in relation to the size of young is also a factor to be considered.

The present results demonstrate that estrogen alone is inadequate to produce a substantial pubic separation and that relaxin plays a necessary role in interpubic ligament formation. It can also be concluded that the action of relaxin and estrogen on the interpubic ligament is similar in this species to that described in the mouse. Hall(9), Crelin(10) and Steinetz *et al*(11) suggest that relaxin in an estrogen environment may activate the catabolic enzyme systems of the osteoclasts and chondrocytes, thus accounting for the resorption of bone and for the swelling and transformation of hyaline cartilage to fibrocartilage. From the gross microscopic observations of the bat pubic ligament, it can be tentatively concluded that the proposed mechanism of action of relaxin appears to explain the observed results in this species.

Summary. A significant elongation of the interpubic ligament occurs in the pregnant little brown bat *(Myotis lucifugus)*. Examined immediately after delivery, the mean ligament length measured 2.01 as compared with 0.52 mm in the non-pregnant bat. Treatment of non-pregnant female bats with estradiol induced a mean pubic separation of 0.78 mm, while treatment with ECP and relaxin induced a mean separation of 1.03 and 1.25 mm with 5 and 10 GPU of relaxin, respectively. It is concluded that separation of the pubic symphysis with the elongation of the interpubic ligament occurs during pregnancy in the little brown bat and after treatment with ECP and relaxin.

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Received August 25, 1966. P.S.E.B.M., 1966, v123.

Influence of *E. coli* Endotoxin on Serotonin Contractions of the Rabbit Aortic Strip.* (31643)

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The mechanisms by which bacterial endotoxins disrupt peripheral vascular function remain largely conjectural(1). Among the explanations advanced for the shock-like collapse of the individual following endotoxin are a release of mediators such as histamine, serotonin, catecholamines, etc.(1), a direct biphasic action on vascular smooth muscle(2), an increased responsiveness to catecholamines(3), and a depressing action on myocardial muscle(2). Data based on *in vitro* experiments to support a direct action of bacterial endotoxins on smooth muscle are for the most part inconclusive(1).

The present report describes experiments with isolated strips of aortic smooth muscle

* This work was supported in part by a grant from Office of the Surgeon General, Dept. of the Army, Contract DA-49-007-MD-993.

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TABLE I. Increased Response of the Isolated Rabbit Aortic Strip to Serotonin After *E. coli* Endotoxin.

Serotonin* (μg/20 ml)	No. of strips	Endotoxin† (μg/20 ml)	% Potentiation Mean	Range	P‡
(.4-1.6)	11	100	18	7-43	<.05
(.4-1.6)	11	400	12	3-24	<.05

* Dose producing 50-60% maximal contractile response.
† All strips pre-exposed to *E. coli* endotoxin for 10 min prior to addition of serotonin.
‡ P = probability of differences occurring by chance according to Student's *t* test.

which show that bacterial endotoxin under *in vitro* conditions potentiates the contractile response of smooth muscle to serotonin (5HT).

Materials and methods. Batches of *E. coli* endotoxin obtained commercially (Difco) were tested for biological activity by their ability to elicit a modified Shwartzman reaction in the abdominal skin of rabbits when a combination of (50 μg) epinephrine and (50 μg) endotoxin was injected intradermally(4). The material used produced positive skin lesions in all of the 6 rabbits so treated.

Helical strips of rabbit thoracic aorta (3-4 mm wide and 35-40 mm long) were prepared, suspended in a 20 ml organ bath containing Krebs-bicarbonate Ringer solution maintained at 37°C, through which a gas mixture of 5% CO_2 and 95% O_2 was bubbled continuously, and reactivity was determined as described previously(5). Complete dose-response curves were established in each case for serotonin, epinephrine and norepinephrine. A concentration of serotonin, epinephrine and norepinephrine was then selected which produced approximately 50-60% of the maximal contractile response and this was used as the standard test dose for potentiation studies with endotoxin. The *E. coli* preparation was added to the bath solution and routinely left in contact with the aortic strip for 10 minutes prior to addition of a test dose of serotonin. Longer periods of exposure (up to 30 minutes) had no additional effect.

