at the Rockefeller University has been described elsewhere (1, 3). Only male mice were used in the present study; they were approximately 4 weeks old when first placed on the experimental diets. They were housed individually on wire grids, or in groups of 3 bedded in sawdust.

The weights of individual animals were determined at the beginning of each experiment and at intervals thereafter; they were determined daily following endotoxin injection. The conditions required for obtaining valid weights of mice have been described elsewhere (1).

Diets.—The diets consisted either of commercial pellets (D & G), distributed by Dietrich and Gambrill, Frederick, Maryland, or of semisynthetic diets. The latter were made up as follows: protein, 15 per cent; Jones Foster salt mixture, 4 per cent; vitamin fortification mixture, 0.4 per cent; inositol, 0.1 per cent; cystine, 0.3 per cent; alphacel, 10 per cent; peanut oil, 5 per cent; and dextrin to 100.[1] The protein used was either "vitamin free" casein or wheat gluten. Amino acid supplementation of the dietary regimen was achieved by adding to the drinking water either *l*-lysine, 1.0 per cent, *dl*-methionine, 0.2 per cent, *dl*-valine, 0.2 per cent, and/or *dl*-threonine, 0.2 per cent. The solutions of these amino acids were acidified with hydrochloric acid to a pH of 2.8 in order to prevent or at least minimize bacterial growth. Control animals received water acidified to the same pH. All animals were maintained on

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[1] Peanut oil (Planters[R]) was obtained locally. All other ingredients were obtained from Nutritional Biochemicals, Inc., Cleveland, Ohio.

their particular dietary regimen until the termination of the experiment. Diets and water were given *ad libitum*.

Endotoxin.—Most experiments were carried out with a commercially available endotoxin prepared from *Escherichia coli* (Difco Laboratories, Inc., Detroit, lot 439277); similar results were obtained when the experiments were carried out with endotoxin prepared in our own laboratory by a modification of Boivin's trichloracetic method. In all cases, the endotoxins were dispersed and diluted in pyrogen-free diluents. Injections (by the intraperitoneal route) were in a final volume of 0.2 ml.

RESULTS

Weight Losses Following Endotoxin Injection.—The design of the experiments to be described was conditioned by the fact that mice have a marked diurnal cycle of feeding. They take neither food nor water during daylight hours; food and water intake begins shortly after dusk and continues throughout the evening and early morning hours. We have observed that this feeding and drinking cycle is interrupted by administration of endotoxin and that, as a result, animals lose 1 gm or more of body weight.

In the first experiment on which the following study was based, 28-day-old NCS mice were fed either commercial pellets, or semisynthetic diets with gluten or casein as protein constituents. Half of the animals on each diet received 30 μg of endotoxin. The weight changes over the following 7 days are given in Table I.

As seen in Table I, the effect of endotoxin administration on the weight curve was essentially the same in mice fed commercial pellets (D & G) or the semisynthetic casein diet (15C). In both cases, there was a marked loss of weight (more than 1 gm per mouse) on the day following endotoxin injection. But in both cases also the animals were well on the way to recovery by the 3rd day and had regained most of the weight loss caused by endotoxin within the next 7 days.

As expected, the administration of endotoxin also caused a marked weight loss in mice fed the gluten diet. But in contrast to what occurred in mice fed the commercial pellets or the casein diet this weight loss persisted in mice fed the gluten diet even 14 days after the administration of endotoxin.

Similar findings are graphically illustrated in Figs. 1 *a* and 1 *b*. In this experiment, 4-week-old mice received 50 μg of endotoxin 5 days after being placed on their respective experimental dietary regimens. For the sake of simplicity, the presentation is limited to the findings with mice fed the casein (15C) or the gluten (15G) diets.

As in the preceding experiment, the growth of casein-fed mice was temporarily impaired by administration of endotoxin, but the animals rapidly recovered their lost weight, even though this required a sustained daily gain of almost a gram per mouse. In gluten fed mice, growth resumed after the initial loss of weight caused by administration of endotoxin, but recovery did not occur within the period of observation.

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Effect of Amino Acid Supplementation on Recovery of Weight Loss Caused by Endotoxin.—In contrast with casein, wheat gluten is deficient in several amino acids, especially lysine, threonine, valine, and methionine. Several experiments were therefore performed to determine whether supplementation of the gluten diet with these amino acids would enable mice to recover the weight lost as a result of endotoxin administration.

