3. Brown, G. C., Maassab, H. F., Veronelli, J. A., Science, 1964, v144, 943.

4. Sever, J. L., Huebner, R. J., Castellano, G. A., et. al., Science, 1965, v148, 385.

5. Cusumano, C. L., Sever, J. L., Schiff, G. M., Huebner, R. J., J. Pediat., 1964, v65, 138.

6. Crawford, L. V., Virology, 1960, v12, 143.

7. O'Connor, T. E., Rauscher, F. J., Zeigel, R. F., Science, 1964, v144, 1144.

8. McCarthy, K., Taylor-Robinson, C. H., Pillinger, S., Lancet, 1963, v2, 593.

9. Parkman, P. D., Buescher, M. S., J. Immunol., 1964, v93, 595.

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Absence of Adrenocortical Stress Responses in Experimental Pyelonephritis.* (31163)

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Infection is a primary threat to life and higher organisms have developed an intricate set of responses to combat invaders and repair resultant tissue damage. By their very nature such responses represent homostatic disturbances likely to elicit corresponding compensatory physiological reactions. Despite the relevance of such a disturbance and response to the theory of stress as an adaptive mechanism, adrenocortical activation has been studied in relatively few diseases and little is known about the adrenocortical stress response in various stages of infection. As Selye(1) has pointed out, such studies are of particular theoretical interest because of the ambiguous survival value of the adrenocortical response in the infectious process. Indeed, the very anti-inflammatory properties of the adrenal corticoids which facilitate tissue repair and alleviate attendant symptomatology also are actions permitting spread of the infection and accentuate the danger to the host.

As part of a larger survey on enzymic and hormonal responses to stress, the present study concerns the endogenous adrenocortical response during stages of an infectious process. Experimental pyelonephritis was selected in this study for several reasons. The disease is readily produced in the laboratory rat by intravenous injection of *Str. faecalis* and its natural history has been studied by bacteriological(2), immunological(3), histochemical (4), and physiological(5) techniques. Injected bacteria are rapidly cleared from the blood by the liver and spleen and are destroyed in these organs. The renal microbial population, however, increases shortly after infection and then remains relatively constant throughout the life of the animal (2+ years). During this time, the infection evolves histologically from an acute phase with abscesses, tissue destruction and polymorphonuclear cell infiltration, through a subacute, to a chronic stage with tissue repair, scarring and mononuclear cell infiltration. The earliest lesions seen are microabscesses in the medullary portion of the kidney 2-3 days after injection. Functional changes occur early and are primarily manifested by an impaired concentrating ability. Clinically, the animal does not appear ill. Indeed, the rat eats normally and rate of weight gain is unaffected(6). Thus, in its insidious chronicity, histological appearance and early functional changes, the disease is similar to renal infection in man.

This model, therefore, permits evaluation of adrenocortical activation accompanying progressive tissue damage without introducing extraneous "non-specific" stress factors such as hyperthemia, anorexia, and anoxia. In addition, the close similarity between the experimental and natural diseases suggested also

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TABLE I. Influence of Infection on Organ Weights and Various Parameters of the Stress Response.

	Control	Infected			
		16 hr	24 hr	1 week	6 weeks
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Body wt (g)	222 ± 39	222 ± 79	200 ± 22	218 ± 21	212 ± 30
Adrenal wt (mg)	33.8 ± 8.9	34.0 ± 8.2	32.4 ± 7.1	32.6 ± 4.6	41.0 ± 12.9
Kidney wt (g)	1.54 ± .34	1.66 ± .33	1.67 ± .10	1.74 ± .18	1.35 ± .34
Adrenal					
Ascorbic acid (mg/100 g)	402 ± 95	351 ± 48	358 ± 22	394 ± 135	379 ± 47
Corticoids (μg)	4.48 ± 2.70	9.28 ± 5.73‡	—	5.18 ± 4.70	3.88 ± 5.08
Liver pyrrolase	3.15 ± 1.30	4.04 ± 1.95	2.66 ± 1.61	—	4.86 ± 1.68‡
" pyrrolase + hematin	9.62 ± 5.10	12.97 ± 8.58	—	—	12.44 ± 4.08
" tyrosine transaminase*	2.59 ± 2.12	2.21 ± 1.34	2.51 ± 1.34	—	2.03 ± 1.32
" tyrosine transaminase†	17.30 ± 8.45	16.54 ± 5.53	12.98 ± 4.43	16.25 ± 5.52	20.87 ± 9.46
" tryptophan transaminase†	8.33 ± 2.63	10.18 ± 3.31	4.49 ± 4.18	8.87 ± 1.92	5.93 ± 2.36
" phenylalanine hydroxylase	57.39 ± 17.56	54.40 ± 5.87	—	—	58.58 ± 11.18
" tryptophan hydroxylase	2.62 ± .64	1.99 ± .16	—	—	2.37 ± .38

Male Wistar rats were intravenously injected with *Str. faecalis* and were sacrificed at the indicated periods after infection.

