early and middle pregnancy in the rat. Where effects are noted, they were, mainly, in that pregnancy did not proceed normally. In some instances there was evidence of abortion, in others of toxic action on the mother, or on the foetuses (with resulting death or malformation) or recurrence of estrus (*e.g.*, ergotoxine) or simple persistence of diestrus. No effort was made in these preliminary experiments to pin-point the site or mechanism of action on the course of pregnancy.

The findings of other workers(1-4) were confirmed. Rats treated with 2 drugs of our series, 1656IS and 933F, revealed significant percent of failure to achieve successful pregnancy, but the mechanism of action remains to be elicited.

It appears that pharmacologically active substances may have significantly selective effects on the sexual cycle and on early pregnancy. This is an area of investigation which until now has remained mainly in the domain of endocrinology.

Summary. Drugs acting on the neurovegetative nervous system, particularly certain derivatives (sympatholytic) of the β-tetra hydronaphthylamine and 2-aminomethylbenzodioxan series, were tested for their action on gestation in the rat. Certain agents showed interference with normal pregnancy, but the mechanism of action remains to be explored.

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Effect of Endotoxin on Gastrointestinal Mucosa of the Rat.* (24696)

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Pathogenesis of the gastrointestinal congestion and hemorrhage caused by endotoxin poisoning(1,2) is not clearly understood. The present investigation was undertaken to study alterations in morphology and biochemical activity of the gastrointestinal tract of the rat during acute endotoxin intoxication. Measurements of succinoxidase activity, an enzyme abundantly distributed in the normal rat gastrointestinal mucosa(3) were used as an indication of aerobic metabolism. An inhibitor of the succinoxidase system in the rat intestinal mucosa has been described by Nakamura *et al.*(4). It was necessary, therefore, to ascertain whether any changes in activity were due to alterations of the inhibitor rather than to changes in enzyme activity *per se*. In addition, parallel studies were carried out to determine if the mucosal barrier to microorganisms was impaired by endotoxin. Invasion by intestinal microorganisms resulting from chemical irritation of intestinal mucosa and other types of gastrointestinal trauma has been observed(5,6) while transmural migration of microorganisms, or products thereof,

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from intestines of experimental animals subjected to hemorrhagic shock has been suggested by Fine and co-workers(7). The failure to culture microorganisms from tissues of rats under the latter condition has been attributed to host antibacterial defense mechanisms. In this study maximal lethal doses of endotoxin were used to minimize host antibacterial defense mechanisms by (1) diminishing efficacy of the reticulo-endothelial system(8,9) and (2) inhibiting diapedesis(1).

Methods. Salmonella typhi endotoxin was prepared according to the method of Levitin *et al.*(10). One ml (6.5 MLD_{50}) was injected into the hearts of ether-anesthetized rats† weighing 150-200 g. This dose produced death within 2 to 4 hours. The small intestine of animals sacrificed after varying intervals was immediately removed, flushed and fixed with 10% buffered formaldehyde. Selected tissues were sectioned and stained with hematoxylin and eosin. The small intestines of animals treated comparably were washed with ice cold saline and a homogenate was prepared by scraping the mucosal surface of the entire small intestine with a razor blade and homogenizing for exactly 90 sec. in 10 ml of water. Succinoxidase activity was measured manometrically in triplicate according to the method of Potter and Schneider(11). The micro Kjeldahl method was used for nitrogen determination. Activity of the succinoxidase inhibitor was measured according to the method of Nakamura *et al.*(4), which consisted of determining the suppression of rat heart succinoxidase activity by addition of rat gut homogenate. Routine cultural technics were used in bacteriological studies.