TABLE I

Effect of Administration of Endotoxin on Weight of Mice Fed Different Diets

Diet*	Endotoxin	Initial weight of mice	Weight changes (gm) on following days after endotoxin‡					Weight on day 7	Net change	Total change caused by endotoxin at day 7
			1	2	3	7	14			
	µg	*gm*						*gm*	*gm*	*gm*
D & G	0	24.2	0.0	0.0	+0.6	+0.7	—	25.5	+1.3	
	30	23.1	−1.1	−0.1	+0.9	+1.2	—	24.0	+0.9	(−0.4)§
15C	0	23.2	−0.1	+0.2	+0.5	+1.4	—	25.2	+2.0	
	30	21.8	−1.8	+0.1	+1.0	+2.4	—	23.5	+1.7	(−0.3)
15G	0	20.2	+0.4	−0.1	+0.4	−0.3	+0.6	20.6	+0.4	
	30	19.2	−1.9	+0.5	+0.2	−0.2	+0.4	17.8	−1.4	(−1.8)

* See Materials and Methods: 15C indicates that the diet contained 15 per cent casein; 15G, 15 per cent gluten.

‡ Values are average changes of weight from the previous day indicated in the table. All weights are for the arithmetical average of 3 or 4 animals.

§ Difference between control and treated groups.

In all experiments designed to test the effect of amino acid supplementation, 4- or 5-week-old male mice were fed either casein (15C) or gluten (15G) diets. In some of the groups, the gluten diet was supplemented with amino acids added to the drinking water. Half of the animals on each diet received 50 µg of endotoxin 2 weeks after being placed on the experimental diets; the remainder received saline instead. The results of a typical experiment are given in Table II.

In confirmation of earlier results, Table II shows that mice maintained on the casein diet recovered rapidly while animals fed the gluten diets did not. However, mice regained their weight promptly when the gluten diet was supplemented with lysine, threonine, methionine, and valine; or with lysine and threonine alone. Supplementation with valine and methionine without lysine or threonine was not effective. Addition of lysine or threonine alone occasionally allowed for moderate to good recovery, as was the case for lysine in the experiment presented in Table II, but such findings were not always reproducible. In contrast, rapid recovery of weight always occurred when the gluten diet was supplemented with both lysine and threonine.

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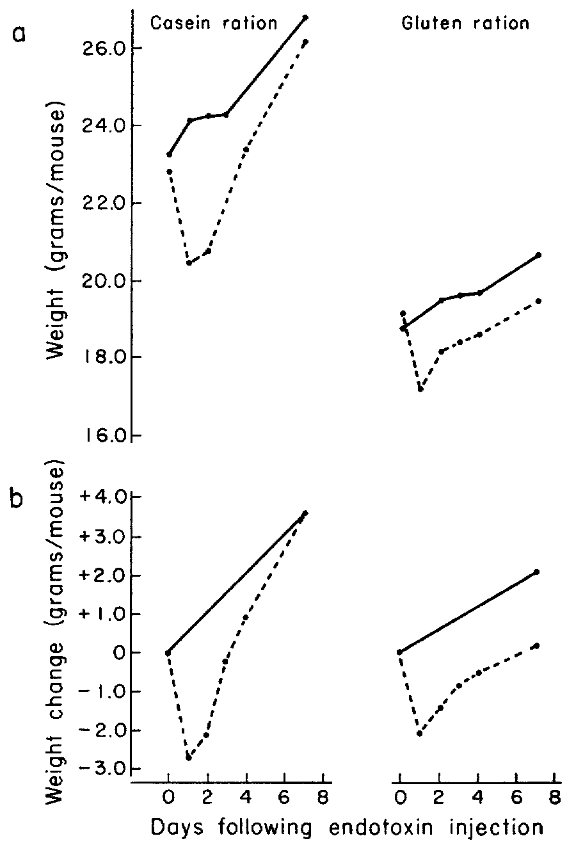

FIGS. 1 *a* and 1 *b*. Fig. 1 *a* illustrates the weight curves of mice fed the casein or gluten diets. The broken line refers to the animals which had received 30 μg of endotoxin at day 0. The unbroken line refers to control mice having received saline.

