

13247

Estrogen Intranasally in Experimental Poliomyelitis.*

EDWIN W. SCHULTZ.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Aycock¹ has reported that castrated immature female monkeys given estrogenic hormone intramuscularly showed a higher level of resistance to intranasal inoculation with poliomyelitis virus than untreated castrates. His studies apparently were prompted in part by endocrinological observations showing that nasal mucosal changes are associated with the changes induced in the vaginal epithelium by estrogenic hormone under experimental conditions. Prominent among the observed genital responses is a thinning of the vaginal epithelium following castration and its restoration to normal with the administration of estrogenic hormone. However, so far as we are aware the nasal changes reported deal only with vascular responses in the region of the inferior and middle turbinates.² Possible changes in the olfactory portion of the nasal mucosa apparently have thus far not been considered.

Inasmuch as it has been established that in monkeys inoculated with poliomyelitis virus by the intranasal route the portal of entry is through the olfactory portion of the nasal mucosa, the results obtained by Aycock suggested the desirability of an experiment in which estrogen would be brought into direct contact with the olfactory mucosa for a number of days, immediately following which some of the animals would be tested for resistance to virus by the intranasal route and the olfactory mucosae of others would be examined histologically for possible anatomic responses.

Fifteen uncastrated female *Macacus rhesus* monkeys were given theelin in oil (10,000 International Units per cc solution[†]) by intranasal instillation, daily, as indicated in Table I. The instillations were made while the animals were under deep ether anesthesia and held in the fully inverted position in a special animal holder. They were made with a Luer syringe, to which was attached a 1-inch

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

¹ Aycock, W. L., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 573; *Am. J. Publ. Health*, 1937, **27**, 575; *Endocrinology*, 1940, **27**, 49.

² Mortimer, H., Wright, R. P., and Collip, J. B., *Canad. Med. Assn. J.*, 1936, **35**, 503.

[†] Supplied through the courtesy of Dr. Oliver Kamm, Parke Davis and Co.

needle with a blunt point. About 0.25 cc of the solution was introduced drop by drop, deep into each nasal passage. During the instillation the mouth of the animal was held open and the tongue pulled forward to reduce nasal breathing and discharge of fluid before it could gravitate to the olfactory area. The condition of the sexual skin was recorded at the time of the first instillation and each day thereafter during the course of the treatments. Animals which showed no appreciable redness and swelling of the sexual skin were recorded as —; those which showed maximal redness and swelling were recorded as ++++.

The animals used in the experiment fall into 3 groups: (1) a group of 8 which received instillations of theelin daily over periods ranging from 1 to 8 days. One animal out of the group was sacrificed each day to determine the onset of any histological change which might occur in the olfactory mucosa, (2) a group of 7 animals which received theelin once daily for 8 days, and which were then tested for resistance to intranasal infection. The resistance test consisted of 2 intranasal instillations of a 10% suspension of virus (MV strain) on successive days. These virus instillations were made with a bulb urethral syringe, while the animals were under ether anesthesia and held in the fully inverted position. The first instillation was made 1 day, the second 2 days following the 8th instillation of theelin, (3) a group of control animals, consisting of 6 females of about the same age and weight as the test animals. None of the group showed appreciable reddening or swelling of the sexual skin.

All of the animals, including the controls, were sacrificed by exsanguination and the tissues fixed by the perfusion method. In most of the animals Bouin's solution was used; in several Bodian's formula No. 2³ was substituted. The olfactory mucosa from both sides was removed, along with the corresponding olfactory nerve filia and bulbs. These parts were removed as one connected mass. After removal of the tissue, the parts were restored as nearly as possible to their normal relationship and put in fixing solution for 18-24 hours. The tissue was then dehydrated in graded alcohols, beginning with 35% solution and finally embedded in paraffin. It was sectioned through the coronal plane, so that each section presented 4 mucosal surfaces, the olfactory nerve filia and the olfactory bulbs. Part of the sections were stained with H and E, part of them by Bodian's method for neurofibrils.³

The results of this experiment are summarized in Table I.

