Further Observations on the Anesthetization of Rabbit's Cornea by Non-Surface Anesthetics. (18667)

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Recently Greig and coworkers(1,2) have reported that the simultaneous or prior administration of topical physostigmine allowed procaine to become an effective topical anesthetic and they hypothecated that this effect was due to the inhibition of cholinesterase by the physostigmine. The theoretical and practical importance of this effect, coupled with the thought that the altered procaine response might be due to a local action of the physostigmine independent of its actions on cholinesterase prompted the present studies upon this phenomenon. On the basis of the Greig hypothesis, the altered procaine response should be independent of the route of administration of the physostigmine. The results obtained in an initial study in which physostigmine was given subcutaneously indicated the advisability of an attempt to repeat and extend the observations of Greig *et al.*(1).

Experimental. The rabbit's cornea *in vivo* was utilized for testing surface anesthesia, and the absence of corneal reflex after touching the cornea with a stiff hair was utilized as a criterion of anesthesia. The drugs (0.5 ml in the concentration and solvent listed in Table I) were instilled into the rabbit's eye and the eyelids held shut for 30 seconds. Subsequently the presence or absence of a corneal reflex was tested at intervals. In the course of these studies a total of 1320 observations were made on the eyes of 44 rabbits. In all cases the observers were unaware of the particular agents given to any one rabbit. In 504 of these observations on 18 rabbits by 11 individuals the observers were completely unaware of the nature of the experiments. The primary data are listed in Table I wherein a plus (+) indicates the presence of corneal anesthesia. The data were statistically analyzed utilizing the method of χ^2 to test the null hypothesis(3).

Results and discussion. The first experiments revealed that subcutaneous physostigmine alone (No. 5) caused significant corneal anesthesia when compared with the control (Nos. 1, 2, 3) ($p < 0.001$) and, further, that the presence or absence of procaine in conjunction with this treatment did not significantly alter the degree of corneal anesthesia (Table I—compare No. 5 vs. No. 6; $p > 0.99$). The presence of significant corneal anesthesia, as tested by this method, in the absence of a local anesthetic suggested further experiments in order to clarify this effect of physostigmine. Accordingly we have tested pilocarpine, a "true" parasympathomimetic agent, and strychnine, a cord stimulant. As may be noted in Table I, strychnine did not alter the action of procaine. Subcutaneous pilocarpine by itself (No. 7) exhibited a significant degree of corneal anesthesia when compared with the control situations (Nos. 1, 2, 3) ($p = 0.035$). As with physostigmine, procaine did not significantly alter the response (No. 7 versus No. 8) ($p = 0.31$).

In view of the above findings it was felt that it would be of value to repeat portions of the work reported by Greig *et al.*(1). In the first of these experiments 0.9% saline solution was utilized as the drug solvent. However, noting the deviations of the present results from those of the previous workers, the experiments were again repeated using water as the drug solvent. Pooling the results, and comparing water as a solvent in all situations (Table I—Nos. 2, 12, 13, 15) against 0.9% saline as a solvent in all situations (Table I—Nos. 3, 11, 14, 16) reveals that there is no significant difference between the solvents as far as the results of this experiment are concerned ($p = 0.99$). In view of this finding,

1. Greig, M. E., Holland, W. C., and Lindvig, P. E., *Brit. J. Pharmacol.*, 1950, v5, 461.

2. Greig, M. E., Holland, W. C. and Lindvig, P. E., *Fed. Proc.*, 1950, v9, 278.

3. Snedecor, G. W., *Statistical Methods*, Iowa State College Press, Ames, Iowa, 4th Ed., 1946.

TABLE I. Incidence of Corneal Anesthesia.

No.	Treatment	+*	—	% showing corneal anesthesia
1	Control (no drug)	1	41	2
2	Water	11	77	13
3	Saline 0.9%	18	92	16
4	Pontocaine 0.5% ophthalmic	37	29	56
5	Subcut. physostigmine 1 mg/kg	61	56	52
6	" " " + procaine (2% in saline)	63	54	54
7	Subcut. pilocarpine 5 mg/kg	13	35	27
8	" " " + procaine (2% in saline)	20	28	42
9	Subcut. strychnine .25 mg/kg	3	45	6
10	" " " + procaine (2% in saline)	4	44	8
11	Procaine (2% in saline)	8	100	7
12	" (2% in water)	8	58	12
13	Physostigmine (.1% in water)	2	64	3
14	" (.1% in saline)	3	90	3
15	" (.1% in water) + procaine (2% in water)	8	58	12
16	Physostigmine (.1% in saline) + procaine (2% in saline)	15	78	16
17	Zephiran 1:3500	3	45	6
18	" 1:3500 + procaine (2% in saline)	9	39	19
	Totals	287	1033	

* (+) indicates absence of corneal reflex.

the data have been regrouped by combining the results with the separate solvents in order to test the effect of the combination of topical physostigmine and procaine against the effects of topical procaine alone (Table I—Nos. 11, 12 vs. Nos. 15, 16) ($p = 0.34$). The result of this analysis reveals that the combination of topical physostigmine with procaine, in these experiments, does not produce a significant difference in corneal anesthesia. Further, Zephiran, an agent used quite commonly to increase the penetration of agents into the eye, does not alter the action of procaine (Table I —No. 17 vs. No. 18; $p = 0.165$). As a control measure, pontocaine was shown to exhibit surface anesthesia (Table I—No. 4 vs. all other situations; $p < 0.001$).

In a further study of the possible mechanism of the production of corneal anesthesia following subcutaneous administration of physostigmine and pilocarpine, the following supplemental observations were made on rats. Carbaminoylcholine produced a similar response and the effects of all three were blocked by atropine given either before the drug or after the corneal anesthesia had been produced. Epinephrine given subcutaneously did not produce corneal anesthesia, and adrenalectomy did not alter the response to physostigmine or pilocarpine. It appears that the corneal anesthesia noted following these autonomic drugs is related to their muscarinic effects.

The results of the present experiments, in which no demonstration of an action of physostigmine, either topically or systemically (Table I—compare No. 5 vs No. 6 and Nos. 15 and 16 vs Nos. 11 and 12), on the topical efficacy of procaine was noted, lead one to question the hypothesis put forward by Greig and her coworkers relating cholinesterase inhibition to the penetration of topical anesthetics. Further, upon examination of the data relating surface anesthesia to the cholinesterase inhibiting powers of the various local anesthetics on dog erythrocytes (Greig *et al.*) (1), it is apparent that the concentrations used *in vivo* are for procaine 53 times, orthoxine 70 times and mesidicaine 25 times the highest concentrations needed in the *in vitro* experiments to produce significant cholinesterase inhibition. In view of the fact that

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.

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