as 1.0 μg/ml were sufficient to markedly inhibit these synthetic processes, and a concentration of 5.0 μg/ml totally inhibited them. DNA synthesis in the same bacterium was unaffected. Synthesis of RNA and proteins in *E. coli*/NSC 87982 was unaffected by 5.0 μg NSC 87982/ml; however, higher concentrations (30 μg/ml) inhibited both of these processes. DNA synthesis was unaffected in this culture.

The precise mechanism by which NSC 87982 inhibits microorganisms is unknown. Based on the lack of cross resistance to other inhibitors of protein and/or RNA synthesis in *E. coli* such as actinobolin(12), puromycin(13), or sparsomycin(5), NSC 87982 may well be inhibitory at a different metabolic site.

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Vascular Leakage Induced by Horseradish Peroxidase in the Rat.* (32504)

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Horseradish peroxidase (HRP), a plant protein of approximately 40,000 molecular weight, has been used extensively during the past decade as a protein tracer in histological studies. Recently a relatively sensitive, specific, and simple method for localization of peroxidase with the electron microscope has been introduced(1) and used in a variety of tissues and experimental animals(1-5).

In the course of experiments on the localization of HRP in the mesothelium of the rat(3), it was observed that increased vascular permeability in venules occurred regularly after local or parenteral injection of the protein. Since one of the main ultrastructural applications of HRP is its use as a vascular tracer, further investigation of this phenomenon was undertaken and this communication presents the results of such studies.

Materials and methods. Adult male Sprague-Dawley rats, white male Hartley guinea pigs, and Swiss mice of both sexes were used. Increased vascular permeability was estimated by the local exudation of intravenously injected Trypan or Evans Blue in intradermal test sites. Rats were given 0.4 ml/100 g of a 1% solution of Trypan Blue into the tail vein. Guinea pigs and mice received 2.5% Evans Blue in a dose of 30 mg/kg. The test substances were injected intradermally, into the clipped abdominal skin, in a volume of 0.1 ml in rats and guinea pigs and 0.05 ml in mice, using a 26 gauge short bevel needle. Thirty minutes later the animals were sacrificed and the diameter of the blue staining on the undersurface of the skin was recorded.

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Four intradermal injections were made in every rat; one was a control injection of Krebs-Ringer phosphate at pH 7.4 (the diluent for HRP), one was an injection of 10 or 100 μg of histamine, and two sites were injected with solutions of HRP.

For the localization of vascular leakage (6), colloidal carbon (0.1 ml/100 g) was injected intravenously immediately before a local of systemic injection of HRP. The animals were sacrificed one hour after the injection of carbon. The tissues were examined either as flat mounts or in histological sections after fixation in 10% formalin. Mast cells were stained with 1% toluidine blue after fixation in 10% alcoholic formalin.

The preparations of horseradish peroxidase tested are presented in Table I. The histamine antagonist used was Pyrilamine maleate, and the serotonin antagonist was BOL-148 (2-brom-d-lysergic-acid-diethylamide; Sandoz). Histamine was injected as histamine phosphate and doses given were calculated as histamine base. All solutions were made in Krebs-Ringer phosphate at pH 7.4.

Results. Experiments in rats. Increased vascular permeability after intradermal injections. All preparations induced increased exudation of Trypan Blue (Table I). With most preparations, including the "electrophoretically purified" Sigma Type VI, a dose of 50 μg of HRP gave a reaction which was equivalent to 10-100 μg of histamine. With one preparation (Sigma Type II) as little as 0.5 μg produced a lesion greater than the control.

Duration of vascular leakage. This was studied by injecting the dye intravenously at various intervals after intradermal injections of HRP. Using HRP Sigma Type II, it was found that exudation was maximal 5-10 minutes after the intradermal injection and began to subside 15 minutes later. There was no bluing in sites tested at 30 minutes to 2 hours. In terms of vascular leakage, therefore, HRP produced an immediate-transient type of vascular response(7).

