at the time of acute intestinal damage is, of course, needed to give substance to this conjecture. The present studies also reopen the question of the extent to which endotoxins may contribute to death from the Gram-negative infections which occur during the first few weeks after irradiation.

Summary. Exposure to radiation, especially in the lethal range, caused a marked increase in sensitivity of mice to *S. typhosa* endotoxin. This was most pronounced on the third day after irradiation. At that time the endotoxin LD_{50} in mice given 1000 rads was 0.5 μg compared to a control value of 480 μg per 20 g mouse. Shielding the hind legs was without effect on endotoxin LD_{50} (2.3 μg for 850 r, 200 KV X-rays). Shielding the back, including the adrenals, was partially effective (LD_{50}, 55 μg). Treatment with cortisone had an effect comparable to that of shielding the back. Shielding the front of the animal, including practically all of the intestinal tract, was very effective in spite of the fact that the adrenals were irradiated (LD_{50}, 410 μg). Shielding a small portion of the abdomen was also quite effective in reducing endotoxin sensitivity in the irradiated mice (LD_{50}, 165 μg).

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Detoxification of Endotoxin by Splenic Extracts.* (28490)

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Bacterial endotoxin is extracted from the circulation by the reticuloendothelial system (1). This system also degrades endotoxin. Experiments reported previously demonstrated that *in vivo* perfusion of the normal dog's spleen with P^{32} labelled endotoxin results in almost immediate extraction and detoxification, with simultaneous release of dialyzable P^{32}(2). Later experiments demonstrated that detoxification also occurs within a few minutes on exposure of endotoxin to saline extracts of fresh spleen from the normal dog(3). Similar extracts of fresh liver from the normal dog are about one-fifth as potent as those from spleen, whereas extracts of kidney, heart muscle and skeletal muscle are inactive. Splenic extracts from animals exposed for some hours to traumatic shock are also inactive. Further data on

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TABLE I. Assay of Detoxification of Endotoxin by Splenic Fractions, Using the Chick Embryo Test.

Incubation mixture	Exp 1	Exp 2	Exp 3
Control—endotoxin and saline	5/6	5/6	5/6
Endotoxin and whole homogenate	2/6	1/6	2/6
Endotoxin and saline soluble supernatant (20,000 × g) of homogenate	0/6	1/6	1/6
Endotoxin and sediment of homogenate	3/6	3/6	4/6

One ml of splenic fraction was incubated with endotoxin at 37°C for 1 hr. Each 0.1 ml of reaction mixture contained 1 LD_{80} of endotoxin prior to incubation.

In this Table, and in Tables II and III, the numerator in the fractions indicates number of chick embryos killed out of a total of 6 (the denominator) tested with each sample.

the nature of this active principle in spleen are reported here.

Method. Samples of fresh or frozen normal dog spleen were homogenized in cold isotonic saline (0.5 g/ml) for one minute in a Waring Blendor. A portion of the whole homogenate was centrifuged at 20,000 × *g* at 4°C for 30 minutes. Following centrifugation the supernatant was decanted, and the residue resuspended in an amount of saline equal to the original volume of the homogenate. The whole homogenate, the supernatant obtained at 20,000 × *g*, and the resuspended residue were assayed for their ability to detoxify bacterial endotoxin. One ml of each sample was incubated at 37°C for one hour with *Aerobacter aerogenes* endotoxin sufficient to give a final concentration of one chick embryo LD_{80} per 0.1 ml of reaction mixture. Controls of endotoxin alone and tissue fractions alone were incubated along with the test samples. Following incubation, the presence or absence of toxicity of each mixture was determined by the chick embryo test(4). Detoxification was considered to have taken place if 4 or more of 6 embryos survived exposure to the reaction mixture. The detoxifying activity of whole splenic homogenate could be localized in the supernatant obtained at 20,000 × *g* (Table. I).

