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QUANTITATIVE EXPERIMENTS ON THE EFFECT OF ESTROGEN ON THE RESPONSE OF GUINEA PIG ILEUM AND UTERUS TO SPECIFIC ANTIGENS

By

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In 1960² we found that the excised uterine tissues from estrous mice are more sensitive to small amounts of some chemical mediators in the Schultz-Dale test than uteri from non-estrous mice. In this study it was also shown that uterine horns from actively sensitized mice in estrus give greater contraction on contact with the specific antigen.

In another study³ we showed that the uterine muscle from guinea pigs in estrus, tested in Tyrode's solution of half strength calcium concentration, serves very well for *in vitro* studies with chemical mediators.

Recently⁴ we investigated the possibility of obtaining uterine horns highly responsive to histamine and acetylcholine by inducing estrus in virgin guinea pigs with one injection of estrogen. In this study we also showed that the hormone has no direct effect on excised tissue to account for any enhancement of response.

The effect of the menstruation period on immediate skin reactions in patients with respiratory allergy was similarly investigated⁵. It was concluded that approximately 35 to 45 per cent of skin reactions showed a 1 ÷ difference during and out of the menstrual period, being more positive during menstruation. We also showed⁶ that uterine and ileum tissues of estrous guinea pigs displayed 15-20 per cent greater con-

tractility on contact with histamine than did those of non-estrous guinea pigs.

The aim of this paper is to study the effect of estrogen on the excised uterus and ileum of female and male guinea pigs to specific antigen, such as bacterial vaccine and crystalline egg albumin, in the Schultz-Dale test.

MATERIALS AND METHODS

1. Active sensitization of guinea pigs (Table 1).

a) Seventy guinea pigs (40 female and 30 male) were actively sensitized by multiple injection of Difco's crystalline egg albumin (CEA) plus Bacto adjuvant complete, Freund (Difco) at 3-day intervals for 25 days.

The antigen was prepared with 1 gram to 100 ml Tyrode's solution, so that 1 ml contained 10 mgm CEA. Twenty-one days after the last sensitization injection, a group of guinea pigs was selected and tested by intravenous injection of the antigen. Typical anaphylactic reactions were observed, including total shock accompanied by the expected drop in rectal temperature.

b) Seventy guinea pigs (40 female and 30 male) were also actively sensitized with bacterial vaccine* plus Bacto adjuvant complete, Freund (Difco). Sensitization was done by CEA schedule, each ml of bacterial vaccine containing 50 million pneumococcus I, II and III; 40 million streptococcus; 500 million staphylococcus; 60 million *M. catarrhalis*; 20 million *M. tetragenes*; 250 million *B. pyocyaneus*; 40 million *Klebsiella pneumoniae* (Friedländer); 40 million *H. influenzae* conservans and 0.4 per cent phenol. This bacterial vaccine was used after being diluted one to 10, and with an equal amount of Freund adjuvant (Difco).

2. Tyrode's solution was made each day by dissolving the following ingredients: NaCl 8 gm, CaCl_2 0.05 gm, KCl 0.2 gm, NaHCO_3 0.5 gm, MgCl_2 0.2 gm, NaH_2PO_4 0.000375 gm, and glucose 0.5 gm per liter of de-ionized distilled water. The pH was adjusted to 7.4.

3. Histamine; a stock solution of histamine dihydrochloride (obtained from E. Merck A. G., Darmstadt) was prepared by dissolving 165.8 mgm (equivalent to a 100 mgm histamine base) in 100 ml Tyrode's solution, which was stored at 4° C. Immediately before use for the tests 1.0 ml of this stock solution was diluted with 100 ml Tyrode's solution to give a concentration of 10 γ histamine base per ml.

4. Induction of estrus of female guinea pigs weighing 300–350 grams were put in estrus by a single intramuscular injection of 25 gamma depot estrogen**

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** Östrogenin (Diethylstil bestrol dipropionate) İbrahim Etem İlâç Fabrikası, İstanbul.

TABLE 1
The Number of Sensitized and Estrogen Treated Guinea Pigs.