Fig. 1 *b* presents the same findings in a different manner. As in Fig. 1 *a*, the broken line indicates the daily changes of the endotoxin group, but represents the daily difference between control and endotoxin animals. The unbroken line indicates the overall weight change of saline controls during the experimental period.

This latter mode of representation will be used throughout the balance of this paper.

Endotoxin Dose and Weight Recovery.—In the experiments recorded so far, a dose of 30 or 50 μg of endotoxin was used to bring out the differences in recovery rate caused by the composition of the dietary regimen. Interestingly enough, the nutritional effect was just as striking, whether the endotoxin dose was much larger or much smaller.

Five-week-old male mice were distributed at random in groups of 3 and fed either D & G pellets, the 15 per cent casein diet (15C), or the 15 per cent gluten diet (15G). After a period

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TABLE II

Amino Acid Supplementation and Weight Recovery following Endotoxin Administration

Diet	Supplement in the drinking water*	Endotoxin	Effect of endotoxin treatment on weight				Difference between control and treated groups
			Initial weight	Initial weight loss	Weight 7 days after endotoxin	Final change	
		μg	*gm*	*gm*	*gm*	*gm*	*gm*
15C‡	0	0	26.4	(+0.2)§	27.2	+0.8	
		50	25.8	(−1.5)	26.6	+0.8	0.0
15G‡	0	0	25.4	(+0.3)	27.6	+2.2	
		50	24.8	(−2.5)	25.0	+0.2	−2.0
15G	Lysine, threonine,	0	25.0	(+0.2)	26.0	+1.0	
	methionine, valine	50	25.2	(−2.2)	26.4	+1.2	+0.2
15G	Lysine, threonine	0	26.8	(+0.3)	28.0	+1.2	
		50	27.0	(−1.7)	28.0	+1.0	−0.2
15G	Methionine, valine	0	24.2	(+0.2)	26.5	+2.3	
		50	26.0	(−2.0)	26.4	+0.4	−1.9
15G	Threonine‖	0	22.8	(+0.7)	25.2	+2.4	
		50	25.9	(−2.0)	25.9	0.0	−2.4
15G	Lysine	0	27.6	(+0.2)	28.4	+0.8	
		50	28.0	(−2.2)	28.2	+0.2	−0.6

* For concentrations of amino acids, see Materials and Methods.

‡ See Materials and Methods: 15C indicates that the diet contained 15 per cent casein; 15G, 15 per cent gluten.

§ Initial weight gain or loss following endotoxin or saline injection. All weights refer to the arithmetical average for 6 animals.

‖ Supplementation was twice as concentrated as in preceding groups.

of adjustment to the experimental diets and determination of initial weight changes, two groups of animals on each diet received one of several doses of endotoxin, and two groups were kept as controls. The results are presented in Figs. 2 *a* and 2 *b*. As for the experiment illustrated in Figs. 1 *a* and 1 *b*, Figs. 2 *a* and 2 *b* present only the results obtained with the casein and gluten diets.

Although the initial weight losses following injection of 5 μg of endotoxin were small, the animals fed the gluten diet did not regain even this small loss for at least 1 week thereafter. In contrast, mice fed adequate diets recovered immediately. Mice given 480 μg of endotoxin showed large weight losses which continued for several days. However, the diet effect was once more demonstrated by the fact that animals fed the casein diet (as well as pellets) eventually regained their losses while animals fed the gluten ration did not.

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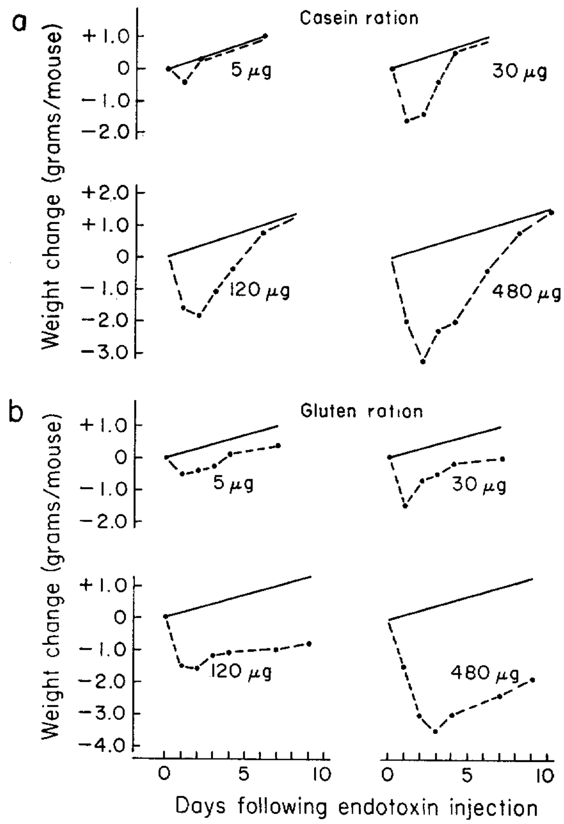
It is of interest to note that, for any dose of endotoxin, the initial weight loss was no greater in the animals fed inadequately than in those receiving pellets. In other words, the gluten-fed mice did not appear to be more susceptible to the primary toxicity of endotoxin than adequately fed animals. Furthermore,



