ject to profound fluctuations during the gestation period and also in response to cortisone treatment. Although very little is known concerning hormonal function in bats during the reproductive cycle, it is suggested that adrenal activity may have played an important role in directing the observed alterations in lipid content of the brown fat of gravid bats.

Summary. The interscapular brown adipose tissue of the bat (*Tadarida b. mexicana*) undergoes significant fluctuations in lipid content during the reproductive cycle. There is a progressive accumulation of lipid in the brown fat cells, beginning in early pregnancy and reaching a maximum level late in the gestation period. In contrast, during and shortly after parturition the brown adipose tissue is essentially deplete of lipid, while 2 weeks following delivery lipid can again be found in the brown fat cells. Cortisone treatment of non-gravid bats results in increased lipid accumulation in the brown fat similar to that observed in the pregnant animal. A gradual increase in weight of brown fat parallels lipid hypertrophy. The role of adrenal activity in directing alterations in the lipid content of the interscapular brown adipose tissue of bats during the reproductive cycle is discussed.

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Localization of Endotoxin in the Walls of the Peripheral Vascular System During Lethal Endotoxemia.* (27824)

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The principal sign of lethal endotoxemia is peripheral vascular collapse. Routine pathologic methods, however, have failed to demonstrate a lesion and provide an explanation for the shock. We are inquiring whether endotoxin is localized in blood vessel walls where it could exert a direct toxic effect and yet be undetected by conventional methods.

Studies using radioactively labeled endotoxin have not permitted precise localization and have been difficult to interpret. For example, Barnes, Lupfer, and Henry(1) studied in rats the fate of intravenously administered I^{131}-labeled *Sh. flexneri* endotoxin and found most of the radioactivity in the liver. They inferred that the hepatic cell was the biochemical target of endotoxin activity. Braude, Carey and Zelesky(2) in a similar study in rabbits with Cr_{51}-labeled *E. coli* endotoxin

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also found most of the radioactivity in the liver. However, they inferred that the data reflected uptake by the reticulo-endothelial system.

Unlike the radioactive labels used in these experiments, the methods of immunofluorescence localize antigens with precision at the cellular level. They have not been used to investigate the phenomenon of endotoxic shock, although they have been successfully employed in endotoxin distribution studies. Thus Cremer and Watson(3) used immunofluorescence to study phagocytosis of intravenously administered *S. typhosa* endotoxin by the reticulo-endothelial system of rabbits. Tanaka, Nishimura, and Yoshiyuki(4) followed in mice the fate of *S. enteritidis* endotoxin administered intravenously and intraperitoneally in sublethal doses. Endotoxin was found consistently in the reticulo-endothelial system as well as in vascular endothelium and, on occasion, in the adventitia of veins.

The present communication describes the localization by immunofluorescence of *E. coli* endotoxin in dogs during acute lethal endotoxemia.

Material and methods. Preparation of animals. A minimal lethal dose of *E. coli* 0111: B_4 endotoxin, 4 mg/kg, was injected intravenously into 4 adult mongrel dogs. Two dogs were killed, one 10 and one 90 minutes later, with intravenous sodium pentobarbital. A third animal died of endotoxemia 13 hours after endotoxin injection. The fourth exhibited prostration, vomiting, and diarrhea, but was alive at the end of 24 hours, the arbitrary survival time, and was not included in our study. A fifth dog, the *control animal,* received no endotoxin and was killed with intravenous pentobarbital.

Endotoxin preparation. The endotoxin used in these experiments was purchased from Difco Laboratories (Detroit, Mich.; Difco #0922, lot #448652, modified method of Boivin). *E. coli* 0111:B_4 was chosen because of its extremely high degree of antigenic specificity(5,6,7).

Preparation of tissues for staining. Representative specimens of all organ systems were promptly removed and, except for the great vessels and the brain, were taken *in toto* or cut into blocks approximately 2 × 6 × 12 mm. Full-thickness strips 2 × 12 mm were taken from the great vessels. Brain specimens were prepared as follows: a parasagittal section from the medial surface of the right frontal lobe of the cerebrum was made; it extended 6 mm on either side of the pre-Sylvian fissure and was 2 × 12 × 3 mm. (The 3 mm dimension given in all brain sections represents depth into the brain, the other 2 dimensions being surface length and width.) A transverse section from the superior vermis of the cerebellum 2 × 12 × 3 mm was made. The pituitary was removed intact. The mamillary bodies were removed just beyond their margins; the section was 2 × 2 × 3 mm. The tuber cinereum was removed directly below the infundibulum; the section was 2 × 2 × 3 mm. For sections of the medulla oblongata, the sharp margins of the trapezoid body were used as transverse borders; in the 10-minute and 90-minute animals, bilaterally symmetrical sections which included both pyramids and the parts of the trapezoid body immediately lateral to them were made; in the 13-hour animal, a unilateral section was taken which extended laterally from the midline and included the left pyramid and the entire left trapezoid body. All medullary sections were 3 × 10 × 3 mm.