140 guinea pigs studied							
80 female guinea pigs actively sensitized by:				60 male guinea pigs activity sensitized by:			
40 CEA		40 Bac. vac.		30 CEA		30 Bac. vac.	
Estrogen treated	Normal	Estrogen treated	Normal	Estrogen treated	Normal	Estrogen treated	Normal
estrous	20	estrous	20	15		15	
20		20					

in 0.2 ml olive oil. To be certain that estrus had been induced, vaginal smears (unstained) from all the animals were studied by the method recommended by Allen¹. The animals selected for study, after vaginal smear examination, were sacrificed, and their uterine horns were separated by midsection from the cervix. These were suspended in Tyrode's solution at pH 7.4 and 37° C, and oxygenation was maintained with 95 per cent O₂ and five per cent CO₂. Male guinea pigs were also subjected to estrogen in the same dose.

5. Operation of the physiograph: Before beginning each experiment, the instrument was calibrated, so that the weight of a metal mass (0.68 gr) would cause a deflection of the recording needles of exactly 30 mm. The two-channel physiograph was arranged to record changes in the contraction of smooth muscle tissue strips in the other bath simultaneously. The response of the muscle, if there was any, was always immediate; if no contraction occurred at once, a period of a few minutes was allowed to elapse before flushing out the test solutions and continuing the experiment.

RESULTS AND DISCUSSION

Experiment I

The experiment on the uterus and ileum of sensitized female guinea pigs with crystalline egg albumin:

Forty guinea pigs were included in this experiment, twenty of which were estrous and twenty non-estrous. The two horns of the uterus of these guinea pigs were suspended in two separate baths, while two pieces of the ileum were stored in another bath filled with Tyrode's solution at 37° C, and oxygenated until used.

TABLE 2

The Results of the Schultz-Dale Reaction on the Uterus of Guinea Pigs Sensitized with Crystalline Egg Albumin.

Experimental animals (sensitized with CEA)			Tested material	Response (muscle contraction) Deflection of recording pen in mm				
Number of guinea pigs	Tested organs	Condition		0	0-10	10-25	25-40	40-70
20	Uterus 40	Non-estrous	Histamine	16	6	2	8	8
20	40	Estrous	CEA	24	5	5	3	3
			Histamine	2	2	2	8	26
			CEA	3	2	3	4	28
	Ileum 40	Non-estrous	Histamine	—	—	—	—	40
			CEA	8	4	2	4	22
	40	Estrous	Histamine	—	—	—	—	40
			CEA	4	—	—	4	32

TABLE 3

The Results of S-D Reaction on the Excised Uterus of Guinea Pigs Sensitized with Bacterial Vaccine.

Experimental animals (sensitized with Bac. vac.)			Tested material	Response (muscle contraction) Deflection of recording pen in mm				
Number of guinea pigs	Tested organs	Condition		0	0-10	10-25	25-40	40-70
20	Uterus horn 40	Non-estrous	Histamine	16	4	8	6	6
			Bac. vac.	30	4	2	2	2
20	40	Estrous	Histamine	4	2	16	8	10
			Bac. vac.	20	2	2	8	8
	Ileum	Non-estrous	Histamine	—	1	1	—	18
10	20		Bac. vac.	10	5	—	3	2
	20	Estrous	Histamine	—	—	—	—	20
			Bac. vac.	7	2	1	6	4

TABLE 4

Summary: The Effect of Estrous on the Response of Guinea Pig Uterus and Ileum.

Guinea pigs (female)	Number of organ tested	Tested reagents					
		Histamine		CEA		Bac. vac.	
		Positive response %	Average con- traction mm	Positive response %	Average con- traction mm	Positive response %	Average con- traction mm
Sensitized G-P	Uterine	160	45.6	40.2	23.4	25	23.2
Sensitized G-P	Uterine horn						
Non-estrous	40	60	32	40	23.4	25	23
Estrous	40	95	45.6	90.2	46.5	50	37.2
Sensitized G-P	Ileum						
Non-estrous	40	100	70	80	43.5	50	23.2
Estrous	40	100	70	90	52.5	65	34

The responses obtained in the above experiments are summarized in Tables 2, 3 and 4, and demonstrated in Figs. 1, 2, 3, 4. Of the 40 uterine horns from 20 non-estrous guinea pigs sensitized with crystalline egg albumine, 24 (60 per cent) responded to histamine, and 16 (40 per cent) to crystalline egg albumin. The uterus gave only a slight response to these reagents, showing a needle deflection averaging 32 mm for histamine and 23.4 mm for crystalline egg albumin.