FIGS. 2 *a* and 2 *b*. Weight recovery of mice fed casein or gluten diets following injection of different doses of endotoxin. See also Fig. 1 *a*.

even though gluten-fed mice did not regain their weight losses for prolonged periods of time, none of them ever died following endotoxin administration.

Acquired Tolerance to Endotoxin and Weight Recovery.—As is well known, animals having received a dose of endotoxin may develop a state of partial "tolerance" to a second dose of this substance. It will be shown in the following experiment that mice can become tolerant to endotoxin while being fed the gluten diet.

Animals fed either gluten or casein diets were given endotoxin and then a second dose was given 14 days later. Two groups of controls were used. One received endotoxin only once,

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at the time the tolerant group received the second dose, and therefore provided a measure of the response of non-tolerant animals. Other animals receiving saline served as absolute weight controls. The results are graphically represented in Fig. 3.

Fig. 3 shows that the mice which had received a prior dose of 30 μg endotoxin, and therefore were in the tolerant state, exhibited a significantly smaller response to a second dose of 50 μg than did mice that had not had a previous experience with this substance. Interestingly enough, this was true whether the animals had been maintained on the gluten rations or casein diets. In other words, the mechanisms responsible for the development of tolerance were not significantly impaired by the inadequate diet.

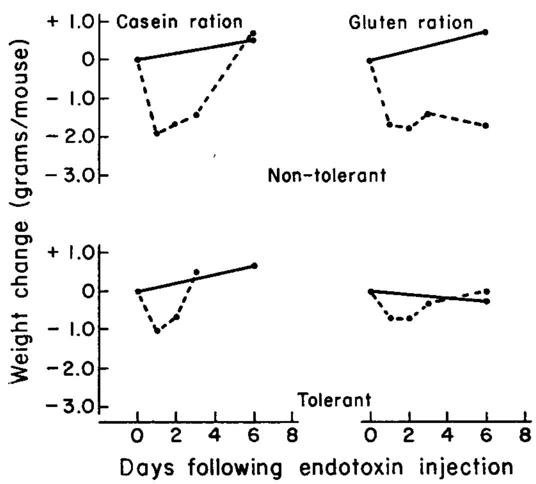

FIG. 3. Effect of prior injection of 30 μg of endotoxin on the response of mice fed gluten or casein diets and rechallenged 14 days later with 50 μg of this substance. See also Fig. 1 *a*.

Irrespective of diet, the "tolerant" mice also regained their small weight losses within 72 hours after administration of endotoxin. It should be noted, however, that prior administration of endotoxin had already profoundly altered the growth rate of gluten fed mice. In Fig. 3, for example, the weight curve for non-tolerant animals shows some weight gain which did not occur for the tolerant animals. Hence, it cannot be unequivocally stated that weight recovery following the second dose of endotoxin preceeded at the same rate on the two diets. Table III presents in greater detail the comparative courses of weight changes in normal and tolerant animals given 30 μg of endotoxin while fed either one of three different diets.

Age of Mice and Weight Recovery.—The purpose of the following experiment was to determine whether the age of the animal affected the extent of weight loss caused by administration of endotoxin and the rate of recovery on different diets.

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Twenty-eight-day-old male mice were randomly grouped and fed either D & G pellets, or 15 per cent casein diet, or 15 per cent gluten diet. At various intervals thereafter, 5 animals on each diet received 30 μg of endotoxin. When first treated with endotoxin (on the 39th day of age) the mice had not reached maturity and were still growing. The animals which received endotoxin 21 days later were 8 weeks old and mature although their weights were still slowly increasing. The third series of animals, about 10 weeks old, had essentially attained their full mature weight and their subsequent weight changes were minimal. The results of this experiment are presented in Table IV. Other experiments performed with mice at similar and markedly older ages gave essentially the same results.