Each block was placed near the bottom of a Wassermann tube, its thickest surface flush against the wall of the tube. The tube was then corked. A tissue block was quick-frozen by immediately plunging the tube into a dry ice—95% ethanol mash where it was kept for approximately one hour. Tubes were then stored at −20°C until time of sectioning.

Sections 3-5 μ were cut in a cryostat at −20°C. Two sections, usually consecutive, were placed on each slide. The slide was then promptly removed from the cryostat, and the sections were quickly thawed by placing a finger on the under surface of the slide immediately below the section. The slides were then placed for one hour before a fan to promote rapid evaporation of tissue moisture. On days when the relative humidity was greater than 50%, the cryostat room was dehumidified to 40-50%. Finally, slides

were stored in a desiccator containing calcium chloride for one to 10 days.

On the day of staining, the desiccator was held at room temperature for approximately one hour before the slides were removed. Sections were fixed in absolute acetone at room temperature for 10 minutes and air-dried for 30 minutes. They were then immersed in staining buffer contained in Coplin jars. The composition of the staining buffer was as follows:

$NaH_2PO_4 \cdot H_2O$, 6.0 g; $Na_2HPO_4 \cdot 7H_2O$, 28.5 g; NaCl, 120 g; sufficient distilled water to make 15 liters; pH was 7.2.

Tissue staining by the indirect fluorescent antibody method. We used the indirect fluorescent antibody method described by Weller and Coons(8). It depends on 2 consecutive antigen-antibody reactions *in which the antibody in the first reaction is the antigen in the second*; and in which the antibody in the second reaction is fluorescent. In our experiments, the first reaction occurs between the endotoxin in the tissue section and rabbit anti-endotoxin antibody. The second reaction occurs between the rabbit anti-endotoxin antibody, now the antigen, and fluorescent horse anti-rabbit gamma globulin antibody. Our controls depend on preventing the first reaction which is highly specific(5,6,7). This may be accomplished in two ways: (1) Eliminating the endotoxin. We stained sections from a normal dog, the *control animal,* with rabbit anti-endotoxin antibody. (2) Eliminating the rabbit anti-endotoxin antibody. We stained sections from endotoxin-treated dogs with rabbit *control serum,* rabbit anti-endotoxin anti-serum from which the anti-endotoxin antibody had been removed by prior absorption. In both controls, the fluorescent horse anti-rabbit gamma globulin antibody is applied in proper sequence; however, in both cases, it has nothing specific with which to react and no specific staining occurs.

After removal of the slides from staining buffer, excess solution around the sections was carefully absorbed with bibulous paper. A drop of specific rabbit anti-endotoxin antiserum (*test serum* or *control serum,* to be described) was placed on each section. The slides were then incubated in a moist chamber for 30 minutes at room temperature. Subsequently, excess serum was gently washed off with cold staining buffer. The slides were returned to the Coplin jar, rinsed with 5 changes of buffer over a 15-minute period, removed from the Coplin jar, and excess moisture again absorbed. This time a drop of horse anti-rabbit gamma globulin antibody conjugated with fluorescein isothiocyanate was placed on each section, and the slides were returned to the moist chamber for another 30 minutes. Sections were flushed as before and again rinsed in cold buffer for 15 minutes, with 5 changes of buffer. Finally, slides were removed from the buffer, excess solution was absorbed, and a drop of buffered glycerol (9 parts glycerol, one part staining buffer) was placed on each section. The sections were then mounted with coverslips. Completed preparations were examined immediately, or stored at 4°C and studied within 4 days.

