tions(7). Male rats developed maternal instincts when injected with testosterone in specific sites in the hypothalamus.

Summary. Male and female turkeys were started on weekly injections of diethylstilbestrol at 6, 8, and 10 weeks of age. Such treatments caused the development of secondary male characteristics consisting of gobbling, strutting, development of thickenings of the neck region, prolapsed rectums, and precocious mating actions. There were also feminizing effects from DES treatments consisting of atrophy of the testes in males and hypertrophy of the oviduct in females. Increased fat deposition and hypercholesterolemia also occurred in treated birds.

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Antiphlogistic Effects of Antiserotonin (SQ 10,643) and Aminopyrine in Rats *Versus* Endotoxin and Other Agents. (30204)

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Rubin *et al* have described(1) the relatively specific and highly potent antiserotonin properties of SQ 10,643 [2′(3-dimethylaminopropylthio) cinnamanilide HCl] on certain smooth muscle organs and tissues as well as the moderate antianaphylactoid activity of this compound. Furgiuele *et al* have reported(2) on some of the central effects of SQ 10,643 in experimental animals.

Antopol and Chryssanthou have reported (3) that pretreatment of rabbits with either SQ 10,643 or with aminopyrine only slightly inhibited the Shwartzman phenomenon, whereas pretreatment with both compounds markedly inhibited the Shwartzman phenomenon. These results tend to support the roles of serotonin and bradykinin in the Shwartzman phenomenon(3) since the combined effect of the antiserotonin compound, SQ 10,643(1), and of the antibradykinin(4), analgesic-antipyretic compound, aminopyrine, appeared to have been more than additive.

This report is concerned with the local anaphylactoid response of the rat's paw to injected serotonin, bradykinin, histamine, or *E. coli* endotoxin and with the degrees of inhibition produced by pretreatment with SQ 10,643, aminopyrine, or with both SQ 10,643 and aminopyrine.

Methods. Male rats of the Sprague-Dawley Strain, ranging in weight from 170 to 275 g, received intraplantar injections (0.055 ml) of an aqueous solution of one of the following phlogistic agents: serotonin creatinine sulfate (10 γ as base), synthetic bradykinin (50 γ), histamine diphosphate (150 γ as base), or *E. coli* endotoxin, (2.0 mg). These injections were made with hypodermic syringes and 26 gauge needles that were directed towards the ankle-joint of the right hind foot. The left hind foot of each rat served as a non-injected control. The latero-medial diameter of each ankle-joint was determined to the nearest 0.1 mm with vernier calipers. Determinations of the widths of the ankle-joints were made 1, 2, 3, 4, 5, 22-24 hours, and in some tests 48 hours after injection of the phlogistic agent.

The endotoxin employed was Bacto lipopolysaccharide W, *E. coli* 0127:B8 (Difco Labs.). Preliminary trials in 40 rats (5 rats

per group) indicated that, relative to the widths of the non-injected left ankle-joints, a 2.0 mg intraplantar dose increased the widths of the right ankle-joints 38 to 50% within the first 2 hours. The corresponding increases after 1.0, 0.5, 0.25, 0.125 and 0.062 mg doses of endotoxin were 25 to 45, 25 to 33, 19 to 32, 17 to 18, and 8 to 11% respectively. The swellings of ankle-joints persisted for at least 24 hours after intraplantar injections of endotoxin, whereas the swellings produced by similar injections of serotonin, bradykinin, or histamine were completely gone within 24 hours. Two groups of rats that received intraplantar injections of physiological saline showed only 4 to 9% increases in ankle-joint widths within the first 2 hours. On the basis of these results, the 2.0 mg dose of endotoxin was selected as the phlogistic dose for the ensuing study.