TABLE III

Effect of Diet on Tolerance to Endotoxin

Diet	Endotoxin administration on day		Average weight effects following second endotoxin administration*				Final weight change
	0	13	13	14	Initial change	21	
	μg	*μg*	*gm*	*gm*	*gm*	*gm*	*gm*
D & G‡	0	0	26.8	27.4	(+0.6)	29.0	+2.2
D & G	0	30	27.0	26.0	(−1.0)	29.2	+2.2 (0.0)§
D & G	30	30	26.6	26.1	(−0.5)	28.4	+1.8 (−0.4)
15C‡	0	0	27.4	28.0	(+0.6)	28.8	+1.4
15C	0	30	28.2	27.0	(−1.2)	29.2	+1.0 (−0.4)
15C	30	30	29.4	29.0	(−0.4)	30.6	+1.2 (−0.2)
15G‡	0	0	23.8	24.2	(+0.4)	26.5	+2.7
15G	0	30	24.4	22.2	(−2.2)	25.0	+0.6 (−2.1)
15G	30	30	24.5	23.6	(−0.9)	26.2	+1.7 (−1.0)

* All weights are the arithmetical average for 6 mice.

‡ See Materials and Methods: 15C indicates that the diet contained 15 per cent casein; 15G, 15 per cent gluten.

§ Difference between control and treated groups.

In Table IV the weight change in any group of mice for the week preceding endotoxin administration is compared with that for the week following such treatment. Each group therefore constitutes its own control.

It will be seen that animals on adequate diets generally recovered completely within the week following administration of endotoxin. Younger or older animals reacted in much the same manner, both as to initial weight loss and as to rate of recovery. Animals fed gluten diets also showed similar initial reactions regardless of age. Although younger animals continued to grow following endotoxin injection, they did not make up for their initial weight losses. On the other hand, some recovery apparently occurred in older animals. Since young rapidly growing animals have greater nutritional requirements than adult animals under normal conditions, it is not surprising that they are also more sensitive to the nutritional disturbances caused by endotoxin administration.

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Water Deprivation and Weight Recovery.—As previously shown, a period of 16 hours of water deprivation causes an initial weight loss in mice similar to that following endotoxin administration, but the animals rapidly regain their initial weight when fed an adequate diet (2). The purpose of the following experiment

TABLE IV

Effect of Diet on Response to Endotoxin at Different Ages

Diet*	Age at administration of endotoxin	Average weights on indicated day before or after administration of endotoxin				Weight change		Difference between pre- and postendotoxin values
		0	7	Initial change	14	0 to 7	7 to 14	
	days	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
D & G	39	22.2	23.7	(−1.3)‡	25.1	+1.5	+1.4	−0.1
D & G	60	28.2	29.1	(−2.5)	29.1	+0.9	0.0	−0.9
D & G	75	29.8	30.0	(−2.3)	30.3§	+0.2	+0.3	+0.1
15C	39	23.0	25.4	(−2.1)	27.0	+2.4	+1.6	−0.8
15C	60	23.2	23.9	(−1.0)	24.7	+0.7	+0.8	+0.1
15C	75	28.1	27.9	(−1.7)	27.7§	−0.2	−0.2	0.0
15G	39	20.8	22.8	(−1.4)	22.8	+2.0	0.0	−2.0
15G	60	22.1	22.9	(−1.3)	22.9	+0.8	0.0	−0.8
15G	75	27.1	27.0	(−1.7)	26.4§	−0.1	−0.6	−0.5
15G‖	40	20.8	23.2	(−1.6)	23.0	+2.4	−0.2	−2.2
15G	47	22.6	24.4	(−1.4)	24.6	+1.8	+0.2	−1.6
15G	68	24.7	25.4	(−1.5)	25.6	+0.7	+0.2	−0.5

* All animals were placed on their respective diets on the 28th day of life.

‡ Initial weight loss following endotoxin treatment. All weights are the arithmetical average for 5 animals.

§ Results for the 13th day instead of the 14th.