Preparation of vaccine for rabbit anti-endotoxin antiserum. A stock culture of *E. coli* $0111:B_4$ on a tryptic digest slant was stored at 4°C. A 50-ml portion of tryptic digest broth (Fields) in a 100-ml Erlenmeyer flask was inoculated with a loopful of organisms, and was incubated at 37°C for 8-10 hours with constant swirling on a platform shaker. After centrifugation at 2800 rpm for 30 minutes at room temperature, the broth was decanted, and the organisms were suspended in 5 ml of staining buffer, pH 7.2, in a 25-ml test tube. The tube was placed in a boiling water bath for one hour. The organisms were again centrifuged after suspension in 15 ml of additional buffer. After 3 washings, sufficient buffer was added to make a suspension of organisms equivalent to the density of a McFarland standard#5(9). Vaccine was freshly prepared each day of injection and was administered within one hour after preparation.

Preparation of rabbit anti-endotoxin antiserum. An albino rabbit weighing approximately 3.0 kg at time of the first injection was immunized over an 8-month period with 7 courses of vaccine injected intravenously into a marginal ear vein. Each course was essentially as follows: day 1, 0.25 ml; day 3,

0.5 ml; day 5, 0.75 ml; day 7, 1.0 ml; day 9, 1.25 ml; day 11, 1.5 ml; day 14, harvest. On day 14 after the last course, approximately 40 ml of blood was harvested from a razor-nicked ear vein. After 2 hours at room temperature, the clot was "rimmed" and the blood was refrigerated at 4°C for 18 hours. Serum with free cells was decanted and centrifuged at 2000 rpm for 30 minutes at 4°C. The cell-free serum was filtered (Seitz-Swinny) into a sterile rubber stoppered bottle and stored at 4°C. All experiments described in this study were performed with this antiserum.

When the antiserum was placed in an Ouchterlony plate† and opposed by a sample of the endotoxin used to produce endotoxemia in the dogs, but at a concentration of 1 mg/ml, 2 bands developed after 4 days.‡ The band near the antigen well was sharp and dense; the band near the antiserum well, diffuse and faint.

Criteria for final selection of the rabbit antiserum used in these experiments were these: that serum was chosen which produced bright specific tissue staining and minimal non-specific staining.

Preparation of rabbit test serum and control serum. A 5-ml portion of rabbit antiserum was absorbed twice with a heterologous boiled vaccine prepared from *E. coli* W. This serum, designated *test serum,* still contained endotoxin antibody active against *E. coli* 0111:B_4. A second 5-ml portion was similarly treated with homologous boiled vaccine from *E. coli* 0111:B_4 to remove the endotoxin antibody. This preparation was designated *control serum.* Approximately 500 ml of tryptic digest broth (Fields) incubated for 10 hours at 37°C provided sufficient organisms for absorption of 5 ml of serum.

The treated sera were preserved with equal volumes of glycerol and stored at 4°C.

For staining purposes, one drop of serum was diluted with 20 drops of staining buffer, representing a final dilution of 1:40. One drop of this diluted test serum was placed on one of the 2 sections on the slide, and a drop of diluted control serum on the other section. The 2 diluted sera were used in parallel in all experiments on endotoxemic animals. Only diluted test serum was used in experiments on the control animal.

Preparation of horse anti-rabbit gamma globulin antiserum.§ Rabbit anti-bovine serum albumin (BSA) antiserum was prepared by immunizing rabbits with tartranilic acid-azo-BSA. Anti-BSA gamma globulin from 112 ml of immune serum was precipitated at equivalence with 19 mg of crystalline BSA. The washed precipitate, containing approximately 100 mg antibody, was suspended in 30 ml of saline and mixed with an equal volume of complete Freund's adjuvant. Approximately 15 ml of this mixture, representing 25 mg of antibody, was injected subcutaneously into a horse. Five months later, a booster of approximately 50 mg of purified rabbit gamma globulin in 15 ml of incomplete (mycobacterium-free) Freund's adjuvant was injected subcutaneously. Two weeks later the animal was bled.

When the horse serum was opposed by rabbit serum in a double diffusion gel plate of Ouchterlony, 3 bands developed in 4 days; one, near the horse serum well, was thick, dense, and laminated; the remaining 2, near the rabbit serum well, were thin, faint, and discrete.

Preparation of fluorescent antibody: Conjugation of horse anti-rabbit gamma globulin

†We have found the Ouchterlony double diffusion gel system a reliable indicator of the effectiveness of rabbit sera for localization of endotoxin by the indirect fluorescent antibody method. All vaccine-prepared antisera which developed discrete bands in 3 or 4 days were capable of producing immunofluorescence with endotoxin by the indirect method. This was true for antisera prepared by a single 2-week course of injections or by multiple courses, like the antiserum used in our study. However, antisera prepared from purified endotoxin, both Westphal and Boivin types (Difco), formed no bands or only poorly developed ones after 7 days and were also ineffective in localization of tissue endotoxin.