* Without addition of pyridoxal phosphate. † With addition of pyridoxal phosphate.

‡ Differs from control at $P < .05$.

that this study could be relevant to further understanding the clinical course of human pyelonephritis.

Materials and methods. Male Wistar rats weighing 150-200 g were divided into control and experimental groups. Experimental animals were intravenously injected with 1.0 ml of *Str. faecalis* in Heart Infusion Broth (Difco) containing 2-5 × 10^8 cells/ml while controls received an equal volume of sterile Heart Infusion Broth alone. Microbial enumeration was performed by standard serial dilution techniques. Animals were maintained on an *ad lib* diet of Purina chow and were handled for several days before sacrifice to minimize the effects of handling as a stress. Groups of control and experimental animals were sacrificed by decapitation 16 hours, 24 hours, 1 week or 6 weeks after infection, organs immediately removed, weighed and placed in appropriate media for assay. Adrenals were homogenized in 5 ml of 20% ethanol saline. Livers were homogenized in 6 volumes of cold, neutral, isotonic KCl and centrifuged at 30,000 × *g* for 30 minutes at 4°C. The supernatant fraction of the liver homogenate was assayed for tryptophan-phenylalanine hydroxylase, tryptophan pyrrolase and α-ketoglutarate-tyrosine and -tryptophan transaminase activities as described previously(7). Adrenals were analyzed for ascorbic acid and corticosterone(7). Protein was determined by ultraviolet absorption(7). Assays were carried out on blocks of matched control and infected animals.

A 72-hour fast was employed in comparing the response of infected and uninfected animals to a superimposed stress. To compare the response of these groups to exogenous steroids, 3.0 mg of hydrocortisone sodium succinate (Solu-Cortef) per kg of body weight was administered intraperitoneally 4 hours before sacrifice of animals. The results were analyzed by an analysis of variance and Duncan's New Multiple Range Test(8).

Results and discussion. The results in Table I show that the only classical measures of adrenocortical activation which appear abnormal over the course of the infection are a small but significant elevation in 16 hour adrenal corticoids and a corresponding suggestive, but nonsignificant fall in adrenal ascorbic acid. These coincide with the induced bacteremia and occurred before apparent tissue destruction.

Indirect measures of adrenocortical activation, such as the activities of the adrenocortical hormone-inducible enzymes, tryptophan and tyrosine-α ketoglutarate transaminase and tryptophan pyrrolase were identical to controls over the 6-week period of this study.

The absence of adrenocortical "stress" re-

TABLE II. Influence of 72 Hour Fast on Stress Responses of Control Animals and Animals Infected with *Str. faecalis.**

	Controls Mean ± S.D.	Infected Mean ± S.D.
Adrenal wt (mg)	38.6 ± 5.2	35.4 ± 7.1
" corticoids (μg/g)	37.4 ± 28.6	46.9 ± 37.4
Plasma corticoids (μg/100 ml)	44.6 ± 4.8	37.5 ± 27.5
Tryptophan pyrrolase†	7.94 ± 4.16	12.46 ± 7.54
Tryptophan pyrrolase + hematin†	14.20 ± 6.62	25.91 ± 11.80

* All animals were infected with *Str. faecalis* 4.5 months prior to fasting. The results are the means and standard deviations for 4 animals per group.
† Rate expressed as μmoles/hr/g protein.

sponses in the face of clear tissue damage to a vital organ could be due to active suppression of adrenocortical activation so as to avoid the deleterious consequences of accompanying anti-inflammatory processes.

In order to test this possibility, control and infected animals were compared for their response to exogenously administered corticoids.

The results in Table II suggest that both infected and control animals responded to the stress of fasting in a normal or even heightened manner.

A similar pattern of responses was observed following acute hydrocortisone treatment. The results in Table III demonstrate that both infected and noninfected animals respond with appropriate enzyme responses following exogenous hormone administration. Further, the responses for infected animals again appeared slightly greater than those for noninfected animals.

These results do not necessarily preclude the existence of active mechanisms suppressing adrenocortical activation during infection. They do demonstrate, however, that any such mechanisms can be overwhelmed by superimposition of additional stressors, and could be functional, therefore, only under relatively restrictive conditions. Nor does any evidence exist suggesting that the absence of a stress response in these infected animals can be attributed to chronic adaptation to an initially acute stress. Rather, it seems most likely that experimental pyelonephritis simply does not elicit a stress response. The course of the clinical and experimental disease is slow and insidious. Tissue damage occurs in minute continuous increments and kidney function may not become insufficient for considerable time, or indeed, not over the entire life of the organism. In this sense, then, the disease process can be compared with the slow continuous decline in tissue function accompanying aging. Because these incremental changes are too small, individually, to require massive restabilization of equilibria, stress adaptation would serve little function since there would be nothing to adapt to. The interpretation would imply that the rate of change of physiological state would be an important determinant of the stress response. Further, it might be anticipated that adrenocortical change will be associated with acute diseases or acute exacerbations of chronic diseases rather than with chronic diseases them-

