

Studies on Cigarette Smoke Irritation. II. The Role of Nicotine.

J. K. FINNEGAN, P. S. LARSON, AND H. B. HAAG.

From the Department of Pharmacology, Medical College of Virginia, Richmond.

That nicotine in the form of its free base is subjectively quite irritating has been known for many years. Thus Vandencorput¹ stated that the least touch of this substance in the mouth caused a burning sensation. Shortly thereafter, Braun² noted that bringing a drop of a solution containing one drop of nicotine per dram of water into contact with the eye of a rabbit seemed very painful to the animal. Similarly, Dworzak and Heinrich³ following self-experimentation with nicotine doses varying in amount from 1/32 drop to 1/16 drop in one dram of water, recorded that the smaller doses occasioned a burning sensation in the tongue, a hot, acrid irritation in the fauces, and when doses somewhat larger were used, the entire length of the esophagus felt as if it had been scraped by an iron instrument.

Hypodermically-injected nicotine also produces smarting at the site of injection,⁴ and inhaled nicotine vapors produce a "scratchy" sensation on the mucous membrane of the mouth and throat.⁵

Bogen⁶ explains this irritation action of nicotine alkaloid on the basis of the caustic effect of its alkalinity, and Mulinos and Osborne⁷ and Weatherby⁸ observed that neutral

solutions of nicotine were nonirritating.

Recently,⁹ we described a method for the quantitative evaluation of cigarette smoke irritation, as judged by its edema-producing properties, based on gravimetric determination of the increase in moisture content of the upper palpebral conjunctiva of the rabbit eye following its exposure to cigarette smoke. For clarity of subsequent presentation of results a brief description of the method follows:

Using a smoking machine of the type described by Bradford, Harlan and Hanmer,¹⁰ which draws a 35 ml puff once per minute, 3 puffs are directed through a suitable eye cup placed over the widely opened eye of a morphinized rabbit. When one eye only of each rabbit is exposed, the other serving as a control, we have named the technic "Procedure A." When both eyes of each rabbit are exposed, thereby permitting comparison of 2 cigarettes on the same animal, the technic is called "Procedure B." In both procedures, following exposure to the smoke, a one-hour period is allowed for edema formation. The rabbit is then sacrificed, the upper lid membrane of each eye excised, weighed, dried in an oven to constant weight, and reweighed. From the wet and dry weights the ratio of moisture to dry weight is calculated and, in the case of Procedure A, the per cent of increase in water in the exposed membrane over that in the control membrane is determined. In a series of such determinations involving 2 or more groups of cigarettes, the differences in the ratios between groups are evaluated statistically for significance by analysis of variance.

In the present study we have applied these procedures to an evaluation of the role of nicotine in cigarette smoke irritation, and, by

¹ Vandencorput, Ed., *Gaz. d. Hop.*, July 1, 1851; abstr. in *Am. J. Med. Sci.*, 1852, **23**, 552.

² Braun, H., *Arch. f. Ophthalmol.*, 1859, **5**, 112.

³ Dworzak and Heinrich, *Mat. Med. d. rein. chem. Pflanzenstoffe*, p. 234; quoted by Hare, H. A., *Fisk Fund Prize Dissertation*, 1885.

⁴ Johnston, L. M., *Lancet*, 1942, **2**, 742.

⁵ Biederbeck, J., *Inaugural Dissertation*, Wurzburg, F. Staudenraus, 24 pp., 1908.

⁶ Bogen, E., *Calif. and West. Med.*, 1936, **45**, 342.

⁷ Mulinos, M. G., and Osborne, R. L., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 241.

⁸ Weatherby, J. H., *J. Lab. and Clin. Med.*, 1939-40, **25**, 1199.

⁹ Finnegan, J. K., Fordham, Doris, Larson, P. S., and Haag, H. B., *J. Pharm. and Exp. Therap.*, 1947, **89**, 115.

¹⁰ Bradford, J., Harlan, W., and Hanmer, H. R., *Ind. Eng. Chem.*, 1936, **28**, 836.

TABLE I.
Effect of Increasing Nicotine Content of Tobacco on the Edema-Producing Properties of Its Smoke.

Cigarette Lot No.	Nicotine content of tobacco (%)	Nicotine content of smoke per cigarette* (mg)	No. of observations	Mean of ratio differences†	Mean of % increase in moisture
1	0.50	0.55	20	2.07	42.28
2	1.02	1.28	20	2.11	47.92
3	1.73	1.83	20	2.46	52.09
4	2.15	2.47	20	2.32	47.96
5	2.62	3.01	20	2.09	41.72

* Represents nicotine content of main stream smoke (that which passes through the mouth end of the cigarette). For these nicotine analyses, two-thirds of the total length of the cigarette was smoked at a rate of one 35 ml puff of 2 seconds duration per minute.¹²

† Ratios for exposed tissues minus ratios for control tissues.

TABLE II.
Results of Tests for Edema-Production by Nicotine Alkaloid.

Nicotine concentrations (M)	No. of observations	Mean of ratio differences*	Mean of % change in moisture
0.001	10	-0.20	-4.75
0.01	10	-0.06	-2.02
0.1	10	-0.15	-3.03

* Ratios for exposed tissues minus ratios for control tissues.

modification of the mode of administration, to an evaluation of the irritant action of water solutions of nicotine.