In contrast to this, the 40 uterine horns from 20 sensitized guinea-pigs in estrus responded much more actively. The reaction to histamine was 95 per cent and to crystalline egg albumin 90.2 per cent and the deflection of contraction averaged 45.6 mm and 46.5 mm respectively.

A similar study was then made using excised ileum pieces from the same guinea pigs as those used in Experiment I. As seen in Tables 2-4, of the 40 ileum pieces, all contracted with histamine to a deflection of the recording pens of 40 to 70 mm. The reaction to histamine was essentially the same in the ileum both of the estrous and non-estrous guinea pigs. Crystalline egg albumin caused contractions in 32 (80 per cent)

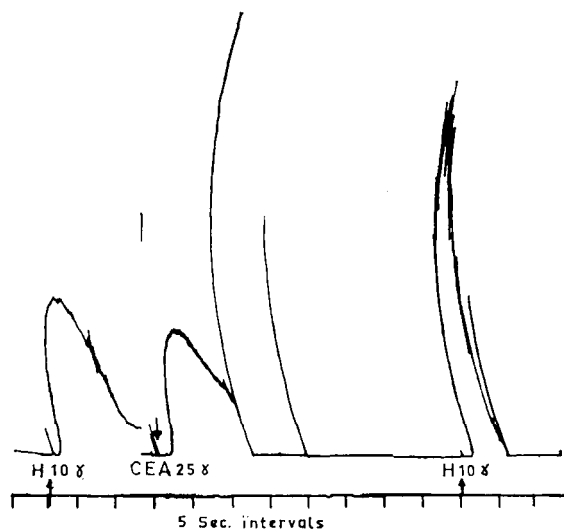


Fig. 1.

Response of uterus from crystalline egg albumin sensitized guinea pigs not in estrus.

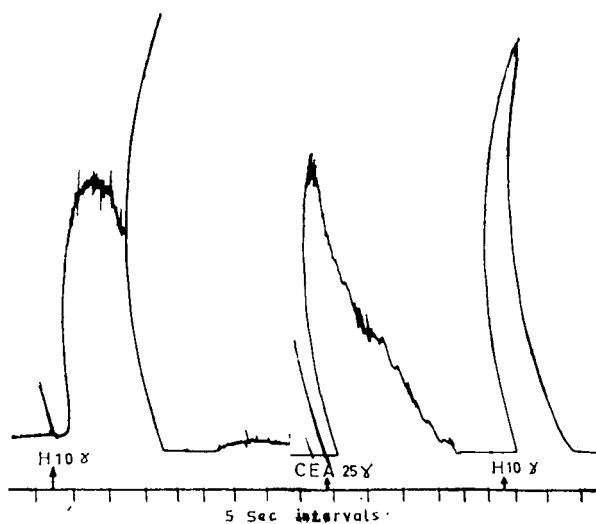


Fig. 2.

Response of uterus from crystalline egg albumin sensitized guinea pigs in estrus.