A total of 240 other rats, divided into groups of 20, was used in the tests described below. Each group of 20 rats received intraplantar injections of one of the 4 phlogistic agents, but 10 of these rats were pretreated subcutaneously with aqueous solutions of SQ 10,643, aminopyrine, or both. The other 10 rats in each group served as no-drug-pretreatment controls which were injected subcutaneously with distilled water in equal volumes/kg. Pretreatment (5 ml/kg) with 25 mg/kg of SQ 10,643, 250 mg/kg of aminopyrine, or of distilled water, was given 20 minutes before the intraplantar injection of the phlogistic agent. When pretreatment included both compounds, the total dose volume per rat was 10 ml/kg for both the drug- and water-pretreated groups, but the compounds were injected into separate subcutaneous sites, one injection immediately following the other.

The significances of differences between means of ankle widths, their standard errors, variances, and variance ratios were determined by "t" tests and "F" tests as described by Bliss and Calhoun(5). Statistical comparisons were made of: (a) average widths of right ankle-joints in the drug-pretreated groups *vs* the water-pretreated groups at the time(s) of the maximum average difference between the 2 groups, (b) left ankle-joints in both groups at the same time as in (a), (c) ratios of widths of right:left ankle-joints in the drug-pretreated group *vs* the water-pretreated groups at the same time as in (a).

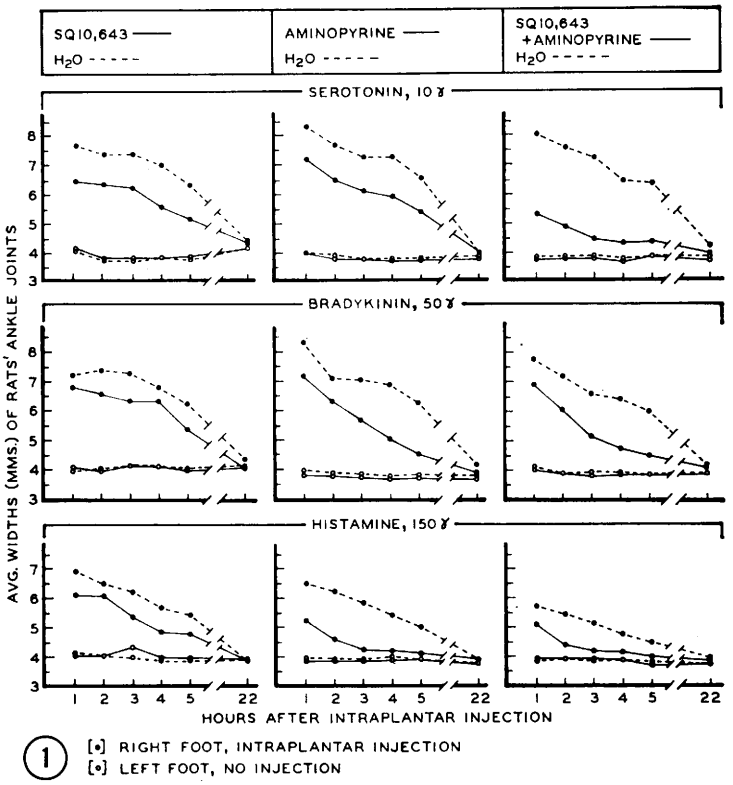
Results. Fig. 1 and 2 show the average widths of the right and left ankle-joints plotted *vs* time for each of the drug- or water-pretreated groups. The maximum widths of the right ankle-joints receiving any one of the phlogistic agents averaged 1.4 to 2 times greater than those of the non-injected left ankle-joints. In all instances maximum edema occurred at, or possibly within, the first hour after intraplantar injection of each phlogistic agent. The average width of the non-injected left ankle-joint in each test was about 4 mm.

Analysis by "t" tests indicated that the mean widths of the non-injected left ankle-joints in the drug-pretreated groups did not differ significantly from those of the water-pretreated groups ($p>0.10$).

As is shown in Table I, the ratios of mean widths of right:left ankle-joints in all but one of the drug-pretreated groups differed significantly ($p<0.01$ or <0.001) from those of the water-pretreated groups. A non-significant difference ($p>0.10$, <0.20) of ratios of mean widths of right:left ankle-joints was obtained only in the endotoxin-treated rats pretreated with SQ 10,643 or with water (Table I). No significant differences were obtained in comparisons of the mean widths of the injected right-ankle-joints in the drug- *vs* water-pretreated groups.

