pansion of vascular volumes by 10% and 25% with dextran caused only slight increases in steroid levels. In the 10% hemorrhagic group, 17-OHCS increased without a significant decrease in mean arterial pressure. In animals which were hemorrhaged 5% of their blood volume every 10 minutes until 35% had been removed, 17-OHCS progressively increased and became significant only after 25% hemorrhage. The alterations in peripheral plasma 17-OHCS which occur during the expansion or reduction of vascular volumes are better described when these values are corrected for circulating plasma volume.

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Received March 18, 1963. P.S.E.B.M., 1963, v113.

Analgesic and Anti-Inflammatory Effects of Orally Given Streptokinase. (28397)

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According to Lewis(1), the nonapeptide bradykinin is an important mediator of the acute inflammatory reaction. It is, therefore, of interest that orally given proteases were recently shown to antagonize 3 pharmacologic actions of bradykinin: vasodilatation, vascular permeability and smooth muscle contraction(2). The objects of the present investigation were: first, to assess the action of orally given streptokinase against the fourth pharmacologic action of bradykinin, namely, pain production; and second, to appraise the anti-inflammatory action of orally given streptokinase in a controlled animal and clinical experiment.

Methods. 1. *Analgesia study.* The cantharidin blister technic of Armstrong *et al.* (3) was slightly modified. This study comprised 30 apparently healthy students and technicians. A band-aid containing 2 drops of tincture cantharidin was applied to the skin of the flexor surface of the forearm the evening before the day of the experiment. When the band-aid was removed the next morning, the area showed a blister with a diameter of about 1.5 to 2 cm. Blisters remained relatively unchanged for 24-48 hours. Intact blisters were opened and the separated

epidermis cut away exposing the blister base, which was bathed in Ringer's solution warmed to 37°C. Before the application of bradykinin, the bathing solution was removed and the area dried with filter paper. 0.2 ml bradykinin (0.1 μg) was applied to the dry blister base and the following information recorded:

1. Length of time for pain to develop.
2. Duration of pain.
3. Intensity of pain. This factor was evaluated by each subject on a 1 to 4 + basis.

Ten control subjects received placebo tablets; 10 treated subjects received tablets containing streptokinase 10,000 units; 10 treated subjects received aspirin, 5 grains. Control and experimental groups received one tablet every 15 minutes for one hour following pretreatment control determinations. Pain evaluations were repeated following the one hour treatment period.

2. *Anti-inflammatory study:*

(A) Animal experiment:

Forty male Sprague-Dawley rats were maintained on Purina-Chow and water. Acute inflammation was produced in one of the hind paws of the rats by injection into the plantar region of 0.05 ml of a 1% Carrageenin** suspension in sterile 0.9% NaCl(4). *Via* stomach tube, 10 rats received 1 ml saline; 10 rats received streptokinase 90,000 units plus human plasma;* 10 rats received 100 mg phenylbutazone; 10 rats received 100 mg sodium salicylate.

Degree of swelling of the foot was determined by the volume displacement method of Adamkiewicz(5). The foot was placed in water containing a wetting agent. The volume of the limb was estimated by measuring the volume of the fluid displaced when the leg was immersed to a known level. The difference in volume of the foot before and after irritation was taken as the measure of inflammatory swelling. Volume measurements were taken every 30 minutes for 6 hours.

(B) Clinical experiment:

This study comprised 20 subjects. A band-aid containing 2 drops cantharidin was applied to one forearm at 8 a.m. Six hour later the band-aid was removed, and the blister area measured and photographed. Individuals who did not develop blisters were excluded from the study. At 8 a.m. the next morning a similar cantharidin application was made on the flexor surface of the other forearm. Enzyme therapy was immediately instituted, one tablet every half-hour for 6 hours; at the end of treatment period, the lesion on this arm was photographed and measured.

3. Kinin activity of Cantharidin injured tissue: Under sterile conditions blister fluid was obtained prior to, and following enzyme therapy. In the absence of blister fluid, a small biopsy was taken of the tissue exposed to cantharidin. Biopsied tissue was immediately homogenized in a mortar and pestle containing Tyrode's solution. Uterus stimulating activity of bradykinin, blister fluid or tissue homogenates was measured on the isolated rat uterus. The uterus was suspended in an isolated organ bath containing 17 ml Tyrode's solution. The temperature was kept at 30°C and 100% O_2 was bubbled through the bath. Bradykinin (0.125 μg) was used as a control. The volume of blister fluid added to the bath was 0.5 ml. In a few instances distilled H_2O was added to bring the final volume to 0.5 ml.

Results. 1. Analgesia study. It appears that bradykinin induces pain in enzyme or aspirin treated patients that is slower in onset, shorter in duration and less intense than placebo treated controls (Table I).

2. Anti-inflammatory study. A. Animal experiment. Inflammation was significantly inhibited following orally given streptokinase, sodium salicylate or butazolidin. The extent of inhibition following oral streptokinase greatly exceeds that following salicylates or butazolidin (Table II). Differences were most marked during the 30-120 minute observation period.

B. Clinical experiment. Blisters developed in each of 20 subjects prior to treatment and in 7 of the following treatment. In the pretreatment groups the blisters measured between 0.5 and 2 cm in 3 subjects and was greater than 2 cm in 17. The blister area

* Varizyme, Lederle.

** Algin Corp. of America.

TABLE I. Evaluation of Pain Following Application of Bradykinin to Blister Base (Mean Values).