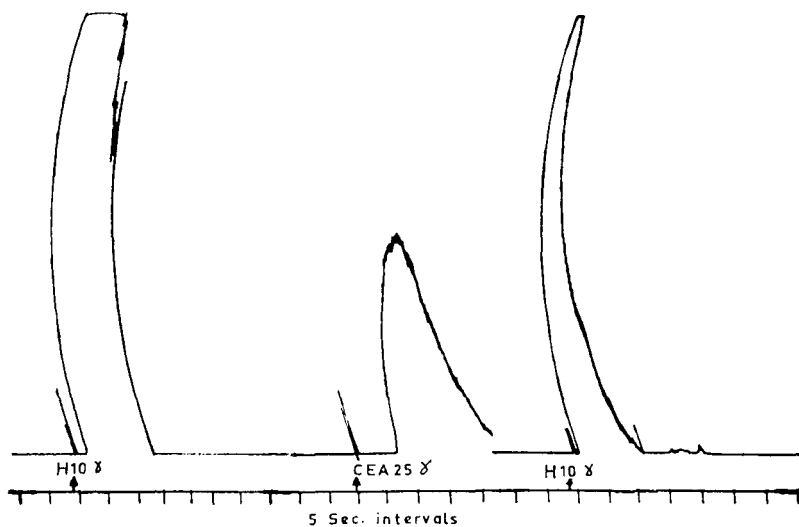


Fig. 3.

Response of ileum from crystalline egg albumin sensitized guinea pigs not in estrus.

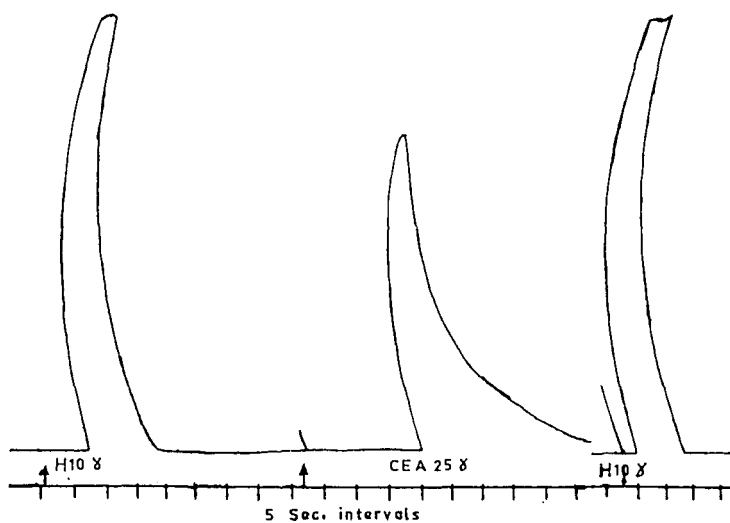
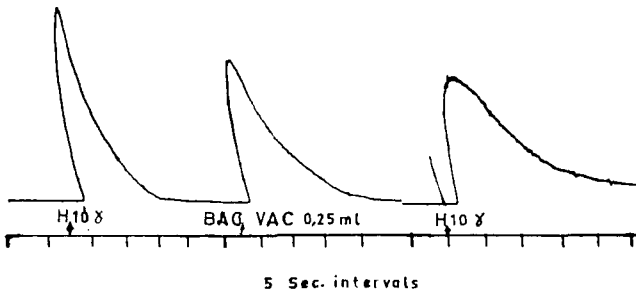
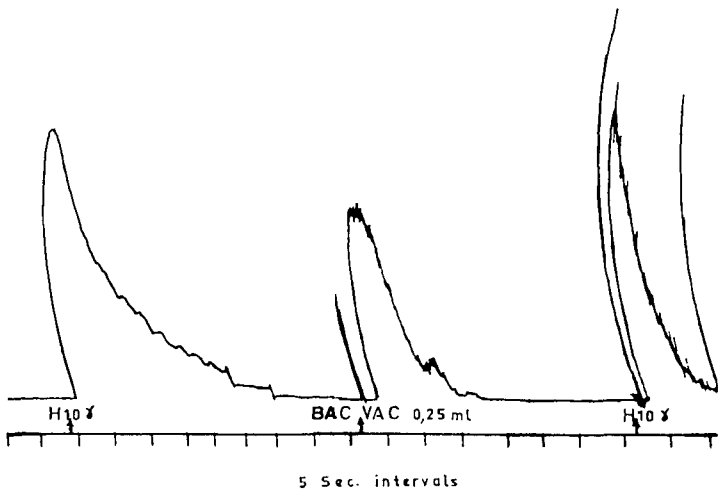


Fig. 4.

Response of ileum from crystalline egg albumin sensitized guinea pigs in estrus.

*Fig. 5.*

Response of uterus from bacterial vaccine sensitized guinea pigs not in estrus.