Inhibition by histamine and serotonin antagonists. Neither Pyrilamine maleate alone (up to 2 mg/kg injected intravenously 5 minutes before intradermal injection of HRP) nor BOL 148 alone (same dose schedule) could inhibit the exudation induced by 50 μg of HRP. When, however, a combination of pyrilamine maleate and BOL 148, each in a dose of 1 mg/kg, was injected intravenously 5 minutes before the HRP, there was virtually total inhibition of the bluing induced by 50 μg of Sigma Type II HRP. These drugs were also effective in inhibiting the vascular labelling of venules with carbon (see below).

Vascular labelling with carbon. The pattern of vascular labelling was studied after injections of 50 and 5 μg of HRP in the skin and into the cremaster muscle. In both sites the carbon deposition was limited to medium-sized and small venules and did not affect the capillaries (Fig. 1). The pattern

TABLE I. Increased Vascular Permeability After Intradermal Injection of Horseradish Peroxidase.

Test substance	Animal	Dose (μg)	Diameter (mm) of bluing
Krebs-Ringer	Rat, mouse, guinea pig	.05-.1 ml	3
Histamine	Rat	10	13
HRP (Sigma Type I)*	"	50	14
HRP (Sigma Type II)	"	.05; .5; 5; 50	3; 6; 11; 15
HRP (Sigma Type II)	Mouse	2.5; 25; 250	3; 3; 2
HRP (Sigma Type II)	Guinea pig	5; 50	8; 14
HRP (Sigma Type VI)	Rat	10; 50	8; 15
HRP (Sigma Type VI)	Mouse	25	6 (faint)
HRP N. B. Co.†	Rat	5; 50; 100	11; 13; 18
HRP Worthington HPOD 6421-21‡	"	50	14
HRP Worthington HPOFF 6550	"	50	13

* Sigma Chemical Co., St. Louis, Mo.
† Nutritional Biochemicals Co., Cleveland, Ohio
‡ Worthington Biochemical Corp., Freehold, N. J.

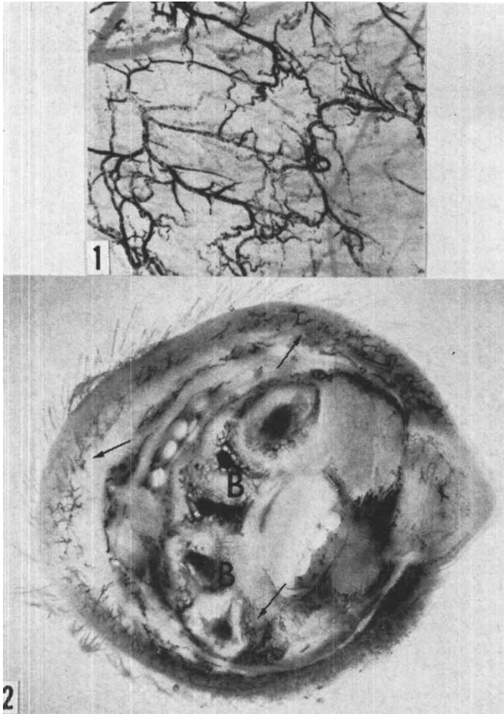

FIG. 1. Carbon labeling pattern in the skin of the rat after intradermal injection of 5 μg of HRP. Only the venules are labeled, a pattern identical with that induced by histamine. $\times$ 22.

FIG. 2. A cross section of the paw of the rat at the level of the metatarsal bones (B) after concurrent intravenous injection of 5 μg of HRP and colloidal carbon. This 3 mm thick section is unstained and has been cleared with glycerin. Note the extensive carbon labeling of vessels (arrow) throughout the soft tissues of the paw. $\times$ 10.

was almost identical with that induced by histamine or serotonin in rats(6). In histological sections the carbon was found to be deposited in the walls of the vessels; intraluminal carbon plugs and thrombi were rare.

Effects of intravenous injections. When HRP (Sigma Type II) was injected intravenously in a dose of 20 mg/100 g body weight in unanesthetized rats, the animals developed the typical syndrome of systemic mast cell damage and vascular injury that can be induced by other known histamine liberators, such as 48/80, or dextran(8). Within 5-10 minutes, there was erythema and edema of the ears, paws, and snout; lacrimation, lethargy and prostration. The effect was maximal between 10-20 minutes after the injection of peroxidase. The animals began to recover within 30-45 minutes, and were apparently normal 4-6 hours later. The syndrome was also produced by a dose of 10 mg/100 g of Sigma Type 11, Worthington HPOD 6421-21 and N.B. Co. preparations; with this dose prostration was less evident. Even with 5 mg/100 g the gross signs of erythema and edema of paws and ears could still be recognized.