TABLE III. Influence of Glucocorticoids on Some Stress Parameters of Control Animals and Animals Infected with *Str. faecalis.*

Group	Infected	Non-infected	
Treatment	Hydrocortisone Mean ± S.D.	Hydrocortisone Mean ± S.D.	None Mean ± S.D.
Body wt (g)	312 ± 32	315 ± 22	340 ± 22
Adrenal wt (mg)	38.9 ± 2.7	43.0 ± 4.8	45.1 ± 9.8
Kidney " (g)	1.202 ± .221	1.161 ± .066	1.322 ± .227
Adrenal corticoids (μg/g)	3.6 ± 2.0	4.4 ± .9	1.1 ± .2
Tyrosine transaminase*	28.73 ± 8.88	22.21 ± 4.65	14.60 ± 1.88
Tryptophan pyrrolase†	33.02 ± 18.20	23.84 ± 12.7	3.48 ± 1.25
" " + hematin†	65.70 ± 16.07	46.20 ± 25.84	7.98 ± 4.19

Control and 6 week infected animals were given 3.0 mg of hydrocortisone (Solucortif)/kg of body weight. Animals were sacrificed 3½ hr after corticoid administration.
* Without addition of pyridoxal phosphate. Rate is expressed as μmoles/min/g protein.
† Rate expressed as μmoles/hr/g protein.

selves regardless of the final extent of tissue damage incurred or the physical impairment involved. For example, it has been reported (9) that 17-keto steroid excretion of rabbits given tuberculous meningitis rose slightly during the early stage of infection but remained at essentially normal levels at the height of the disease. If this is correct, then chronic illnesses such as emphysema, arthritis and possibly certain cancerous states might be unaccompanied by adrenocortical effects. On the other hand, some diseases of acute onset, such as certain infections (pneumonia, meningitis) or myocardial infarction may have concomitant adrenocortical activation and such activation could, indeed, contribute to their outcome. These possibilities are under examination.

1. Selye, H., Proc. Soc. Exp. Biol. and Med., 1959, v102, 403.
2. Guze, L. B., Goldner, B. H., Kalmanson, G. M., Yale J. Biol. Med., 1961, v33, 372.
3. Kalmanson, G. M., Hubert, E. G., Guze, L. B., Proc. Soc. Exp. Biol. and Med., 1963, v113, 918.
4. Breslau, A. M., Gonick, H. C., Sommers, S. C., Guze, L. B., Am. J. Pathol., 1964, v44, 679.
5. Gonick, H. C., Foos, R. Y., Rubini, M. E., Guze, L. B., J. Clin. Invest., 1963, v42, 937.
6. Guze, L. B., Kalmanson, G. M., Proc. Soc. Exp. Biol. and Med., 1961, v108, 496.
7. Geller, E., Yuwiler, A., Schapiro, S., Biochim. Biophys. Acta, 1964, v93, 311.
8. Duncan, D. B., Biometrics, 1957, v13, 164.
9. Buskina, V. A., Prob. Endocrin. Hormonal Therap., 1962, v4, 23.

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Response of the Bear to Endotoxin.* (31164)

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This laboratory has been particularly interested in responses of different species of animals to endotoxin(1-5). Marked dissimilarities in vascular responses and tissue alteration have been observed between dogs, cats, rabbits, monkeys† and chickens(1-5). The response of the monkey to endotoxin is in marked contrast to that of the dog since hepatosplanchnic pooling in the monkey is not observed while shock in the dog is characterized by a sharp rise in portal pressure and extensive abdominal visceral pooling. Marked vascular changes were noted in the pulmonary bed of the monkey, cat, and rabbit(2). Gross differences found between animal species in response to endotoxin create difficulties in comparing data from a given species with the suspected responses in man(5). Information gained about responses in various species of animals to endotoxin would be of benefit in determining mechanisms operating in shock and in understanding the etiology of changes occurring in man. The present study was concerned with vascular and tissue responses of the bear to endotoxin.

Methods. Three adult American black bears (*Ursus americanus*) weighing between 96 and 173 kg fasted 24-48 hours were carefully restrained in a gorilla squeeze cage and administered sodium pentobarbital intraperitoneally followed by an intravenous injection. The total dose varied from 25-40 mg/kg body weight.

The animals were then placed in the supine position on a large operating table, and catheters were placed in the aorta (*via* the femoral artery), portal vein (*via* the splenic vein following a laparotomy) and right ventricle or pulmonary artery (*via* the jugular vein). Pressures at these 3 sites were continuously recorded by means of Statham pressure transducers on a Sanborn direct writing recorder. Heart rates were also monitored. An indwelling catheter was placed in the femoral vein for the injection of anesthetic, endotoxin and for procurement of blood samples required for pH and hematocrit determinations. *E. coli*

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† Cercocebus torquatus atys; rhesus.