‡We have not observed less than 2 bands between a serum effective in immunofluorescence and purified Boivin and Westphal antigens (Difco), although we have observed as many as 5 bands.

§Dr. A. M. Pappenheimer, Jr., Dr. R. R. Porter, and Mr. Leo Levine prepared the horse anti-rabbit gamma globulin antiserum.

antibody with fluorescein isothiocyanate. The globulin fraction of the horse antiserum was salted out with half-saturated ammonium sulfate(10). Protein content was determined by Nesslerization(11). The globulin was conjugated with fluorescein isothiocyanate and dialyzed according to Marshall *et al.*(12).

The fluorescent antibody was filtered (Seitz-Swinny) into a sterile rubber stoppered bottle and stored at 4°C.

Five-ml portions were removed as needed and absorbed twice with mouse liver powder, 100 mg/ml antibody, to reduce non-specific staining(10). After centrifugation at 16,000 rpm for 60 minutes at 4°C, the antibody supernate was mixed with an equal volume of glycerol and stored at 4°C.

Immediately prior to staining, the fluorescent antibody was diluted 2:3 with staining buffer, providing a 1:5 final dilution.

Fluorescence microscopy. Observations were made with the following system: an Osram HBO 200 W mercury vapor arc light source with heat filter, a Corning 5840 filter of standard thickness, a Zeiss darkfield achromatic substage condenser with a n.a. 1.1-1.2, a Zeiss apochromatic 40X oil objective lens or a Zeiss achromatic 100X oil objective, Zeiss barrier filters –65/41, and Zeiss 12.5X oculars. At times phase contrast microscopy was helpful, especially in distinguishing between capillaries and cells; for, with dark field microscopy, both lumens and nuclei sometimes appear black.

Results. Gross pathology. The 2 animals which were killed with pentobarbital, one 10 and the other 90 minutes after endotoxin injection, showed no gross anatomical lesions. On the other hand, the animal which died 13 hours after receiving endotoxin had a large basilar subarachnoid hemorrhage with bloody spinal fluid, but without evidence of bleeding into the brain substance. The intestinal contents were bloody, and petechial hemorrhages were seen in the serosa of the bowel. Petechiae were also observed in the adrenal cortices, in the myocardium, and in the liver, spleen, and lungs. The kidneys were normal.

Microscopic examination by immunofluorescence. The histologic findings were essentially the same in all 3 animals. Immunofluorescent material was found in all organ systems, both extracellular, especially in connective tissue and in the lumens of vessels, and intracellular within cytoplasm. (We use the words *immunofluorescent* and *fluorescent* to mean immunologically specific staining, as opposed to *autofluorescent* and *non-specific staining.*)

Fluorescent material was widespread throughout the reticulo-endothelial system: In Kupffer cells of the liver,‖ greater amounts in the periphery of the lobule than near the central vein; in sinus lining cells of the spleen, lymph node, adrenal cortex and medulla, and of the *pars distalis* of the pituitary; and in connective tissue histiocytes, including adventitial cells of veins and septal cells of lungs.

Fluorescent material was widely distributed throughout the walls of the peripheral vascular system. It was frequently detected in the endothelium of capillaries and of small and medium-sized veins (Fig. 3), including the *vasa vasorum* of the portal vein and inferior *vena cava,* and extracellular as well as intracellular in the walls¶ of veins of all sizes (Fig. 7); on occasion it was seen extracellular or intracellular in the media of muscular arteries and arterioles, including branches of the coronary, pulmonary, and renal arteries (Fig. 2). Fluorescent material in blood vessels, although wide-spread, was patchy, both in terms of over-all distribution (Table) and in terms of distribution within a particular vessel (Fig. 1). Capillary and venule endothelium was frequently involved, but arteriolar endothelium, only rarely; and the endothelium of the great arteries and veins was consistently spared. While fluorescent

‖ The Kupffer cells were difficult to recognize and could be identified with certainty only in the animal which was killed 10 minutes after administration of toxin (Fig. 6); because the sinuses, especially at the periphery of the lobule, were frequently distended with aggregates of polymorphonuclear leukocytes and bizarre masses of fluorescent material, often as large as 20 to 30 μ in diameter. Similar aggregates (Fig. 4) and masses (Fig. 5) were found in the other 2 animals in which Kupffer cells could not be recognized at all.