Thus, pretreatment with either SQ 10,643 or aminopyrine slightly or moderately suppressed serotonin-, bradykinin-, or histamine-induced edema; the degrees of inhibition were statistically significant ($p<0.01$ or <0.001) in each instance. SQ 10,643, however, produced only very slight, equivocal, inhibition of endotoxin-induced edema; the inhibitory effect was not significant ($p>0.10$, <0.20). Aminopyrine, on the other hand, produced moderate and significant ($p<0.001$) inhibition of the endotoxin-induced edema.

Simultaneous pretreatment with both SQ 10,643 and aminopyrine resulted in additive, marked, and statistically significant ($p<0.001$) inhibition of the edema induced by



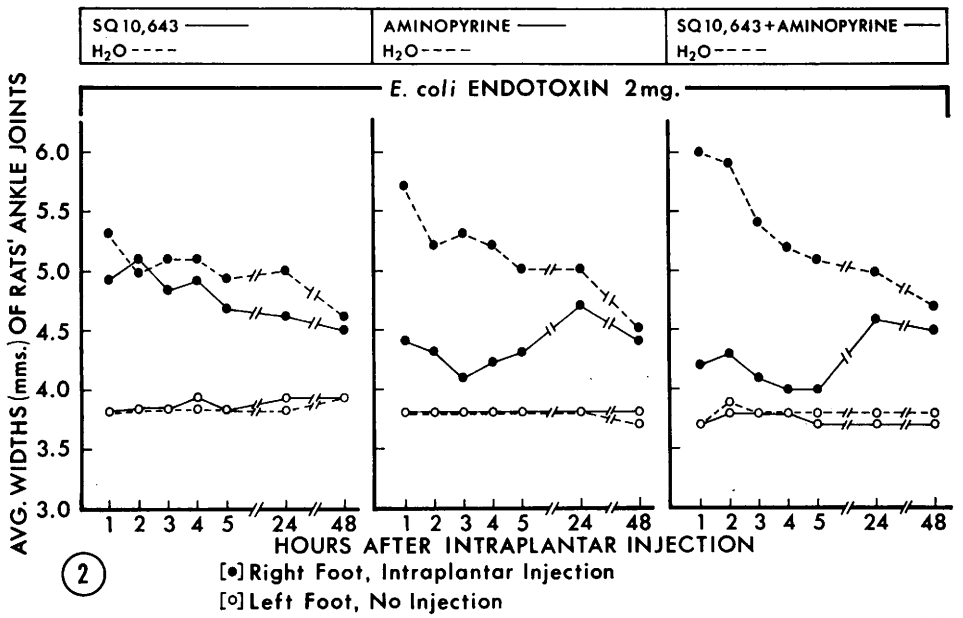


FIG. 1. Antiphlogistic effects of SQ 10,643 and of aminopyrine *vs* serotonin, bradykinin, and histamine.

FIG. 2. Antiphlogistic effects of SQ 10,643 and aminopyrine *vs* *E. coli* endotoxin.

TABLE I. Antiphlogistic Effects of SQ 10,643 and Aminopyrine in Rats.