Results. Morphologic changes. After endotoxin injection, the rats exhibited generalized weakness, ataxia, and respiratory distress typical of endotoxin poisoning. Under pentothal anesthesia 6 rats were injected by intracardiac route with endotoxin, laparotomy was performed and visceral changes observed. Onset of edema and congestion of small intestine varied from 20 to 80 minutes after endotoxin injection. This variability was further corroborated by observation in sacrificed rats that 20 of 40 animals had edema and congestion within 60 minutes after endotoxin injection. Benzidene tests for blood in the stool were positive in 6 of 9 rats within 40 minutes after endotoxin injection; all had positive stools at the end of 100 minutes. Grossly bloody stools were not seen nor was diarrhea a constant finding. Fourteen of 16 rats autopsied immediately after death showed marked edema and congestion of small and large intestine and dilatation and engorgement of mesenteric blood vessels. Microscopic examination‡ revealed congestion and hemorrhage into the lamina propria with disruption of glandular architecture. Migration of red blood cells into the interepithelial spaces and, in some instances, escape of red blood cells into the lumen of intestine were also visualized.

TABLE I. Effect of Rat Serum on Succinoxidase Activity of Normal Rat Intestinal Mucosa.

Animal #	Q_{O_2}/N	Animal #	Q_{O_2}/N
1	202	8	244
2	303	9	150
3	267	10	211
4	256	11	401
5	389	12	103
6	276	13	395
7	154	14	305
		Mean ± S.D. = 261 ± 93	

Effect of endotoxin on succinoxidase activity. Previous studies demonstrated that succinoxidase activity of the normal rat intestinal mucosa is exceedingly variable ranging from zero to 400 microliter O_2/hr/mg N. Similar difficulties were encountered by Davenport *et al.*(12) in the mouse stomach. When 0.3 ml of rat serum was substituted for distilled water in Warburg vessels, less variability in respiration (Table I) was observed. Whether rat serum acts in the same capacity as various "activating agents" of the succinoxidase system described by Keilin and Hartree(13) has not been determined. However, Nakamura *et al.*(4) suggested that rat serum may remove or inactivate an 18 carbon fatty acid inhibitor by binding with serum albumin and thus allowing a higher level of oxygen up-

† Obtained from Charles River Breeding Labs, Boston, Mass.

‡ We are indebted to Dr. Earle Hellerstein who provided the microscopic descriptions.

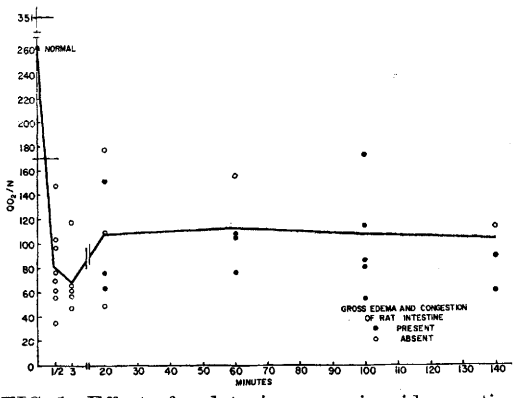

FIG. 1. Effect of endotoxin on succinoxidase activity of rat small intestinal homogenate.

take. Accordingly, measurements of succinoxidase activity in endotoxin treated animals was performed in the presence of 0.3 ml of normal rat serum. Succinoxidase activity decreased 30 seconds after intracardiac injection of endotoxin and remained depressed for 140 minutes (Fig. 1). This decrease was apparent whether the intestine appeared edematous and congested or was normal by gross observation. There was no decrease in heart succinoxidase activity of the same animal throughout endotoxin poisoning (Table II). Average degree of inhibition of heart succinoxidase upon addition of intestinal homogenate in normal rats was 82% (Table II). Endotoxin treated rats exhibited the same degree of inhibition up to 140 minutes after injection (Table II). Addition of varying amounts of endotoxin (from 0.01 ml to 0.5 ml) to Warburg vessels containing normal rat intestinal mucosal homogenates was without effect on succinoxidase activity.