¶ We could not recognize the media of veins. Consequently, when describing veins we use the word *wall* to mean adventitia and possibly media.

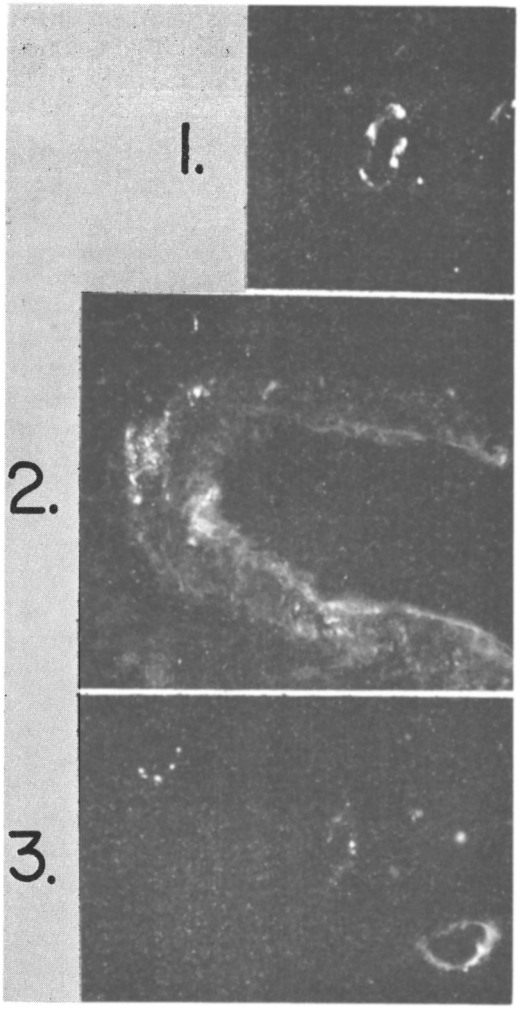

FIG. 1. Myocardium, 13 hr dog. Small vessel, probably a venule. Dense immunofluorescent patches are in the wall. × 250.

FIG. 2. Lung, 13 hr dog. Pulmonary artery branch. A large patch of particulate immunofluorescent material is in the media, at left in picture. A fluorescent polymorphonuclear leukocyte is immediately above and to the right of patch just described. Vessel lumen is black; internal elastic membrane is the thin gray band (bright blue in stained section); media is gray (dull green in stained section); adventitia is gray-black (blue-black in stained section). × 250.

FIG. 3. Medulla oblongata, 13 hr dog. Three large capillaries or small venules in brain substance. Vessel in upper left quadrant resembles a constellation, a common appearance of immunofluorescent capillaries. Large extravascular spots are not immunofluorescent. × 600.

particles were usually uniformly dispersed in endothelium; in the media and adventitia, patches of particles or scattered particles were typical. Many organ vessels had no detectable fluorescent material, and the great arteries usually had none.

Fluorescent material was abundant in polymorphonuclear leukocytes both in the peripheral circulation and in the tissues. Individually or in groups, granulocytes were occasionally detected in unusual sites: in vessel walls; in the epithelium and lumens of distal convoluted tubules and collecting tubules of the kidney; between axones of the celiac ganglion; in the connective tissue of the kidney and other organs; and in the white pulp of the spleen. Large numbers were seen in the septa and alveoli of the lungs, scattered throughout the red pulp of the spleen, and agglomerated in masses in liver sinuses (Fig. 4). Many granulocytes were distorted or partially ruptured.

Generally, fluorescent material was scattered free in the lumens of vessels, but at times it was adherent to the endothelium of capillaries and veins. Sometimes it completely filled a capillary lumen. This was particularly characteristic of renal glomeruli and of sinuses of the adrenal medulla and the liver.

Particulate, extracellular fluorescent material was present in connective tissue throughout the body, frequently in circumscribed but irregular patches 15-20 μ in diameter.

An occasional hepatic cell, even in the 10-minute animal, contained a few particles of fluorescent material.

In the kidney, fluorescent material was frequently seen in glomerular capillary endothelium and/or in Bowman's visceral epithelium; the basement membrane could not be identified. Fluorescent material was seen frequently in stromal capillary endothelium; rarely in Bowman's parietal epithelium, Henle's loop, the distal convoluted tubule and collecting tubules; and not at all in the proximal convoluted tubule. The medulla of the 90-minute animal was noteworthy because the stroma between tubules was a sea of fluorescent particles.