*Fig. 6.*

Response of uterus from bacterial vaccine sensitized guinea pigs in estrus.

of the guinea pigs not in estrus, and in 36 (90 per cent) of those in estrus, their average contractions being 43.5 mm and 52.5 mm respectively. Similar experiments with bacterial vaccine revealed that the uterine horn of guinea pigs not in estrus contracted in 10 (25 per cent), while of those in estrus 20 (50 per cent) contracted. The average contraction, given

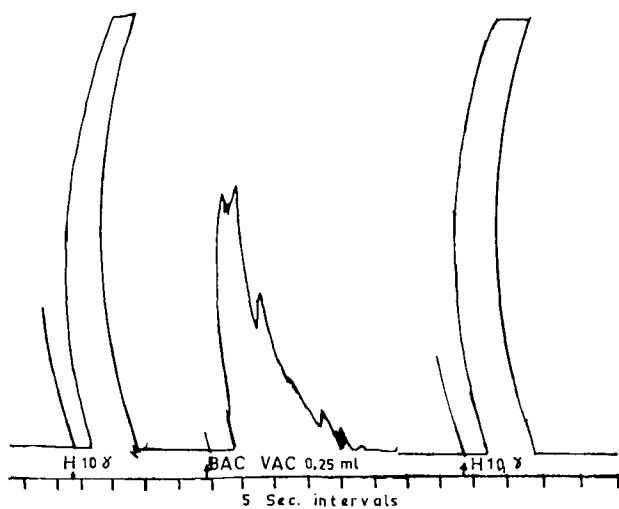


Fig. 7.

Response of uterus from bacterial vaccine sensitized guinea pigs not in estrus.

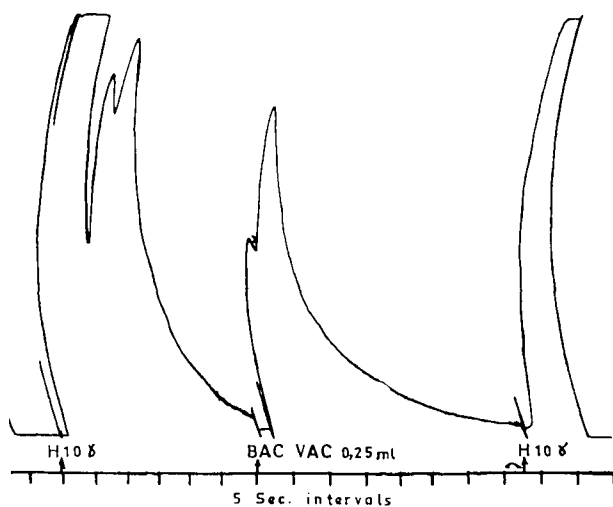


Fig. 8.

Response of uterus from bacterial vaccine sensitized guinea pigs in estrus.

TABLE 5

The Results of S-D Reaction on Excised Ileus of Guinea Pigs Sensitized with Crystalline Egg Albumin Antigen.

Experimental animals (sensitized with CEA)			Tested material	Response (muscle contraction) Deflection of recording pen in mm				
Number of guinea pigs	Tested organs	Condition		0	0-10	10-25	25-40	40-70
15	30	Non-estrogen- treated (control)	Histamine	-	-	-	-	30
			CEA	-	1	-	1	28
15	30	Estrogen treated	Histamine	-	-	-	-	30
			CEA	1	1	1	1	26

as mm deflection, was 23 mm and 37.2 mm. The ileum of these animals showed only a 15 per cent difference in positive response, and 11 mm in average contraction (Figs. 5, 6, 7, 8).

Experiment II

The findings from the results on the ileum of the estrogen-treated guinea pigs are summarized in Tables 5-6 and 7, and demonstrated in Figs. 9, 10, 11, 12.

Thirty sensitized normal male guinea pigs, and 30 sensitized guinea pigs treated with estrogen were used in this experiment. Histamine caused contractions of the ileum of all the guinea pigs both in and not in estrus, and the average needle deflection was 40-70 mm.

The ileum from those sensitized with CEA and treated with estrogen gave similar results in number and magnitude.