Effect of endotoxin on transmural migration. Since colon and caecum adequately served as their own reservoirs for microorganisms, it was necessary to insure sufficient numbers of microorganisms in the small intestine. Thus, a gastric inoculation technic was used. A 24-hour culture of *Serratia marcescens,* not indigenous to the rat gastrointestinal tract, was grown in nutrient broth (Difco), at room temperature, centrifuged and washed twice with 0.85% saline. The saline washed culture (approx. 3 x 10^8 orgs./ml) was resuspended in 0.1 M potassium phosphate buffer. A series of 25 fasted rats was sacrificed 20, 40, 60 and 100 minutes after endotoxin injection; 15 minutes prior to sacrifice the rats were given 1 ml of the bacterial suspension by stomach tube. A 15 minute interval was used since it was previously determined that *S. marcescens* could be cultured at the ileo-cecal valve 15 minutes after gastric inoculation. Sections of liver, kidney, spleen, lymph nodes and heart were removed aseptically and cultured for 48 and 72 hours in nutrient broth at room temperature The small intestine was also removed and the luminal contents were cultured for *S. marcescens.* These cultures were streaked on nutrient agar plates and incubated for 48 and 72 hours at 25°C.

Neither *S. marcescens* nor intestinal microorganisms could be isolated from heart, liver, kidney, and spleen during acute endotoxin poisoning. Although mesenteric lymph nodes were negative for *S. marcescens,* 40% yielded positive cultures of other microorganisms. Positive cultures for *S. marcescens* were uniformly obtained from the lumen of small intestine. That the failure to grow *S. marcescens* was not due to action of systemic antibacterial defense mechanisms was shown by the following experiment. Eight rats were given 1 ml of endotoxin by intracardiac injection. Forty-five minutes later, they received minimal numbers of these organisms

TABLE II. Per Cent Inhibition of Heart Succinoxidase by Intestinal Homogenate in Endotoxin Treated Rats from 20 to 80 Min. after Inj.

Time after endotoxin inj. (min.)	Q_{O_2}/N Heart	Gut	Heart and gut	% inhibition
20	1031	108	113	89
	898	189	91	99
	960	62	144	85
40	658	76	171	74
	676	51	112	78
	931	233	385	59
60	1366	103	224	83
	729	78	204	72
	900	157	90	90
	1043	104	32	92
80	882	60	94	83
	978	97	345	65
	755	102	79	87
			Mean	= 81 ± 3*
			Control mean†	= 82 ± 4

* Stand. dev. † 5 animals.

in 0.5 ml of saline, *via* the intracardiac route, (equivalent to approximately 150 orgs./ml of circulating blood). The rats were sacrificed 15 minutes after inoculation with *S. marcescens*. Positive cultures of *S. marcescens* were obtained consistently from heart, liver, kidney, spleen and mesenteric lymph nodes. Thus, at a time when 50% of endotoxin treated rats had gross changes in the small intestine, and all had decreased succinoxidase activity, the animals were unable to clear an intracardiac injection of only 1500 organisms in 15 minutes. Since positive cultures were not obtained under similar conditions when large numbers of *S. marcescens* (3×10^8 orgs./ml) were administered by stomach tube, it is unlikely that *S. marcescens* in numbers greater than 150 orgs./ml of circulating blood passed through the intestinal walls.

Discussion. Although edema and congestion occurred from 20 to 80 minutes following injection of maximal lethal doses of endotoxin, depression of succinoxidase activity was noted 30 seconds after injection of endotoxin. Thus, it appears that the decrease in succinoxidase activity may not have resulted wholly from tissue anoxia, secondary to the vascular reaction, but suggests a more direct effect of endotoxin on the gastrointestinal tract. The fact that inhibitor concentration was not altered while intestinal succinoxidase activity was reduced following endotoxin injection, further supports a direct effect of endotoxin on the enzyme system. These findings are similar to those of Kun and Miller(14), who reported decreases in liver and muscle succinic dehydrogenase activity in rabbits given maximal doses of intravenous endotoxin. Endotoxin, *per se*, had no effect *in vitro* on succinoxidase activity of normal gut homogenate. Furthermore, heart homogenate from endotoxin treated animals did not exhibit a like decrease in succinoxidase activity. Additional study is necessary to clarify this effect. Although the small intestine of intoxicated rats was damaged grossly and altered biochemically, bacterial transmural migration in measurable numbers by viable organisms from gut lumen did not occur. Though not all the defense mechanisms are rendered inoperative by large amounts of endotoxin, it is reasonable to assume there is sufficient impairment of the rat antibacterial defense mechanisms to account for the observed minimal numbers of *S. marcescens* in the tissue. Had viable organisms traversed the intestinal wall into the portal system of the intoxicated rats, these would have been cultured from the visceral organs. The fact that transmural migration did not occur despite morphologic damage and enzymatic impairment may be one indication of the effectiveness of the mucosal barrier.

