for most cholinesterase inhibitors the *in vitro* concentrations are much higher than those required *in vivo*, it would appear that even in the absence of physostigmine the local anesthetics should produce significant cholinesterase inhibition in the concentrations used *in vivo*. Following Greig's hypothesis, therefore, these agents, in the concentrations used should produce significant topical anesthesia. However, the validity of the comparison of quantitative data relating the cholinesterase of dog erythrocytes with that of rabbit cornea may be questionable. Nevertheless, in the light of the experimental data presented in this study and the above considerations, it is felt the probability of cholinesterase playing a role in the penetration of topical anesthetic agents is quite small.

Summary. In the present experiments, utilizing 1320 observations on the eyes of 44 rabbits, physostigmine, either topically or subcutaneously, did not alter the topical anesthetic activity of procaine. Subcutaneous administration of physostigmine or pilocarpine produced corneal anesthesia in the rabbit eye as measured by loss of corneal reflex. The role of cholinesterase inhibition in the penetration of non-surface anesthetics is discussed.

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Mammary Spreading Factor and Relaxin.* (18668)

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In a previous paper(1) data were presented indicating the presence of a factor (enzyme?) in the growing mammary gland of the rat which was believed to facilitate the forward advance of the duct and alveolar structures into the subcutaneous tissue in which the glands are located. This factor, which had spreading properties, was not identical with hyaluronidase.

Hamolsky and Sparrow(2) reported that relaxin synergized the action of estradiol and progesterone in stimulating mammary gland growth and Van der Meer(3) suggested that relaxin might act as a spreading factor. It seemed of interest to determine whether there might be a relation between the spreading factor present in the growing mammary gland during pregnancy and relaxin. If so it would aid in explaining the possible role of relaxin in mammary gland growth and further define the properties of the mammary gland spreading factor.

Methods and Materials. The relaxin used in these experiments was prepared as an aqueous extract of swine ovaries containing 75 guinea pig units per ml.† Extracts(1) of mammary glands from albino rats pregnant 8 to 14 days were employed to determine the ability of the mammary spreading factor to cause pelvic relaxation. Immature female guinea pigs (pretreated with estrogen) were employed as assay animals. Pelvic relaxation, used as the index of relaxin activity, was determined by palpating and floroscopically examining the guinea pigs preceding and 7 or 8 hours following injection of the test material.

Experimental and results. In testing the spreading properties of the relaxin sample eight portions of 0.2 ml were injected together with 0.1 ml of dye. The average area of spread was 231.0 sq mm. Testicular extracts and pH 6.0 buffer solutions used as controls

* Contribution from the Department of Dairy Husbandry, Mo. Agr. Exp. Station, Journal Series No. 1253. Approved by the Director of the Mo. Agri. Exp. Station.

1. Elliott, J. R., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 384.

2. Hamolsky, M., and Sparrow, R. C., PROC. SOC. EXP. BIOL. AND MED., 1945, v60, 8.

3. Van der Meer, C., *Acta Endocrinologia*, 1950, v4, 325.

† The relaxin used in this investigation was generously provided by Dr. R. L. Kroc, Chilcott Laboratories, Morris Plains, N. J.

TABLE I. Spreading Tests.*

Assay material	Initial bleb, mm	After 30 min., mm		Area of spread, sq mm
pH 6 buffer	14 × 14	16 × 17		59.7
	14 × 14	16 × 16		47.1
	14 × 14	16 × 16		47.1
	14 × 14	16 × 17		59.7
			Avg	53.4
Testicular extr.	13 × 14	24 × 24		309.6
	14 × 14	24 × 24		298.6
	14 × 14	25 × 25		317.4
	14 × 14	23 × 25		298.6
	14 × 14	24 × 24		298.6
			Avg	304.6
Relaxin†	14 × 14	21 × 22		210.6
	13 × 14	21 × 23		237.3
	14 × 14	21 × 22		210.6
	14 × 14	22 × 22		226.3
	13 × 14	21 × 22		221.6
	14 × 14	21.5 × 22		235.7
	14 × 14	22 × 23		245.1
	14 × 14	23 × 23		260.8
			Avg	231.0
Relaxin after 10 min. at 40°C‡	14 × 14	22 × 23		245.1
45°C	13.5 × 14	22 × 22		232.6
50°C	14 × 14	17 × 18		90.0
55°C	14 × 14	16 × 18		72.8

* Injections included 0.2 ml assay material + 0.1 cc dye.
† Releasin 31-81DSF-75GPU/ml.
‡ 5 samples used at each temperature.

gave areas of spread of 304.6 and 53.4 sq mm., respectively.