Similar experiment with bacterial vaccine showed few differences. Against a 27 (90 per cent) reaction of ileum from guinea pigs not treated with estrogen, 29 (96.6 per cent) reacted with estrogen treatment. Their average contractions were similar (45.1 mm and 45.2 mm).

TABLE 6

The Results of S-D Reaction on Excised Ileum of Male Guinea Pigs Sensitized with Bacterial Vaccine.

Experimental animals (sensitized with Bac. vac.)			Tested material	Response (muscle contraction) Deflection of recording pen in mm				
Number of guinea pigs	Tested organs	Condition		0	0-10	10-25	25-40	40-70
15	Ileum 30	Non-estrogen-treated (control)	Histamine	—	—	—	—	30
			Bac. vac.	3	2	2	4	19
15	30	Estrogen treated	Histamine	—	—	—	—	30
			Bac. vac.	1	4	1	2	22

TABLE 7

Summary: The Effect of Estrogen on the Response of Male Guinea Pig Ileum.

Guinea pigs (male)	Number of organs tested	Tested materials					
		Histamine		CEA		Bac. vac.	
		Positive response %	Average contraction mm	Positive response %	Average contraction mm	Positive response %	Average contraction mm
Sensitized G-P	Ileum						
Non-estrogen-treated	30	100	70	93.3	56.4	90	45.1
Estrogen-treated	30	100	70	90	57	96.6	45.2

In this experiment, using a standardized Shultz-Dale physiograph, it was possible to make a valid comparison of the relative degree of contractive response. These tests also allowed us to compare the tissue strips directly in terms of their length in millimeters and the excursion made by the recording needles.

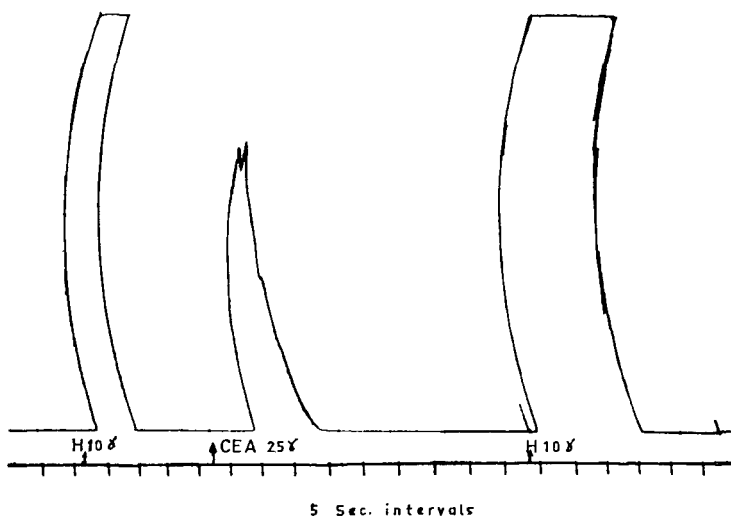


Fig. 9.

Response of ileum from crystalline egg albumin sensitized normal male guinea pigs.

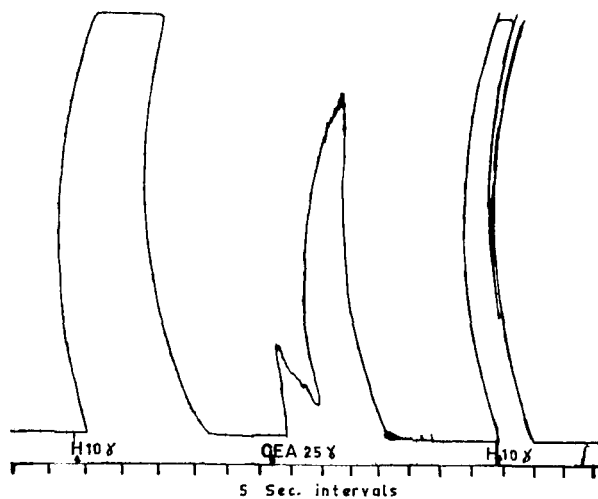


Fig. 10.