Summary. 1. Intracardiac injection of maximal lethal doses of endotoxin resulted in grossly apparent edema and congestion and microscopic hemorrhages of small intestine within 20 to 80 minutes. 2. Succinoxidase activity of small intestinal mucosal homogenate decreased within 30 seconds after endotoxin injection and remained depressed until animal was moribund. 3. By assay procedure used, there was no change in amount of succinoxidase inhibitor in the intestinal homogenate. 4. Transmural migration of bacteria through the bowel wall was not apparent despite the effect of endotoxin on gut morphology and enzyme activity.

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Adsorption Characteristics of Influenza Viruses to Cholesterol and Fatty Acids. (24697)

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A new and interesting phenomenon, viral combination with cholesterol(1) and other lipids(2) in chromatographic columns was recently discovered by Youngner and Noll. This reaction might be applicable to preparation of improved influenza vaccines since virus was reported to be adsorbed to cholesterol from infected allantoic fluids, whereas nonviral material was not. Thus, passage of infected chorioallantoic fluid through suitable columns of cholesterol resulted in 15-fold purification of virus. Addition of cholesterol to the biologic might be desirable also since Youngner and Noll presented evidence that inoculation of animals with the cholesterol-influenza complex induced elevated antibody responses by adjuvant action. Our work amplifies these basic studies and in particular describes marked differences in combining capacity with cholesterol which exist for several strains of influenza virus.

Methods. Chromatographic columns were prepared by grinding cholesterol to fine powder with mortar and pestle. Weighed amounts of cholesterol (5 g) were then made into a paste-like consistency with phosphate buffered saline, pH 7.2 (PBS); quantitatively transferred to glass columns and packed with slight positive pressure and repeated washings with PBS. Care was exercised to prevent drying or cracking of columns. Virus suspensions of known concentration were then introduced into the columns and effluent fractions assayed for hemagglutinin (HA) titer with standard pattern tests using 0.5% chicken erythrocytes(3). Cholesterol-virus slurries were prepared using weighed amounts of powdered cholesterol to which virus suspensions of known concentration and quantity were added with grinding in mortar and pestle. The mixture was transferred to Erlenmeyer flasks and incubated at room temperature 30 minutes; spun in refrigerated centrifuge at 1500 rpm 30 minutes and supernate assayed for HA titer. Adsorption efficiency was compared on the basis of either total HA units/g cholesterol or % retained.

Results. Early experiments with crude infected allantoic fluid made it clear that the virus-cholesterol system contained complex variables since the affinity of adsorption to cholesterol differed markedly among strains of influenza virus (Table I). Among type A strains, a gradient was found with Asian isolate, "Formosa," readily combining with cholesterol, whereas other A strains showed decreased affinity. Type B strain, Great Lakes (B/GL), did not combine with cholesterol to any measurable degree. When this type of experiment was repeated with strains partially purified by centrifugation and resuspension in PBS, the virus preparations behaved quite differently In particular, a significant increase was observed in retention value with A/PR 301 and B/GL strains

Additional investigations were carried out with these 2 strains which had demonstrated a marked difference in adsorption rate when partially purified Formosa strain allantoic fluid was separated from virus by high-speed centrifugation and used as suspending medium for sedimented B/GL strain virus. Similarly, sedimented B/GL virus was resuspended in its own allantoic fluid and in PBS. The same rate of retention was found for B/GL virus when suspended in any of above