In the central nervous system, fluorescent material was present in the capillaries of the brain substance and the arachnoid where it was also seen in the walls of larger vessels and in cells that could not be identified with con-

fidence, but resembled histiocytes. In rare instances, a capillary lumen was filled with a fluorescent mass.

Fluorescent material was found in unidentifiable cells, possibly marked distorted granulocytes and histiocytes, in the *pars distalis*

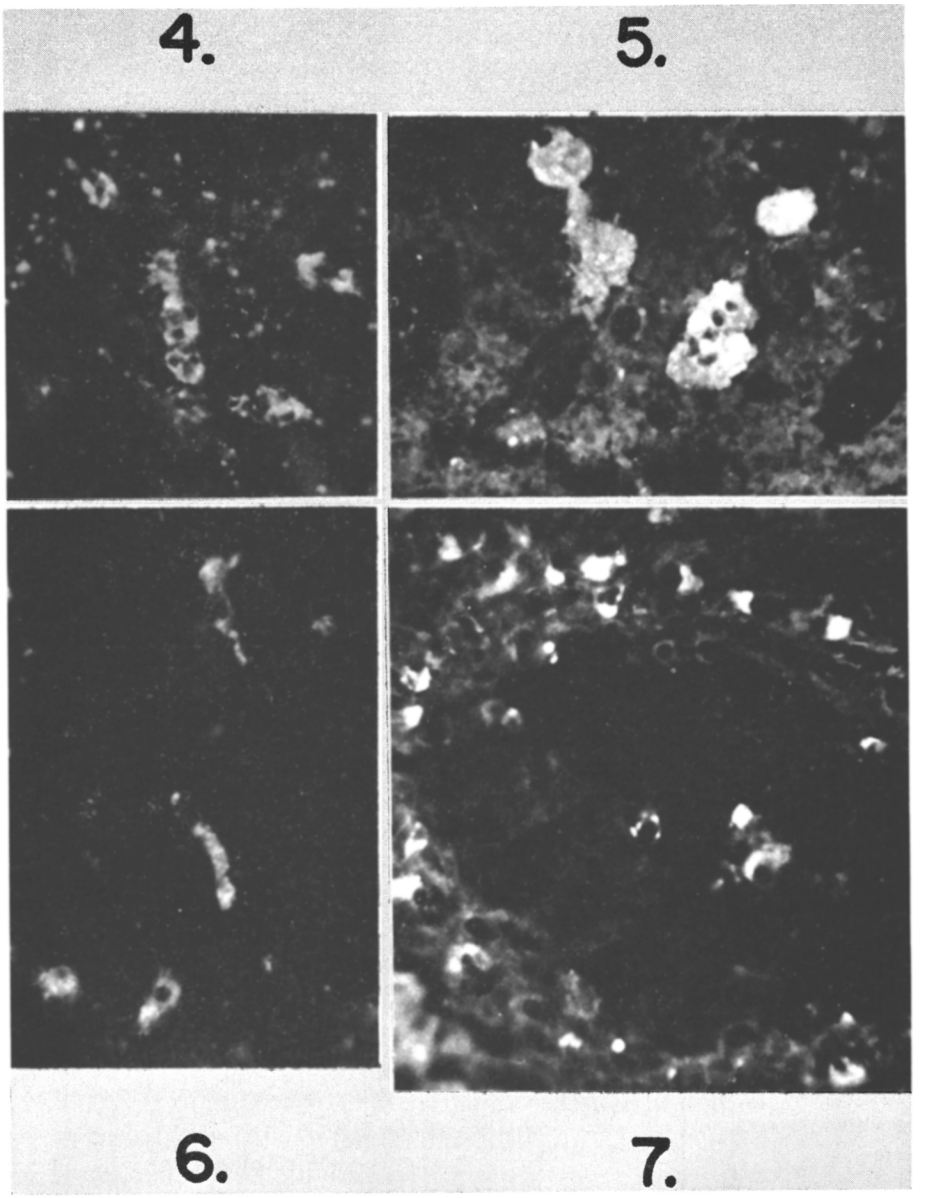

FIG. 4. Liver, 90 min dog. Granulocytes sequestered in liver sinuses. Granulocytic cytoplasm is filled with immunofluorescent material. Some cells have agglomerated and are hardly recognizable. Flecks (in the liver cords) are autofluorescent. × 250.