−20 min S.C. pretreatment	"0 Time" intraplantar phlogistic agent	Time of max inhibition (hr)	Ratios of mean widths ± S.E., right : left ankle-joints	Difference of ratios	Significance P
SQ 10,643 H_2O	Serotonin	3	1.63 ± .05 2.02 ± .04	.39	<.001
Aminopyrine H_2O		4	1.59 ± .07 1.95 ± .05	.36	<.001
SQ 10,643 + aminopyrine H_2O		2	1.28 ± .03 1.96 ± .05	.68	<.001
SQ 10,643 H_2O	Bradykinin	3	1.54 ± .04 1.78 ± .04	.24	<.001
Aminopyrine H_2O		4	1.39 ± .06 1.84 ± .04	.45	<.001
SQ 10,643 + aminopyrine H_2O		5	1.18 ± .02 1.56 ± .03	.38	<.001
SQ 10,643 H_2O	Histamine	4	1.22 ± .03 1.45 ± .05	.23	<.01
Aminopyrine H_2O		2	1.19 ± .04 1.60 ± .06	.41	<.001
SQ 10,643 + aminopyrine H_2O		2	1.13 ± .02* 1.39 ± .05	.26	<.001
SQ 10,643 H_2O	Endotoxin	1	1.29 ± .05 1.38 ± .04	.09	<.20, >.10
Aminopyrine H_2O		1	1.15 ± .02 1.49 ± .03	.34	<.001
SQ 10,643 + aminopyrine H_2O		1	1.11 ± .02† 1.61 ± .05	.50	<.001

* n = 12 instead of 18,
† n = 11 instead of 18,
} recalculation of n when variances are unequal(5).

either serotonin or endotoxin. Similar pretreatment resulted in only moderate, but nevertheless significant ($p<0.001$) inhibition of the edema induced by bradykinin or histamine; this inhibition, however, was equal to or less than that produced by aminopyrine alone, thereby suggesting the occurrence of antagonism of aminopyrine's effect.

The maximum inhibitory effects of SQ 10,643 and of aminopyrine occurred at some time between the 2nd and 5th hours in the serotonin-, bradykinin-, or histamine-injected rats and at the 1st hour in the endotoxin-injected rats (Fig. 1 and 2, Table I).

Discussion. Endotoxins can produce profound and diverse effects in animals. These effects include fever; dermal inflammation; Shwartzman reactions; release, formation, or breakdown of serotonin, epinephrine, histamine, heparin; release or activation of proteolytic enzymes including fibrinolysin, bradykinin, or other vasoactive polypeptides; depletion of lysosomal enzymes from cellular elements which normally participate in the tissue-injury response; leukopenia followed by leukocytosis; hypotension; shock, and lethality(3,6-13).

As others have indicated(3,7), some of the reactions to endotoxin probably involve a sequence of several vasoactive factors and an interaction between several components. A given reaction to endotoxin theoretically should be subject to modification if one or more inhibitors or potentiators are administered at the right time(s). Although the febrile response to endotoxin has been effectively suppressed by analgesic-antipyretics such as aminopyrine, salicylates, etc., the local Shwartzman reaction has been only partially suppressed by such drugs(3,12). Most of the other classical effects of endotoxin have been

almost wholly unaffected by analgesic-antipyretics(12).

Antopol and Chryssanthou reported(3) that antiserotonin compounds, including SQ 10,643, and aminopyrine, designated as an anti-bradykinin compound, inhibited the local Shwartzman reaction in a more than additive manner. The relatively specific anti-bradykinin activities of the analgesic-antipyretics such as aminopyrine or salicylates, however, have been largely restricted to inhibition of (a) bronchoconstriction induced by i.v. bradykinin in the guinea pig(4) and (b) pain or vocalization induced by intra-arterial injections of bradykinin into dogs (14). The inhibition of bradykinin-induced writhing in mice(15) by these analgesic-antipyretics was apparently less specific. These analgesic-antipyretics, furthermore, did not specifically antagonize the actions of bradykinin on the capillaries of guinea pig skin or on excised intestinal muscle of the guinea pig or rat(4).

Our results tend to indicate either that systemic pretreatment of rats with aminopyrine alone or with SQ 10,643 produced either (a) relatively non-specific inhibition of the localized edema induced by injected serotonin, bradykinin, histamine, or endotoxin, or more speculatively, (b) inhibition of one or more of a series of these or other amines, peptides, etc. simultaneously or sequentially released or activated by injection of endotoxin. As shown in Fig. 2, pretreatment with aminopyrine alone or in combination with SQ 10,643 resulted in marked inhibition of endotoxin-induced edema; this inhibition persisted for at least the first 5 hours. The inhibitory effect, however, partly wore off by the 24th to 48th hours; this could possibly be attributed to the sequential or prolonged release or activation by endotoxin of edema-producing substances, long after the metabolic disappearance of inhibitory concentrations of aminopyrine, SQ 10,643, or both.