The active supernatant fraction was purified further by ammonium sulfate precipitation. Precipitates were collected between the following per cent saturations of ammonium sulfate: 0-30, 30-50, 50-70, and 70-100, by addition of the solid salt to the supernatant. After standing for 30 minutes the solutions were centrifuged at 10,000 rpm for 30 minutes, the supernatants decanted, and the residues resuspended in one-half the original volume of isotonic saline. After dialysis against saline to remove excess ammonium sulfate, the endotoxin-detoxifying potency of these samples was determined. The most active preparation was that obtained from precipitation at 30% $(NH_4)_2SO_4$ saturation (Table II). This fraction was as potent as the whole homogenate, and the supernatant from which it was prepared. One ml of this preparation ("30F"), with a nitrogen content equivalent to some 4 mg of protein, was the standard amount used in the following experiments.

Since measurements of the kinetic properties of this system are dependent on the bioassay of endotoxin, the results could not be defined in the precise terms of a biochemical method. Nevertheless, the control data in each experiment are sufficiently uniform and reproducible to make the assay data reliable.

TABLE II. Detoxification by Fractions of Spleen Obtained at Increasing Concentrations of $(NH_4)_2SO_4$.

$(NH_4)_2SO_4$ fractions (% saturation)	Exp 1		Exp 2	
	Precipitate	Supernatant	Precipitate	Supernatant
0-30	1/6	6/6	0/6	5/6
30-50	3/6	3/6	3/6	3/6
50-70	6/6	3/6	6/6	3/6
70-100	6/6	6/6	3/6	6/6
Endotoxin plus saline	5/6		6/6	

The $(NH_4)_2SO_4$ precipitates were resuspended in 0.1 M NaCl and dialyzed against same. These suspensions and the corresponding supernatants were assayed for their detoxifying potency by the chick embryo method.

TABLE III. Detoxification of Endotoxin Incubated for One Hour with Several Dilutions of the 30F Preparation.

Dilution of 30F*	Amt of endotoxin 0.2 γ (1)	2 γ (10)	12 γ (60)	20 γ (100)
None	0/6	1/6	1/6	3/6
3:4	0/6	—	—	—
1:2	2/6	—	—	—
1:4	3/6	—	—	—
Endotoxin control for 1 LD_{80}	6/6	5/6	6/6	6/6

Numbers in parentheses represent number of chick embryo lethal doses per 0.1 ml.

* 30F = spleen fraction insoluble in 30% saturated $(NH_4)_2SO_4$.

One ml of the 30F fraction of the normal spleen degraded up to 3 times the chick embryo LD_{80} dose of endotoxin in as little as 3-5 minutes. Longer incubation yielded greater detoxification. Thus one ml of the 30F fraction detoxified at least 60 chick embryo MLD's if allowed to act for one hour (Table III). Detoxifying activity was proportional to the concentration of the 30F fraction.

The detoxifying component remained stable after storage for at least 3-4 months at −20°C either as whole spleen or as the 30F preparation. The latter was stable at 37°C for 3 hours or longer. It was inactivated at 50°C in 3 hours and at 60°C in 30 minutes. These data suggest that the active fraction is not a γ-globulin. Full activity was present from pH 5.5 to 8.5. Exposure of one ml of the 30F fraction to 1 mg of crystalline trypsin (Worthington, 2 × crystallized) in 0.1 M phosphate buffer at pH 7.4 for 30 minutes at 37°C resulted in complete inactivation of the preparation. Therefore, it is likely that the active principle is a protein. Complete inactivation by tetraethyl pyrophosphate at a concentration of 10^{-6} M suggests that an esterase type of enzyme is involved in detoxification.

The active substance was found to remain in solution when the 20,000 × *g* supernatant was further purified by centrifugation for one hour at 144,000 × *g*. Preliminary studies indicate that fresh spleen of the rat, rabbit, cattle and hog contains a similar active principle.

Discussion. In vitro degradation of endotoxin by serum has been reported by many investigators(5-10). This was determined in terms of (a) release of P^{32} from P^{32} labelled endotoxin (Rowley(5)); (b) tumor necrotizing activity and pyrogenicity (Hegemann (6); Goodale(7)); (c) reduced lethality in the rat and chick embryo (Ho and Kass (8)); (d) alteration in antigenicity by various immunologic techniques (Landy(9) and Skarnes(10)). The time required to effect degradation by fresh serum varied from 40 minutes to 24 hours depending upon conditions of the reaction.