FIG. 5. Liver, 13 hr dog. Bizarre immunofluorescent conglomerates filling sinuses. Nuclei are evident in the masses. Are these masses agglomerated granulocytes or engorged Kupffer cells? Liver cords exhibit rather bright blue autofluorescence. × 250.

FIG. 6. Liver, 10 min dog. Four Kupffer cells. Cytoplasm of Kupffer cells contains particulate antigen. Liver cords are not discernible. × 250.

FIG. 7. Liver, 13 hr dog. Portal vein branch. Many immunofluorescent polymorphonuclear leukocytes and histiocytes are present in wall of vein and a few granulocytes are in the lumen. Large numbers of cells in a vessel wall was a rare finding. × 250.

of the pituitary and in the *zonae fasciculata et reticularis* of the adrenal cortex.

Qualitatively, the distribution of fluorescent material was similar in all 3 dogs (Table). There were, however, quantitative variations. For example, in the 90-minute dog, the renal stroma was heavily infiltrated; and in the 13-hour animal, the coronary and pulmonary vessels were more heavily involved than comparable vessels of the other 2 animals.

Texture of specific staining. Immunofluorescent material was particulate as well as brilliant apple green. Particles in the endothelium of a capillary cut in cross section often looked like a constellation. Its particulate nature made possible its identification against the dull green non-specific staining of the media of blood vessels, but it could not be detected against a bright green non-specific background, such as that of mucous secretions in the intestine.

Non-specific staining. In general, non-specific staining was homogeneous, confluent, and dull green. To our surprise, the polymorphonuclear leukocyte, notorious for non-specific staining, was not troublesome under the conditions of this experiment; cells laden with immunofluorescent material were readily recognizable by brilliant green pleiomorphic particles, so numerous as nearly to obscure the cytoplasm. In the control serum system, such cells were negative, exhibiting a blue autofluorescence. On the other hand, in blood smears of the 3 animals made immediately prior to endotoxin injection, granulocytes exhibited slight non-specific staining of equal intensity in both the test and control serum staining systems. However, the cytoplasm of these cells, unlike that of their endotoxemic counterparts, was homogeneous, dull green and void of matter except on occasion for a few green isomorphic granules.

Non-specific staining was usually non-particulate. In certain tissues, however, particulate matter usually stained in controls; for example, in sweat glands, between and/or in skeletal and cardiac muscle fibers, in the muscularis of the intestine, in bronchial epithelium, and in cardiac capillary endothelium. Thus, if immunofluorescent material was present in any of these areas, it could not be identified.

Discussion. Immunofluorescent material was found in patchy distribution throughout the walls of the peripheral vascular system, providing clear evidence that endotoxin is deposited in blood vessel walls during the course of endotoxemia.

Fluorescent antibodies react only with specific determinants of antigens. Therefore, any alteration in the molecular structure of an antigen apart from one in the specific determinant would not be recognizable. If detoxification of endotoxin involves such a change, fluorescence would not shed light on toxicity.

The distribution of endotoxin within all 3 dogs was essentially the same. Although the pulmonary and coronary vessels of the animal which died 13 hours after injection of endotoxin were more severely involved than the comparable vessels of the other 2 animals, no significant quantitative differences were established in our study.

Diapedesis of endotoxin-bearing granulocytes was occasionally observed. The polymorphonuclear leukocyte is apparently, therefore, a mechanism for transport of endotoxin from the intravascular compartment out into the extravascular space. On occasion, particulate, fluorescent patches measuring 15-20 μ in diameter were encountered; for example, in vessel walls and in connective tissue. Were these the remains of ruptured granulocytes?

Within the central nervous system, endotoxin was found as elsewhere in blood vessel walls. In our study, however, organ systems were only sampled. Extensive mapping of the hypothalamus and reticular substance was not performed.

Summary and conclusion. A minimal lethal dose of *E. coli* 0111:B_4 endotoxin was injected intravenously into 3 dogs. Two were killed, one 10 and one 90 minutes after endotoxin administration. The third died of endotoxemia in 13 hours. By immunofluorescence, endotoxin was found in patchy distribution throughout the walls of the peripheral vascular system: frequently in the endothelium of capillaries and venules and, extra-

TABLE. Immunofluorescent Material in Vessel Walls of 3 Dogs with Lethal Endotoxemia.