Tests were then made to determine if the spreading factor was heat labile. Ten portions of the relaxin sample, in two equal groups, heated to temperatures of 40°C and 45°C for 10 minutes showed the spreading factor still active while 2 other groups of 5 portions each showed no spreading activity after being heated to 50°C and 55°C for the same length of time (Table I).

Determination of the hyaluronidase content of relaxin was accomplished by assaying three portions turbidimetrically. Results of the assays indicated that in no case was the substrate, hyaluronidate, hydrolyzed by relaxin.

As the relaxin preparation indicated the presence of a heat labile spreading factor a portion of the sample was heated to 60°C for 10 minutes. After spreading type tests had shown the spreading factor to be inactivated, the ability of the heated portion to cause pelvic relaxation was determined. Ten pretreated guinea pigs were injected with 5.0 G.P.U. of the heated relaxin. Ten other pretreated guinea pigs were injected with 5.0 G.P.U. of the unheated sample. Apparently all or nearly all of the original activity was retained for the pelvic relaxation was as great in guinea pigs injected with heated portions of the relaxin preparation as in guinea pigs injected with the unheated solution.

Finally the mammary spreading factor was tested for its ability to cause pelvic relaxation. In all, 21 samples containing mammary spreading factor were tested. The first trial involved injection of 5 samples of 0.3 ml into 5 pretreated guinea pigs. In the second series of 5 samples the amount injected was raised to 0.5 ml while the amount injected in the last 11 trials was 1.0 ml. Examination of the injected guinea pigs showed little or no relaxation in response to all levels of the mammary spreading factor which were tested.

Discussion. While the experimental results show rather conclusively that the mammary spreading factor did not cause pelvic relaxation in the guinea pigs, the results obtained with the relaxin preparation are not so easily

evaluated. These data indicate the presence of a heat labile spreading factor in the relaxin preparation.

Heat inactivation (60°C for 10 minutes) of the spreading factor failed to diminish the ability of the relaxin preparation to cause pelvic relaxation. Kroc(4) employed even higher temperatures in investigating the heat resistance of relaxin. He found that extracts similar to those employed in these experiments retained all or nearly all their "relaxin" ability after being heated to 90°C-95°C for 10 minutes or autoclaving at 15 lb pressure for 15 minutes.

These data indicate that the mammary spreading factor and relaxin differ both in heat tolerance and biological activity. Further it appears that the spreading factor present in the relaxin preparation plays no part in the relaxation phenomena and that the hormone possessed little or no ability to increase dermal permeability. Therefore, it is probable that the ovarian extract spreading factor was merely present in the relaxin extract and had no connection with the hormone.

It seems possible that the relaxin extract employed by Hamolsky and Sparrow might contain a spreading factor similar to the one observed in this study. There are also possibilities that ovarian extracts of relaxin now in use might contain other biologically active components. Therefore, it is suggested that in future experiments care should be taken to determine whether the synergism of relaxin preparations with estrogen and progestin which produced increased growth of the mammary is due to relaxin, the spreading factor or an undetermined substance.

Summary. 1. It was observed that a relaxin preparation extracted from swine ovaries contained a spreading factor which was inactivated by heating at 50°C for ten minutes. The inactivation of the spreading factor did not diminish the effectiveness of the relaxin preparation to cause pelvic relaxation. 2. The spreading factor extracted from the mammary glands of pregnant rats had no ability to cause pelvic relaxation in the amounts employed.

4. Kroc, R. L., Personal Communication.

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Effect of Administration of Intermedin upon Melanin Content of the Skin of *Rana pipiens.* (18669)

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The relation of humoral factors, especially of the pars intermedia, in controlling the color responses of amphibia have been clearly elucidated by the investigations of Hogben, *et al.*, (1,2). Although the melanophore hormone* is also considered to be involved in melanin synthesis(4), evidence for its role in influencing the actual production of melanin is less direct. Odiorne(5) observed significant decreases in total numbers of melanophores and melanin granules in fundulus maintained for long periods on light backgrounds, as well as a reversal of this effect when the animals were placed on a black background. Sumner (6,7) measured changes in melanin content

* Although some doubt exists as to the identity of intermedin, the name given by Zondek(3) to the erythrophore-expanding substance of the pituitary, with the melanophore hormone, the two terms are used interchangeably.

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7. Sumner, F. B., *Biol. Bull.,* 1943, v84, 195.