After concurrent intravenous injection of 5-20 mg/100 g of HRP and colloidal carbon, flat mounts and histological sections showed abnormal carbon deposition in small blood vessels in the following locations: soft tissues of paws (Fig. 2), ears, snout; in the pericardial fat (but not myocardium), in the mesentery, omentum and serosa of the intestinal tract; in the peribronchial and peritracheal and periesophageal connective tissue (Fig. 3); in the renal pelvis, and in many

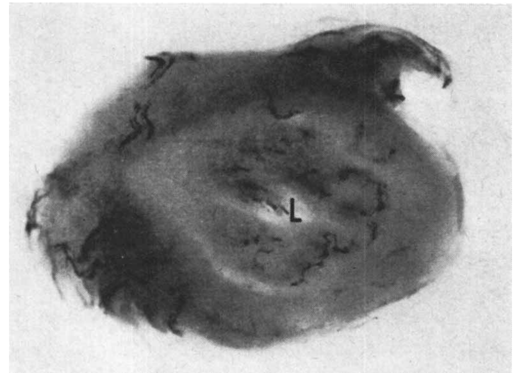

FIG. 3. Slightly oblique section of the esophagus of the rat (L is the lumen) treated in same fashion as Fig. 2. Note the blackened vessels in the wall of the esophagus and in the periesophageal soft tissue. $\times$ 12.

thoracic, abdominal and peripheral muscles.

Effects of intraperitoneal injections. Intraperitoneal injections of HRP in a dose of 0.5 mg per ml, caused focal labelling of venules in the omentum, mesentery, serosa of the viscera and the parietal peritoneum. When the peroxidase was injected directly into the scrotal sac, the peroxidase diffused into the cremaster muscle which exhibited carbon labeling of venules. Carbon labeling after intraperitoneal injection was virtually completely abolished by previous treatment of the animals with a combination of BOL 148 and pyrilamine maleate as mentioned above.

Mast cell degranulation. Subcutaneous and

cremasteric spreads as well as histological sections stained with toluidine blue showed extensive degranulation of mast cells after local injection of horseradish peroxidase. Controls injected with Krebs-Ringer phosphate showed focal and minimal degranulation.

Effect of dialysis. Solutions of HRP (Sigma Type II, Worthington HPOD 642121 and N. B. Co.) were dialyzed in Visking cellulose tubes against Krebs-Ringer phosphate (3 changes, each 100 × volume) for 2 days. When tested intradermally or intravenously these solutions gave the same results as those obtained with undialyzed solutions.

Experiments in guinea pigs. An intradermal injection of 50 μg of Sigma Type II HRP in the guinea pig induced local bluing equivalent to that produced by 10 μg of histamine. Five μg produced a lesion significantly greater than the control (Table I).

Experiments in mice. From Table I it is seen that even large doses of HRP failed to induce an appreciable degree of increased permeabiltiy. With one preparation (Type VI), there was minimal increase in bluing. After intraperitoneal injections of HRP (0.5 mg/cc), and intravenous carbon there was a rare labeled venule in the parietal peritoneum.

Discussion. The findings reported here indicate that commercially available preparations of horseradish peroxidase induce an increase in the permeability of small blood vessels when injected locally or parenterally in rats. This vascular leakage involves predominantly the venules, is associated with degranulation of mast cells, occurs within minutes after injection, lasts for less than half an hour, and can be depressed by histamine and serotonin antagonists. These reactions are identical with those induced by other "mastcell damaging" agents, such as 48/80, dextran, and dextrin. Limited experiments in guinea pigs show a similar effect. Copley and Carol(9) using a quantitative method for assessing vascular permeability with the use of peroxidase concluded that vascular permeability was affected significantly by peroxidase in the guinea pig. Their data indicate that the maximal change in vascular permeability occurred 10-15 minutes after intravenous injection of peroxidase, a finding consistent with the results in rats presented here. Mice appear to be resistant to the action of HRP since neither intradermal nor parenteral injections produced significant vascular leakage. This is in line with the relatively slight effect that other mastcell damaging agents have in mice.