Patients	Treatment	Onset of pain		Duration of pain		Pain intensity	
		Pre-Tr.	Post Tr.	Pre-Tr.	Post Tr.	Pre-Tr.	Post Tr.
		(sec)		(sec)			
10	Placebo	1.45 (1–2.5)* .3†	1.5 (1–2) .35	111.5 (85–135) 14.6	133 (105–180) 15.7	4+	4+
10	Oral Streptokinase	1.85 (1–2.5)* .2†	13 (2–23) 2.3 <.01‡	113.0 (90–136) 15.5	69.3 (50–110) 19.6 <.01	4+	2+
10	Aspirin	1.65 (1–2.2)*	11 (2–19) <.01‡	126 (85–140)	76.2 (57–122) <.01	4+	2+
						Sign test ($\frac{1}{2}$)[8]	

* Range. † Standard deviation. ‡ P value.

TABLE II. Foot Swelling Changes.

Observations	Treatment	Mean increase in foot vol (ml displaced H_2O) ——Min. after injection of irritant—— 30	90	150	210
10	Placebo	.6 (.5 –.7)*	1.0 (.8 –1.1)	1.1 (.9 –1.3)	1.1 (.9 –1.3)
10	Streptokinase	.15 (.12–.19)* <.01†	.38 (.31– .42) <.01	.42 (.38– .48) <.01	.58 (.5 – .62) <.01
10	Sodium salicylate	.38 (.33–.51)* <.01†	.76 (.68– .84) <.01	.76 (.71– .79) <.05	.77 (.72– .79) <.05
10	Phenylbutazone	.49 (.42–.56)* <.01†	.82 (.76– .85) <.05	.84 (.78– .85) <.05	.86 (.81– .89) <.05

* Range. † P value.

was less than 0.5 cm in each of the 7 enzyme treated patients.

3. Uterine stimulating activity of cantharidin injured tissue. In each instance, blister fluid showed evidence of uterine stimulating activity as measured by the rat uterus contraction technic. This was noted in the 20 pre-treatment controls and 7 of 20 treated subjects. Tissue homogenates, however, failed to contract the rat uterus. This was so in 13 of 20 treated subjects.

Discussion. In this study orally given enzymes inhibited significantly Carrageenin inflammation of the rat paw and either inhibited (65%) or significantly modified (35%) the blister response to cantharidin ($P<0.01$). Blister fluid from both treated and untreated individuals induced uterine stimulating activity. The exquisite responsiveness of the rat uterus to minute quantities of kinin-like polypeptides precluded any possibility of demonstrating less uterine stimulating activity in treated than in non-treated blister fluid. Tissue homogenates from 13 of 20 treated subjects without blisters, however, failed to elicit this response.

In addition to blister modification, significant antagonism to the algesic action of bradykinin was observed in enzyme treated subjects in this study. This analgesic action is similar to that following aspirin and constitutes the fourth documented anti-bradykinin effect of orally given proteases. Whether this antagonism toward bradykinin is due to direct enzymatic action, the elaboration of polypeptides with pharmacologic activity op-

posing bradykinin, or some other factors, cannot be determined from this study.

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Received March 18, 1963. P.S.E.B.M., 1963, v113.

Sensitivity of Thorotrast-Treated Monkeys to Staphylococcal Enterotoxin.* (28398)

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Preliminary evidence has been adduced that pretreatment of monkeys with stabilized colloidal thorium dioxide (Thorotrast)† sensitizes the animals to the emetic action of staphylococcal enterotoxin. Previously untreated monkeys not vomiting to an ED_{50} dose (eliciting vomiting in 50% of animals tested) of enterotoxin were challenged one week later with the same enterotoxin dose after intravenous injection of Thorotrast 18 hours previously. All of 7 such animals vomited, even though the dose without Thorotrast had been ineffective previously and some resistance to the enterotoxin should have developed as a consequence of the previous enterotoxin feeding(1).

The present communication extends the initial observation. Thorotrast pretreatment rendered monkeys hypersusceptible to the emetic action of both of the antigenic types of staphylococcal enterotoxin tested. The degree to which this colloidal agent enhanced the sensitivity to these distinct enterotoxins is reported.

Materials and methods. Young rhesus monkeys *(Macaca mulatta)* of 1.5 to 2.4 kg body weight were employed. These animals were conditioned to laboratory environment for about one month and had not been exposed experimentally to enterotoxin previously.

Enterotoxin was fed by stomach tube in a volume of 50 ml of water and the animals observed for emesis continuously over a 5-hour period. Thorotrast, at a level of 3 ml per kg body weight, was injected intravenously 4 or 18 hours prior to the challenge with enterotoxin. Thorotrast alone did not provoke vomiting. Wherever possible control monkeys administered enterotoxin alone were used; they were of the same shipment as the experimental animals.

The enterotoxin preparations used were lyophilized samples which had previously been assayed in monkeys. In accordance with a recent proposal for the designation of staphylococcal enterotoxins(2), the naming of the preparations used has been changed from that employed in previous communications from this laboratory. Enterotoxin A (previously called 196E type) was of approximately 20% purity with an ED_{50} level of 50 μg per animal by the intragastric route. Enterotoxin B (formerly called S-6 type) was of estimated 95% purity with a 50% emetic dose of 20 μg per animal. Although the S-6 strain produces both Enterotoxins A and B, only the B remains at this stage of purification. These enterotoxins, while producing the same symptoms, do not cross-react serologically.

Results. Intravenous injection of Thorotrast 18 hr before intragastric challenge with enterotoxin increased the sensitivity of the monkeys to the emetic action of Enterotoxins A and B (Table I). The data represent totals obtained from several different tests using different shipments of animals. The dosage of Enterotoxin B required to provoke

* Supported by grant from U. S. Public Health Service, E-77.

† Testagar & Co., Inc., Detroit, Mich.