³ Bodian, D., *Anat. Record*, 1936, **65**, 89; 1937, **69**, 153.

TABLE I.

Group 1.

Animals given 1 to 8 instillations of theelin, daily. One animal was sacrificed each day for histological observations.

Animal No.	Wt, lb	Sexual skin			Olfactory mucosa
		No. daily instillations of theelin	At beginning of treatment	End of treatment	
E 602	6¼	1	—	—	} No distinctive epithelial changes recognized
E 603	5¼	2	—	—	
E 604	4	3	—	—	
E 605	6¼	4	+	+++	
E 606	7¼	5	++++	++++	
E 607	6½	6	++	++++	
E 608	6	7	+	++++	
E 609	6	8	+	++++	

Group 2.

A group of animals given 8 instillations of theelin, daily, followed 1 and 2 days later by intranasal instillations of 10% suspension of virus.

Animal No.	Wt, lb	Sexual skin		Onset of poliomyelitis, days after 1st inoculation with virus	Time sacrificed, days after 1st inoculation with virus	State of sexual skin at time sacrificed	Olfactory mucosa
		Beginning of treatment	End of treatment				
E 610	5¾	+	++++	8	12	±	} No distinctive epithelial changes recognized
E 611	5½	+	++++	10	12	±	
E 612	6⅞	+++	++++	10	12	+	
E 613	5	+	++++	8	9	±	
E 614	6½	+	++++	8	12	—	
E 615	5¾	+	++++	9	10	±	
E 616	4⅞	+	+++	10	12	+	

Group 3.

Control group. Two instillations of virus as in group 2.

Monkey No.	Wt, lb	Sexual skin	Onset of poliomyelitis, days after 1st inoculation with virus	Olfactory mucosa
E 618	5⅞	—	8	These and other "normal" mucosæ served as basis for comparison
E 619	6	—	10	
E 620	5	—	8	
E 621	6	—	10	
E 624	5¼	—	10	
E 625	6¼	—	7	

It is apparent that under the conditions of this experiment theelin-treated animals showed about the same susceptibility to infection by the intranasal route as the untreated control animals. Whether a difference might have been demonstrated had the theelin treatments been continued over a longer period of time, or a lower concentration

of virus had been employed in testing the resistance to infection, is hard to say. There is, however, nothing in these results to suggest that this might have been the case.

Other than a slight degree of leucocytic infiltration the olfactory mucosae from theelin-treated animals showed no structural features which failed to fall in with variations met with in "normal" olfactory mucosae. While individual mucosae in Group 1 showed epithelia which were above average in thickness, others obtained from animals treated about as long with theelin, showed epithelia which were distinctly below average. There was no evidence of unusual proliferative activity. In 2 mucosae there was evidence of some antemortem detachment of epithelial cells. Whether there is normally some detachment of the olfactory epithelial cells with relation to the menstrual cycle remains a question. In a later paper, in which we shall deal with variations in the olfactory mucosae of normal monkeys, reference will be made to observations suggesting that the olfactory epithelium is not infrequently called upon to repair losses due to natural causes.

The group (Group 2) tested for resistance to poliomyelitis virus showed about the same degree of round cell infiltration along the olfactory pathway⁴ as was present in the untreated control group.

What impressed us most in the results of this experiment was the striking response of the sexual skin to the intranasal instillation of estrogen, confirming an already recognized fact that physiologically active drugs are readily absorbed from the nasal mucosa.

13248

Failure of Sulfanilamide in Treatment of Experimental Vaccinia Rabbits.

JOHN A. KOLMER AND HERMAN BROWN. (With the technical assistance of Anna M. Rule.)

From the Research Institute of Cutaneous Medicine, Philadelphia, Pa.

McCammon¹ reported that the early administration of sulfanilamide to 4 cases of benign smallpox appeared to have a beneficial

⁴ Sabin, A. B., and Olitsky, P. K., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 300; *J. A. M. A.*, 1937, **108**, 21.

¹ McCammon, W. O., *J. A. M. A.*, 1939, **112**, 1936.