Electron microscopic studies on the distribution of intravenously injected HRP in the rat, now in progress, indicate that leakage of HRP and carbon in venules occurs across intercellular gaps in the endothelium, in a manner similar to that of histamine(10). In the myocardial capillaries, however, peroxidase is localized in apparently intact intercellular junctions which do not allow the passage of concurrently injected carbon particles. These observations are consistent on one hand with the lack of effect of histamine on capillaries in general(6,7) and on the other with the studies of Karnovsky on the distribution of HRP in the mouse(11). In a detailed study Karnovsky has shown that intercellular junctions in myocardial capillaries of the mouse are permeable to horseradish peroxidase and are identical ultrastructurally with junctions from uninjected control animals(11).

Several other vascular tracers have also been reported to induce vascular leakage. Thorotrast (Testagar and Co.) produces mast cell damage due to its content of dextrin, which is used as the suspending medium(12). Several colloidal iron preparations contain dextran or dextrin and cause vascular injury (13). Grant *et al*(14) have recently reported that cadmium-free ferritin, in rabbit skin, induces increased permeability to intravenously injected Evans Blue. In the rat(12), Rowley has shown that leakage induced by thorotrast could be inhibited by a combination of histamine and serotonin antagonists, but not by either antagonist alone. The inhibition studies with peroxidase are similar to those with thorotrast and are compatible with the observation that mast cell granules in rats conatin both amines(8,15).

Whether vascular leakage induced by HRP preparations is due to an impurity or to the peroxidase itself is unknown, but the substance does not appear to be dialysable. Nevertheless, our studies emphasize the neces-

sity for rigid controls in the use of peroxidase as a vascular tracer, especially in the rat, since both histamine and serotonin affect not only permeability but numerous other biological phenomena.

Summary. Intradermal, intraperitoneal and intravenous injections of horseradish peroxidase solutions induce increased vascular permeability in rats. The vascular leakage is confined to venules, is of short duration, can be inhibited by a combination of histamine and serotonin antagonists and is associated with mast cell degranulation. This phenomenon occurs also in the guinea pig but is minimal or non-existent in the mouse.

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Effect of Tension on Lysis of Collagen.* (32505)

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The possibility that tension might accelerate the lysis of collagen or elastin by endogenous or exogenous enzymes is of interest in periodontology, where tensions caused by faulty occlusion are associated with the breakdown of the collagenous supporting tissues of the teeth. Reasons for believing that such an effect may occur are found in the reports that stretching modifies mloecular adsorption on rubber(1) or collagen(2), that mechanical extension denatures the collagen helix and that denatured collagen fibers(3) are digested more rapidly by trypsin as heavier loads are put on them(4). Since there does not seem to be any information on the effects of tension on the enzymatic breakdown of undenatured collagen, we put the matter to test.

To provide undenatured collagen fresh bovine Achilles tendon was processed by the method of Einbinder and Schubert(5) as modified by Mandl *et al*(6). That this material was suitable for our purpose is indicated by triplicate estimates of available hydroxy proline (Leach(7)) on 5 samples of our collagen and commercial undenatured collagen (Sigma). After 48-hour hydrolysis at 100°C in 6M HCl the mean amounts of hydroxy proline were, respectively, 1308 ± 161 and 1397 ± 174 μg/100 mg. In addition, our collagen, even when shredded, was less rapidly broken down than the commercial undenatured collagen when incubated with 200 μg/ml collagenase in pH 7 Tris buffer (Fig. 1).

In testing the effect of tension on collagen breakdown pieces of processed Achilles tendon were divided into matched strips and exposed to the action of collagenase in either a stretched or relaxed state. To do this, tendon strips were passed under a stainless steel hook fixed in the base of a plastic test tube and the ends sutured with surgical silk, which, in turn, was attached over a pulley to a 250

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