In vitro degradation of endotoxin by tissues has been reported by Collins and Wood (11) who observed loss of pyrogenicity on exposure to leucocytes for 4 hours.

Trapani *et al.* reported inactivation of endotoxin by tissue extracts of rabbit liver, adrenal, and lymph nodes. Similar fractions of spleen, heart and brain were inactive(12). Of these tissues only liver was consistently active. In other experiments Keene reported an active principle in spleen, liver, kidney and heart, which was inhibited by certain divalent cations(13). Degradation was demonstrated by Trapani *et al.* and by Keene by the loss of pyrogenicity in rabbits, and of tumor-necrotizing capacity in tumors of mice.

The results herein reported demonstrate an endotoxin-detoxifying factor in spleen, and in lower concentration in liver, which is not found in other tissues (*e.g.*, kidney, skeletal muscle, heart muscle or brain (unpublished data)). This factor is present in a non-particulate fraction of spleen, is independent of the presence or absence of cations, and is a protein with an esterase type of enzymatic behavior.

The endotoxin-detoxifying factor in tissue reported herein differs from those reported by Trapani *et al.* and by Keene in its location in tissues, in the method of assay of detoxification, and in its rapidity of action. The time required to effect detoxification by the tissue extracts of Trapani *et al.* was 10 minutes, and by Keene *et al.*, 1-2 hours. In our hands the healthy spleen *in vivo* and fresh saline extracts of healthy spleen detoxified endotoxin within 3 minutes.

Summary. A fraction which rapidly de-

toxifies bacterial endotoxin has been prepared from normal dog spleen. The detoxifying system is thermolabile, precipitable with ammonium sulfate, completely inactivated by trypsin and by tetraethyl pyrophosphate. These findings suggest that the active component is a protein with the properties of an esterase type of enzyme.

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Comparative Expiratory and Oxidative Effects of Glucagon and Epinephrine in Mice. (28491)

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Subcutaneous injection of glucagon has been reported to increase metabolic rate of rats, similarly to epinephrine(1). In fact, it has been proposed that in rats and dogs, glucagon may cause secretion of epinephrine from adrenal glands(2,3). Recent experiments in our laboratory have been concerned with the general metabolic effects of glucagon and epinephrine in mice. The present report suggests that, in contrast to epinephrine, glucagon did not increase metabolic rate of mice. However, glucagon appeared to increase oxidation rate of C^{14}-glycerol by nonfasted mice.

Materials and methods. Ad libitum fed male albino rats (Charles River) and mice (Upjohn colony-Rockland Farm ancestry or Carworth Farm CF_1) weighing 165-175 g and 25-28 g respectively were used. Certain mice (Upjohn colony) were fasted 15 hours (overnight) before use. Diabetes was induced in mice by intravenous (IV) injection of 70 mg/kg alloxan-monohydrate as a 1% aqueous solution(4). Typical fasting blood glucose values obtained from diabetic mice were about 300 mg% when placed on experiment 48 hours after alloxan injections. Blood for glucose analysis was obtained by cardiac puncture of mice under light ether anesthesia. Blood glucose was determined by an Auto-Analyzer* utilizing a method reported previously(5) but modified in that protein-free filtrates were obtained by dialysis. Liver glycogen was determined by an anthrone method (6). Fresh preparations of hormones were made daily and injected as indicated; control animals received appropriate vehicle alone. Glucagon† was suspended in 0.25% methyl cellulose aqueous vehicle and 0.2 ml of the appropriate concentration injected subcutaneously (SC) or intraperitoneally (IP) into rats or mice. Epinephrine‡ was given SC (0.37 mg/kg) in 0.1 ml 0.9% saline to rats or mice.

For expiratory experiments, a hormone or vehicle alone was injected 40 minutes before measurements of expired CO_2 were begun. In

* Technicon Instrument Corp., Chauncey, N. Y.
† Eli Lilly & Co., Lot 258-234B-167-1.
‡ Parke-Davis & Co., Adrenalin 1:1000.