The apparently additive inhibitory effects obtained by simultaneous pretreatment with both SQ 10,643 and aminopyrine suggest that serotonin may have been one of the endogenously released mediators following intraplantar injection of endotoxin. Perhaps the lipopolysaccharide endotoxin of *E. coli*, like some other polysaccharides such as dextran or ovomucoid, produced local edema in the rat largely through the anaphylactoid release of serotonin(16). In the absence of biochemical and histological analyses, however, other mediators such as histamine, bradykinin, as well as any one of a wide variety of substances that are capable of increasing vascular permeability in the rat(17), cannot be ruled out as contributors to or mediators of the local effect of endotoxin.

Finally, there may have been an even more complex relationship between endotoxin and the 3 other phlogistic agents used. Janoff and Kaley(7) have noted that certain inflammatory responses to serotonin, bradykinin, or histamine have been unexplainably inhibited by pretreatment of rodents with endotoxin; in the case of the rat, mast cell depletion did not occur. This inhibitory effect of endotoxin may be based upon depletion of lysosomal enzymes from cellular elements which normally participate in tissue injury responses (7). Further work is indicated in attempting to ascertain the mechanism of action and the identity of the mediator(s) in the local inflammatory response induced by the intraplantar injection of endotoxin into the rat.

Summary. Pretreatment of rats with the antiserotonin agent SQ 10,643 [2′-(3-dimethylamino-propylthio) cinnamanilide HCl] or with aminopyrine produced slight to moderate inhibition of the localized edema of the ankle-joints induced by intraplantar injections of serotonin, bradykinin, histamine, or *(E. coli)* endotoxin. Simultaneous pretreatment with both SQ 10,643 and aminopyrine resulted in (a) slight to moderate, but less than additive inhibition of the response to bradykinin or histamine, and (b) marked and additive inhibition of the response to serotonin or endotoxin.

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Effect of Large Dose of Gastrin I on Pepsin Secretion.* (30205)

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Gillespie and Grossman(1) showed that rapid intravenous injection of large doses of a partially purified gastrin extract inhibited secretion of acid from a vagally denervated pouch. Gregory and Tracy(2), using a pure gastrin polypeptide, confirmed this action of gastrin and showed that secretion of pepsin was markedly stimulated at the same time that secretion of acid was inhibited. The present study describes the effect of a pure gastrin polypeptide, gastrin I of Gregory and Tracy(2), on histamine stimulated secretion of acid and pepsin in dogs with both a vagally denervated Heidenhain pouch and a vagally innervated gastric fistula.

Methods. Three dogs with gastric fistula and Heidenhain pouch were prepared as previously described(3). Several months after the surgical procedures and after an 18-hour fast, the tests were performed. Histamine dihydrochloride was given by continuous intravenous infusion (Harvard peristaltic pump) at a dose of 0.4 mg per hour throughout the tests. After 7 15-minute periods of collection from both the pouches and the fistulas, a single rapid intravenous injection of 50 μg of gastrin I was given. Collections were continued for an additional 10 15-minute periods. Concentration of acid was measured by titration of samples of juice with an Autoburet Titrator (Radiometer, Copenhagen). Pepsin concentration was measured by a method that uses radio-iodinated albumin as substrate(4).

Results. Gastrin I inhibited secretion of acid by more than 90 per cent in both the pouch and the fistula (Fig. 1). The inhibition was maximal immediately after the injection but full recovery had not occurred even 150 minutes later. The concentration and output of pepsin from the pouch rose markedly (Fig. 2), confirming the findings of Gregory and Tracy(2). The concentration of pepsin in the juice secreted by the gastric fistula did not change when gastrin I was injected and therefore the output fell to the same extent as did the output of acid. At the time when the maximal effect on output of pepsin from the pouch occurred, 60 to 90

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