9239 P

Tobacco Sensitization in Rats.

JOSEPH HARKAVY. (Introduced by Louis Gross.)

From the Laboratories of Mount Sinai Hospital, New York City.

Friedlander, Silbert and Laskey[1] reported the occurrence of gangrene of the toes in male albino rats injected daily with denicotinized tobacco by the intraäbdominal route. In order to elucidate the nature of these lesions it was deemed important to determine whether the tissues of the animals thus injected had been sensitized to tobacco. We therefore repeated their experiments and, having confirmed their observations, selected those animals in whom we had succeeded in reproducing the lesions described by Friedlander, *et al.*, for this investigation.

The present report deals with results obtained by testing intestinal strips of 6 injected animals and 5 controls.

Of the 6 treated animals one was injected with tobacco only; 4 with horse serum, eggwhite, and tobacco; and one with ragweed only. Injections were given daily until the toe-lesions appeared. This usually occurred in 6 to 10 weeks. After the appearance of gangrene, injections were stopped for about 10 days and the animal sacrificed for our experiments. The extracts used in injecting the animals represented a mixture of tobaccos prepared from the cured leaves of Burley, Maryland, Virginia and Xanthis tobaccos, denicotinized and extracted according to a technic previously described.[2] This extract contained 0.085 mg. N per cc. The ragweed-pollen extract contained 0.42 mg. N per cc.; the eggwhite 1.2 mg. N per cc. The horse serum was furnished by the Board of Health and used in undiluted form. The amount of the total material injected never exceeded one cc.

The animal to be studied was killed by a blow on the head and about one inch of the duodenum or intestine excised. This was suspended in a bath containing 75 cc. of oxygenated Tyrode's solution. When the movements of the intestinal strip had stabilized, the extract with which the animal had been injected was introduced into the bath, and its effect on the gut recorded.

The amount of tobacco-extract used was determined in each instance by preliminary standardization on the suspended intestinal

[1] Friedlander, M., Silbert, S., and Laskey, N., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 156.

[2] Harkavy, J., and Romanoff, A., *J. Allergy*, 1934, **6**, 56.

strip obtained from a normal rat. This enabled us to learn the effect of our tobacco extract on the excised intestine of normal rats and also to determine the optimal amount that we could use in studying the reaction of the tissue obtained from the injected animal.

No phenol was used in preparation of the test-extract, because we found that it had a paralyzing effect on the intestinal strip.

The preëstablished quantity of the tobacco-solution (usually between 0.2 and 0.5 cc.) was introduced into the bath containing the intestinal strip of the injected animal. In each one of the 5 tobacco-injected rats it caused a prompt contraction. After the preparation was washed, subsequent addition of the same quantity of tobacco-extract was without effect. This indicated desensitization of the gut. (Illustration.)

None of our 5 non-injected animals reacted to tobacco-extracts, even in doses of 1.6 cc. (Illustration.)

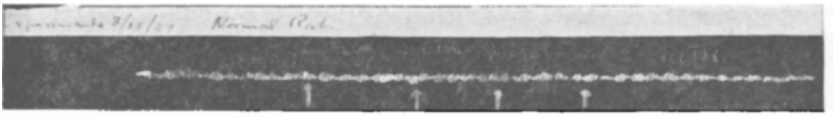

FIG. 1.

Negative response of intestinal strip of normal rat to repeated introduction of 0.2 cc. of tobacco extract. A total of .8 cc. of extract present in bath.

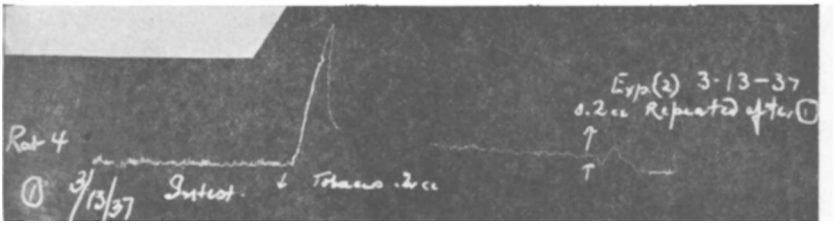

FIG. 2.

Exp. 1. Positive anaphylactic reaction of gut from rat 4, injected with denicotinized tobacco, following the introduction of .2 cc. of tobacco extract into bath.

Exp. 2. The same intestinal strip following the reaction in Exp. 1 giving a negative response to .2 cc. of tobacco extract indicating that the gut had been desensitized.

No evidence of sensitization to ragweed, horse serum, or eggwhite was obtained in animals 1, 2, 4, and 5, which had been injected with ragweed, horse serum and eggwhite in addition to tobacco. Animal 6, one of 3 injected with ragweed only, without having developed any lesions, when tested with tobacco and ragweed gave negative reactions to both. (Table I.)

These results indicate that 5 animals injected daily with tobacco

TABLE I.
Reactions of Intestinal Strip of Normal Rats and Rats Injected with Tobacco, Horse Serum, Eggwhite, and Ragweed.

Rat No.	Injected daily with	cc.	Toe lesions	Intestinal strip tested with	Results
1	a. Horse serum	0.5		0.1 cc. horse serum 1:100	Negative
	b. Tobacco	0.5	Positive	0.2 cc. tobacco*	Positive
2	a. Ragweed-pollen	0.5		0.3 cc. ragweed-pollen 0.42 mg. N per cc.	Negative
	b. Tobacco	0.5	"	0.2 cc. tobacco*	Positive
3	a. Tobacco	1.	"	0.5 cc. tobacco*	"
4	a. Horse serum	0.33		0.3 cc. horse serum 1:100	Negative
	b. Eggwhite	0.33	"	0.3 cc. eggwhite 0.1 mg. N per cc.	"
	c. Tobacco	0.33		0.2 cc. tobacco*	Positive
5	a. Horse serum	0.33	"	0.3 cc. horse serum 1:100	Negative
	b. Eggwhite	0.33		0.3 cc. eggwhite 0.1 mg. N per cc.	"
	c. Tobacco	0.33		0.2 cc. tobacco*	Positive
6	Ragweed-pollen	1.		0.3 cc. ragweed-pollen 0.42 mg. N per cc.	Negative
			Negative	0.4 cc. tobacco*	"
7-11	Non-injected		"	Tobacco	"

*Tobacco extract contained .085 mg. N per cc.

alone, or tobacco plus ragweed, horse serum or eggwhite, who had developed gangrenous lesions of the toes within 6 to 10 weeks, gave definite evidence of sensitization to tobacco but no sensitization to any of the other extracts used.

Our inability to demonstrate sensitization to the other injected substances such as ragweed, horse serum or eggwhite is not without precedent. With the exception of Arthus in 1903 and J. T. Parker and F. Parker in 1924, numerous investigators, including Longcope who used horse serum, have failed to sensitize rats.

Experiments involving passive transfer, anaphylactic shock and cross-desensitization are being carried out in order to elucidate further the nature of the reaction described.*

* I wish to express my appreciation to George Brooks for his technical assistance.