‖ This additional experiment is added to show the consistency and reproducibility of the results. Animals were caged in groups of 3 in sawdust.

was to determine whether the rate of recovery from the weight loss caused by water and food deprivation is as dependent on the composition of the nutritional regimens as is the rate of recovery from the effects of endotoxin.

Five-week-old mice were fed D & G pellets, or 15 per cent casein diet, or 15 per cent gluten diet. One third of the animals in each group received 50 μg of endotoxin, or were deprived of water and food for 16 hours, or were kept as untreated controls. The results of this experiment are shown in Table V.

The results presented in Table V confirm those of earlier experiments in showing that mice fed the gluten diet recovered from the effect of endotoxin

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administration much more slowly than did animals fed either pellets or the casein diet. In contrast, the dietary regimen had no influence on the rate at which the animals regained the weight loss caused by water and food deprivation. This loss was as great as or greater than that following endotoxin administration, but it was made up for within 24 hours after water and food were again made available, even in the case of mice fed the gluten diet.

TABLE V

Comparative Effects of Food Deprivation and Endotoxin on Rate of Weight Recovery

Diet*	Treatment	Average weights on indicated days following treatment‡ (gm)				Weight differences between control and treated group on indicated days§		
		0	1	2	7	1	2	7
						gm	*gm*	*gm*
D & G	None	28.8	29.0	29.4	30.2			
D & G	16 hours food and water deprivation	28.8	25.2	30.0	30.2	−3.8	+0.6	0.0
D & G	50 μg Endotoxin	29.2	27.6	28.0	30.2	−1.8	−1.8	−0.4
Casein	None	28.2	28.6	28.4	28.6			
Casein	16 hours food and water deprivation	28.8	25.4	29.0	29.2	−3.8	0.0	0.0
Casein	50 μg Endotoxin	27.4	26.0	26.6	28.0	−1.8	−1.0	+0.2
Gluten	None	29.2	29.2	29.0	30.0			
Gluten	16 hours food and water deprivation	27.8	24.2	28.0	28.6	−3.6	+0.4	0.0
Gluten	50 μg Endotoxin	28.4	26.6	27.2	28.2	−1.8	−1.0	−1.0

* See Materials and Methods. Diets were fed for 18 days prior to treatment.

‡ All weights are arithmetical averages for 6 animals.

§ Differences (in grams) between control and treated groups as calculated after adjustment for initial weight differences at day 0.

Physiological Disturbances and Weight Loss.—The mice used in the present study respond to a variety of environmental changes by irregularities in their intake of food and water. For this reason, they often lose weight when regrouped, or placed in another type of cage, or fed a different dietary regimen, or even simply handled. Fortunately, such effects are usually transient. Care was taken in the experiments reported in the present paper to exclude these disturbances from those caused by administration of endotoxin and the composition of the diets.

Other influences exert more lasting and significant effects. We have observed, for example, that exposure of young NCS mice to the intestinal contents of ordinary Swiss mice results in a smaller weight at weaning time and a slower

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rate of subsequent growth (3). As already mentioned, these effects can be traced to an alteration of the gastrointestinal microbiota and are markedly increased by dietary deficiencies (1). The administration of certain antibacterial drugs also causes a weight loss in NCS mice; in this case recovery is rapid if the animals are maintained on a well balanced regimen, but it does not occur, or occurs only slowly, if they are fed the gluten diet. There is evidence that the growth-depressing effect of antibacterial drugs in NCS mice is mediated through changes in the gastrointestinal microbiota (1).

In the cases mentioned above, the disturbances in the gastrointestinal microbiota always include a large increase in the population of Gram-negative bacilli and presumably result in the production of more endotoxin *in vivo*. It is not unlikely therefore that the so called indigenous flora plays an important role in determining the nutritional requirements of its host.

DISCUSSION

Albino mice of the so called Swiss colony fail to grow when wheat gluten is their sole source of dietary amino acids. In contrast, their growth is normal if the gluten diet is supplemented with cystine, lysine, and threonine. Interestingly enough, NCS mice, although derived from the Swiss colony, grow well on the gluten diet without lysine or threonine supplementation (3). The results of earlier studies strongly suggest that their ability to grow on this deficient diet is related to the simplicity of their intestinal flora, for example, to the fact that they harbor only very small populations of enterococci, coliforms, and clostridia.