Response of ileum from crystalline egg albumin sensitized, estrogen treated male guinea pigs.

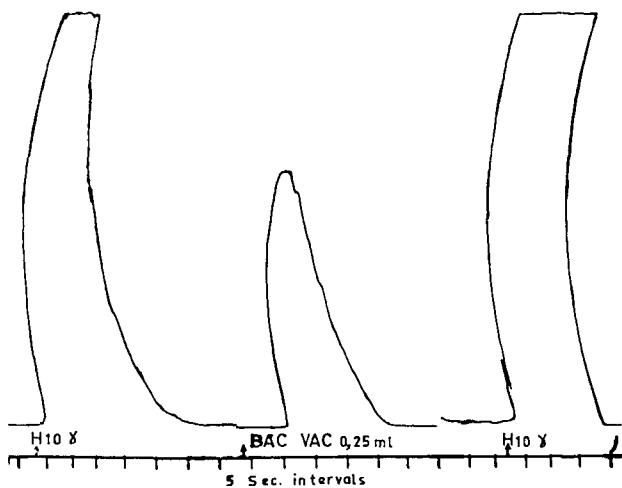


Fig. 11.

Response of ileum from crystalline egg albumin sensitized normal male guinea pigs.

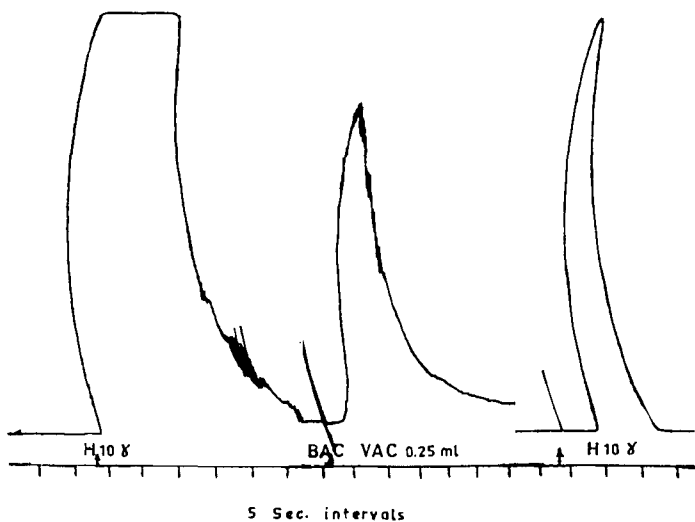


Fig. 12.

Response of ileum from crystalline egg albumin sensitized, estrogen treated male guinea pigs.

Using these measurements, the estrous guinea pig uterus was found to respond, on the average, with contractions about twice as great as the non-estrous uterus. This effect of the estrous state on the ileum was essentially the same when the test reagent was histamine, but in the case of sensitized crystalline egg albumin and bacterial vaccine there was some difference (see Table 4). About 10 per cent more ileum strips responded positively from estrous guinea pigs than from non-estrous one. The difference in the average contraction was also found to be 10–11 mm.

In estrogen treated male guinea pigs these differences were not at all significant (see Table 7). All of the ileum strips, whether from estrogen treated or non-treated guinea pigs, responded to histamine, and over 90 per cent of them reacted to specific antigens.

CONCLUSION

The effect of estrogen on the response of guinea pig ileum and uterus to specific antigens were investigated. The excised uterine tissues from sensitized estrous guinea pigs were significantly more sensitive to specific antigen, and the ileum strips taken from them displayed somewhat greater contractility on contact with the specific antigens than did those from similarly sensitized non-estrous guinea pigs.

Excised ileum strips of similarly sensitized and estrogen treated male guinea pigs revealed no significant difference in response from ileum strips taken from non-estrogen-treated male guinea pigs.

These comparisons between the relative sensitivity of uterine and ileum strips from estrous and non-estrous guinea pigs were made under strictly controlled conditions, made possible by the use of the physiograph, a sensitive electronic recorder, which permits a reliable quantitative evaluation of results.

The conclusion is that in Schultz-Dale tests both the female guinea pig ileum and the uterine tissue, show increased reactivity during estrous.

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