Results and discussion. When *E. coli* endotoxin was added to the bath in concentrations up to 800 μg per 20 ml, no observable effect could be detected in the basal tone of the aortic muscle strip. The endotoxin had no *in vitro* effect on the contractile responses to test doses of epinephrine and norepinephrine. Essentially similar results have been reported by Vargas and Beck(6).

In contrast, as seen in Table I, the contractile responses of the aortic strip to serotonin were potentiated after addition of *E. coli* endotoxin (100 μg and 400 μg) to the bath. The degree of potentiation was expressed as the percentage by which the contractile response was increased in relation to the control contractile height. When the muscle strip was exposed to *E. coli* endotoxin for longer than the standard 10-minute period (up to 30 minutes), there was no further increase in the potentiating effect.

The enhanced reactivity of aortic smooth muscle to serotonin following exposure to *E. coli* endotoxin was completely reversible; contractile responses returned to control levels when endotoxin was washed out of the bath.

Many investigators believe that the profound hemodynamic effects seen after injection of endotoxin leading to death in experimental animals are the result of an indirect action, mediated secondarily either by a pharmacological action or through an immune mechanism(1-3,7).

Evidently *E. coli* endotoxin has the capacity to potentiate the contractile response to serotonin in isolated arterial smooth muscle of the rabbit. Such evidence does not rule out an immunological basis for the response to endotoxins since antibody may be present on the smooth muscle cells or other constituents of the aorta. Inasmuch as the response to other contracting agents is not altered, the data point to a specific change in the effector muscle unit which may be a

contributing factor in the pathogenesis of endotoxin shock.

The authors acknowledge the able technical assistance of Mrs. F. Callahan.

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Received August 30, 1966. P.S.E.B.M., 1966, v123.

Ribonucleic Acid of Foot-and-Mouth Disease Virus: An Ultrasensitive Plaque Assay. (31644)

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In 1960(1) the writer carried out a detailed study on the preparation, stability and plating efficiency of ribonucleic acid (RNA) from relatively crude foot-and-mouth disease virus (FMDV). Other workers(2) have recently shown the importance of hypertonic salt solutions for obtaining higher titers of FMDV RNA. While these methods are useful, recent developments indicated that a highly improved procedure might be possible. One such development has been the production of pure FMDV and FMDV RNA(3) possessing well-characterized physical and chemical parameters(4,5). Progress has also been made in the use of additives to improve the plating efficiencies of some viruses and RNAs of the picorna group. For example, Takemoto and Fabisch(6) observed that polycations such as diethylaminoethyl-dextran (DEAE-dextran) enhanced the size and number of plaques of wild-type EMC virus by interfering with the absorption of virus to sulfated polysaccharides present in autoclaved agar. More recently, Pagano and Vaheri(7) obtained a 3- to 4-fold increase in poliovirus plaques in the presence of DEAE-dextran, but did not propose the same mechanism of enhancement as did Takemoto and Fabisch. More significantly, DEAE-dextran increased the sensitivity of the assay of poliovirus RNA by 20- to 100-fold. This enhancement was thought due to a protective action of DEAE-dextran on the RNA and possibly to a direct effect of DEAE-dextran on the host cell.

The present work was begun to increase the sensitivity of the plaque assay of FMDV RNA prepared from purified FMDV. Facilitators of the types described by Dubes and Klinger(8) were ineffective because of limited and non-reproducible enhancements (Bachrach, unpublished results). However, using DEAE-dextran, the increase in sensitivity was even greater than for poliovirus RNA(7). Enhancement of FMDV RNA infectivity ranged from 10^3- to 10^7-fold with good reproducibility in replicate titrations of any given lot of RNA. The number of plaques due to RNA in some experiments exceeded those due to virus by as much as 100-fold. Explanations for these striking results are discussed.

Materials and methods. Virus. Foot-and-mouth disease virus from cattle, type A119, was used after 1 passage in mice, 150 passages in calf-kidney (CK) cultures and one passage in a line of baby hamster kidney cells (BHK).* The BHK cell sheets were grown in rotating 2-liter Baxter bottles in tris (hydroxymethyl)-aminomethane-buffered cell growth medium(3). After infection and harvest, the virus was purified by methods de-

* BHK cells derived from cell line 21, clone 13, were obtained from the American Type Culture Collection Cell Repository, Rockville, Md.