Experimental. Two sets of experiments were performed. In the first of these, a single batch of Burley tobacco,* naturally low in nicotine (ca 0.5%), was divided into 5 lots. One lot was untreated and the remaining 4 were treated successively with pre-selected increasing amounts of nicotine malate solution. Subsequent analyses by the AOAC method¹¹ showed that after this treatment the 5 lots ranged in nicotine content from about 0.5 to 2.5% in increments of approximately 0.5% (Table I).

For the irritation tests 20 cigarettes from each lot were selected on the basis of uniform weight ($\pm 5\%$), brought to uniform moisture content (11.5-12.5%) by exposure to an atmosphere of suitable humidity, and their smoke analyzed for edema-production

by Procedure A. The results are summarized in Table I.

In the second set of experiments, the edema-producing properties of nicotine alkaloid (0.001, 0.01 and 0.1 M in a 0.9% sodium chloride solution) were tested by Procedure A modified to the extent that 3 drops of solution instead of 3 puffs of smoke were held in contact with the tissues of the eye for a period of 3 minutes. Ten rabbits were used at each of the above 3 concentrations of nicotine. The results are summarized in Table II.

Discussion. Statistical analysis of the results summarized in Table I show that no significant differences exist between the edema-producing properties of the smoke from the 5 lots of cigarettes tested. It may be noted that the greatest mean difference in moisture to dry weight ratios resulted between the tissues exposed to lots 1 and 3 and 3 and 5, yet the probability of encountering a more divergent sampling was .45 and .51 respectively. Therefore, on the basis of these studies, it may be concluded that neither nicotine nor its combustion products contribute significantly *per se* to the edema-producing irritants in cigarette smoke.

* Kindly supplied to us by Dr. W. D. Valleau, University of Kentucky Agricultural Experiment Station, Lexington, Ky.

¹¹ Assn. Official Agri. Chem., *Official and Tentative Methods of Analysis*, 5th ed., p. 64, 1940.

¹² Bradford, J. A., Harlow, E. S., Harlan, W. R., and Hanmer, H. R., *Ind. Eng. Chem.*, 1937, **29**, 45.

This conclusion does not hold for the subjective sensations of irritation produced by cigarette smoke. In blind tests, the average smoker had no difficulty in arranging these cigarettes in the order of increasing nicotine content on the basis of increasing irritation of the smoke.

The results obtained with nicotine solutions were quite similar. As seen in Table II, edema failed to result at any of the nicotine concentrations used. Tests at higher concentrations were not completed owing to the appearance of rather severe symptoms of systemic nicotine poisoning.

Again the lack of edema production bore no relation to the evidence of subjective irritation. Even though the rabbits were well depressed by morphine, the struggling that followed application of the higher concentrations of nicotine indicated a severe subjective irritation far exceeding that which in our experience results from application of smoke in amounts sufficient to produce massive edemas.

This lack of edema formation following

¹³ Bardy, H., *Skand. Arch. Physiol.*, 1915, **32**, 198.

nicotine application brings to mind the observation by Bardy¹³ that local application of nicotine to the eye of the rabbit will block further production of conjunctivitis on administration of mustard oil. From this and other evidence, Bardy arrived at the conclusion that conjunctivitis produced by mustard oil was reflex in character and that nicotine interrupts the reflex path through acting on peripheral synapses. Carrying this argument further could lead to the postulate that nicotine tends in this manner to inhibit its own production of conjunctivitis. However this may be, it seems certain that while nicotine may markedly influence the subjective sensations of irritation from cigarette smoke it does not significantly contribute *per se* to its edema-producing properties.

Conclusions. Neither nicotine nor its products of combustion contribute significantly *per se* to the edema-producing properties of cigarette smoke, although it may definitely increase the subjective sensations of irritation.

We are indebted to Mrs. Sarah Guerry for technical assistance in performing the reported experiments.

15910

Studies on "Vitamin P." I. Topically Applied "Vitamin P"-like Substances on the Mammalian Capillary Bed.*

THOMAS J. HALEY, WILLIAM G. CLARK,[†] AND T. A. GEISSMAN.

From the Department of Pharmacology, School of Medicine, University of Southern California, and the Department of Chemistry, University of California at Los Angeles.

Since the introduction of the concept of "vitamin P" by Szent-Györgyi and his collaborators,¹ an extensive and controversial

literature has appeared on the subject.² Recently, interest has been reawakened in the subject by the observations of Griffith, Couch and Lindauer³ that rutin, a flavonol glycoside, decreases capillary fragility in hypertensive patients; and by the work of Lavollay, Par-

* Supported in part by grants from U. S. Public Health Service, and Hoffmann-La Roche, Inc., Nutley, N.J., to the second author.

[†] Present address: The Scripps Metabolic Clinic, La Jolla, Calif.

¹ Armentano, L., Bensáth, A., Bères, T., Rusznyak, I., and Szent-Györgyi, A., *Dtsch. med. Wchschr.*, 1936, **62**, 1326.

² Anon., "Bibliography of Vitamin P" (256 refs.), *Nutrition Research*, 1945, **5**, No. 1 and 2.

³ Griffith, J. Q., Jr., Couch, J. F., and Lindauer, M. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1944, **55**, 228.