Organs studied	Artery or arteriole (media)			Capillary (endothelium) or sinus (reticular cell)			Venule or vein (endothelium or wall)					
	10 min	90 min	13 hr	10 min	90 min	13 hr	10 min		90 min		13 hr	
							Endo.	Wall	Endo.	Wall	Endo.	Wall
Spleen	+	+	0	+	+	+	0	0	0	0	0	0
Liver	0	0	+*	+	?	?	0	+	0	+	0	+
Kidney	+	+	+	+	+	+	0	0	0	0	0	0
Adrenal	0	0	0	+	+	+	0	+	0	0	0	0
Left ventricle	0	0	+	?	?	?	+	0	0	0	0	0
Right "	0	+	+	?	?	?	+	0	+	0	0	+
Lung	0	0	+	?	?	?	0	+	0	0	+	0
Jejunum	0	0	0	+	0	+	+	+	+	+	+	+
Colon	0	0	0	+	0	+	+	+	+	+	+	+
Celiac ganglion	0	0	0	+	+	+	0	0	0	0	0	0
Splanchnic nerve	0	0	0	0	+	+	0	0	0	0	0	0
Vagus "	0	+	0	+	+	+	0	+	+	+	0	0
Cerebrum	0	+	0	+	0	0	0	+	+	+	+	+
Cerebellum	0	0	0	+	0	0	+	0	0	0	0	+
Pituitary	0	0	0	+	+	+	0	0	0	0	0	0
Mamillary bodies	0	+	+	+	+	+	0	+	0	+	0	0
Tuber cinereum	N.S.	N.S.	0	N.S.	N.S.	+	N.S.	N.S.	N.S.	N.S.	+	+
Medulla oblongata	0	0	0	+	+	+	+	+	+	+	+	+
Lymph node	+	+	0	+	+	+	+	0	0	+	0	+
Skin	0	+*	0	?	?	?	0	+	0	+	0	0
Skeletal muscle	0	+	+	?	?	?	0	0	+	0	0	+
Uterus	N.S.	0	0	N.S.	+	+	N.S.	N.S.	+	0	+	0
No. of organs in which a stained vessel was found	3	9	7	14	11	14	7	10	8	9	7	10

This table is qualitative; it shows for each organ whether or not a vessel wall was ever seen containing fluorescent material. Hepatic sinus lining cells are indicated "?" when they were not recognizable. Lung capillaries are indicated "?" because lung morphology is badly distorted in frozen sections and capillaries cannot be identified with certainty. Skin capillaries could not be adequately studied because of bright blue dermal autofluorescence. Myocardium and skeletal muscle capillaries are "?" because of non-specific staining.

* Endothelium involved as well.

N.S. = not studied.

cellular as well as intracellular, in the walls of veins; and, on occasion, extracellular and intracellular, in the media of arterioles and muscular arteries.

Endotoxin was also detected in polymorphonuclear leukocytes, both free in the peripheral circulation and in unusual tissue sites; for example, within vessel walls and aggregated in the sinuses of the liver. Particles of endotoxin were frequently seen free in the lumens of blood vessels; in some instances endotoxin appeared to be fixed to the intima of vessels; and masses of endotoxin occasionally completely filled a vascular lumen, most often in renal glomerular capillaries, in the adrenal medullary sinuses, and in liver sinues. Endotoxin was widely distributed throughout the reticulo-endothelial system, including histiocytes. Qualitatively and quantitatively, the distribution of endotoxin within the 3 dogs was similar.

We infer from the presence of endotoxin in blood vessel walls that endotoxin may be acting directly upon these structures to produce the peripheral vascular collapse of lethal endotoxemia.

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Immunochemical Identification of Salivary Proteins.* (27825)

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Immunochemical technics, employing antisera specific for individual human plasma proteins, have been used successfully to identify proteins in human oral fluids. Using immunoelectrophoresis, Brill and Brönnestam (1) demonstrated γ-globulin, transferrin and α_2-macroglobulin in gingival pocket fluid. Ellison, Mashimo and Mandel(2) detected albumin and γ-globulin in 10 times concentrated saliva, also by immunoelectrophoresis. Mandel and Ellison(3) similarly identified the same 2 proteins in 20 to 50 times concentrated parotid gland secretion. Kraus and Sirisinha(4) measured γ-globulin in saliva and parotid gland secretion by specific precipitation.

The present study attempts to demonstrate

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