The finding, described in the present paper, that NCS mice develop more exacting nutritional requirements when treated with endotoxin also supports the view that the indigenous bacterial flora can affect the nutritional state. In brief, it has been found that NCS mice which have lost weight as a result of endotoxin administration recovered rapidly when fed an adequate diet, but not when gluten and cystine constituted their sole source of amino acids; on the other hand, weight recovery was rapid when the gluten diet was supplemented with lysine and threonine.

It might be assumed that the more lasting effects of endotoxemia in NCS mice fed the gluten diet was due to the fact that amino acid deficiency rendered the animals more susceptible to the initial toxic effects of the endotoxins. However, several facts militate against this hypothesis. One is that the minimum effective dose of endotoxin, and the extent of weight loss for any given dose, was the same irrespective of the amino acid composition of the diet. Another is that tolerance to endotoxin, as evidenced by smaller weight loss following administration of a second dose, seemed to develop as well with the gluten diet as with the more complete regimens.

The failure of mice fed the gluten diet to regain the weight loss caused by

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endotoxin appears therefore to be the expression of the fact that the toxic effects of this substance increased the nutritional requirements, either directly or indirectly. The mechanism of this nutritional disturbance may simply be greater amino acid requirements, such as for tissue repair; on the other hand, it may be more indirect and result from an interference with absorption of food, or changes in nitrogen metabolism.

Endotoxin does indeed cause a variety of physiological disturbances. In mice, for example, it retards markedly the evacuation of stomach contents (4), and also alters the pattern of nitrogen excretion (5); in chickens, it decreases the blood levels of several amino acids (6).

Awareness of the nutritional disturbances caused by endotoxemia contributes to an understanding of the interplay between nutrition and infection. As shown elsewhere, mice fed gluten diets exhibit heightened susceptibility to various types of pathogenic bacteria. Yet, there is no evidence that the multiplication of these pathogens in the organs is more rapid, or their elimination less efficient, in mice fed gluten diets than in those receiving a more complete regimen (7). In other words, the increase in susceptibility to infection caused by the amino acid deficiency does not result from a loss of resistance to the infectious agent but rather from a decrease in the animal's ability to overcome the effects of the infectious process. It is probable that a similar mechanism is at work in human beings suffering from protein malnutrition. In these persons, there is no evidence that the nutritional deficiency actually increases the chance of contracting infection. It appears instead that the effect of the deficiency is to aggravate and prolong the infectious process (8). In an analogous manner, children on a low or poor protein diet commonly show protracted depression of growth following the usual respiratory diseases, whereas well nourished children are more likely to regain weight rapidly.

Studies to be reported in a subsequent publication have revealed that mice nursed by mothers fed gluten diets are smaller at weaning time, if they survive at all, than mice nursed by dams fed more complete regimens; moreover, they remain underdeveloped throughout their life span even if they are given an optimum diet continuously after weaning. As in the case of the endotoxin effect, the growth depression can be corrected by supplementing the gluten diet of the mother with lysine or threonine. In other words nutritional deficiencies occurring during lactation (as well as during gestation) can exert lasting deleterious effects on the young. These unpublished observations extend the ones reported in the present paper in demonstrating that certain biological stresses (administration of endotoxin) and physiological states (lactation) affect profoundly the nutritional requirements and that diets which are adequate under certain circumstances may prove grossly insufficient in others.

SUMMARY

Albino mice lose weight within 24 hours following administration of bacterial endotoxin. The initial weight loss is proportional to the dose of endotoxin in-

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jected only when this dose is very small. The loss during the 1st day reaches a maximum with 10 to 30 μg of endotoxin; larger doses increase the duration of the overall effect.

The rate at which mice regain weight after administration of endotoxin is markedly influenced by the composition of the diet. Recovery was rapid and complete within a few days when the animals were fed commercial pellets or a semisynthetic diet containing casein. In contrast, recovery was slow and incomplete when wheat gluten was used instead of casein in the diet. The deleterious effect of the gluten diet was less marked in older than in younger animals, probably because the latter have less exacting nutritional requirements.

It was postulated that the failure of endotoxin-treated mice to regain weight when fed the gluten diet was due to the fact that this protein is low in certain amino acids. In fact, rapid and complete recovery from the weight loss uniformly occurred when the gluten diet was supplemented with proper amounts of lysine and threonine.

The composition of the diet did not influence the extent of the initial loss of weight caused by endotoxin, nor did it prevent the animals from developing tolerance to this substance.

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