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Alleged Influence of Glycerine and Diethylene Glycol upon the Irritating Qualities of Cigarette Smoke.

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Mulinos and Osborne[1] have reported that the addition of small amounts of glycerine (1 to 5%) to cigarette tobacco definitely increases the irritation by the smoke, while the addition of similar amounts of diethylene glycol markedly decreases this irritation. For criteria they used the degree and duration of edema and redness produced in the mucous membrane of the rabbit eye after instilling into the conjunctival sac water presumably saturated with cigarette smoke. This method is not quantitative. Flinn[2] has since reported that in patients suffering from irritation associated with the smoking of cigarettes (coughing, irritation of the tongue, congested pharynx and larynx) such conditions were cured or improved by smoking cigarettes containing diethylene glycol. Prompt return of the throat congestion occurred in 80% of the cases when glycerine was substituted for diethylene glycol, and in nearly all cases the tongue condition returned with this change. This method is also not quantitative.

It is known that within physiological limits irritation of the mucous membranes of the mouth gives rise to increased salivation. Tobacco smoke is an irritant known to stimulate salivary flow. If glycerine added to tobacco increases the irritating properties of the smoke, one would expect a greater flow of saliva from the smoking of tobacco treated with this hygroscopic agent, and if diethylene glycol markedly decreases the irritative properties, the reverse would be expected. With these considerations in mind, experiments were undertaken to measure the salivary responses of 28 persons (26 men, 2 women). This method of measuring the degree of irritation caused by smoke was employed because the buccal cavity is normally the place of entrance of tobacco smoke, and because it gives an objective quantitative measurement.

Thirteen one-hour tests were conducted upon each human subject; the first one acquainted the person with the procedure, but was not included in the final calculations. Each experiment consisted of 4 parts in this order: (1) 13 minutes of light reading while sitting in

1 Mulinos and Osborne, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 241.

2 Flinn, *Laryngoscope*, 1935, **45**, 149.

a comfortable chair, at the end of which time any accumulation of saliva was removed from the mouth by a Dewitt dental saliva ejector and discarded; (2) 15 minutes of similar reading while saliva was being collected; (3) 15 minutes of puffing through a short resin or glass cigarette holder, each draw lasting 2 seconds and each exhalation about the same, a puff every half-minute, while in the interim the saliva was being collected continuously; (4) a final 15 minutes the same as under (2). The removal of saliva at the end of part (1) served also to moisten the inner walls of the collecting tube, insuring the smooth flow of saliva from the mouth to the 30 cc. graduated centrifuge tube. At all times during the collection the subject sat with head inclined forwards at an angle of about 45° so that the saliva would flow to the point of insertion of the ejector.

The apparatus used in these experiments of the salivary ejector connected with a short piece of pressure tubing with the measuring tube; suction was provided by a water suction pump, and a trap was inserted to catch any back-flow of water. The ejector and the cigarette holder were cleaned with boiling water between tests.

The tests upon each subject were run at the same time of day and under quiet conditions. It was agreed upon that there would be no smoking on the day of a test before the trial. There was no knowledge on the part of subject or experimenters as to the contents of the cigarettes. If during a test there occurred any mishaps, the results of that trial were discarded and another test substituted on another day. The 3 kinds of cigarettes used were made from the same tobacco, but one contained only tobacco, a second tobacco containing 2.61% of glycerine, and the third tobacco with 2.24% diethylene glycol, according to determinations made by the Miner Laboratories.

We classified the subjects into 3 groups based upon the amount of tobacco they were accustomed to smoke under ordinary circumstances. There were about equal numbers of non-smokers, of light and moderate, and of heavy smokers; an explanation of the latter 3 terms is given in Table I.

The average responses of the 28 subjects to the 3 kinds of cigarettes agreed very closely, so that there was not the slightest indication that one cigarette was more irritating than another. That the smoke did stimulate salivation is seen in the fact that smoking evoked an average of 50% greater flow of saliva than the puffing of air. Considering the averages for each of the 3 sub-groups, the variations are naturally greater, but in none are there significant differences in the response to the 3 tobaccos; very large variations in resting and in stimulated saliva are seen among individual subjects.

TABLE I.

Cubic Centimeters of Saliva Collected from Human Beings During Smoking Tests. Each Figure Represents the Average of 3 Fifteen-Minute Periods.

Subject	Pre-control	Air	Post-control	Pre-control	Glyc. tobacco	Post-control	Pre-control	Tobacco	Post-control	Pre-control	Diet.-Glyc. tobacco	Post-control
						Non-Smokers.						
WmL	2.5	4.0	3.5	3.1	14.0	3.3	2.7	13.6	4.2	3.9	15.1	5.4
YO	3.9	6.4	4.2	4.5	10.8	5.7	3.3	10.5	4.0	3.4	9.1	4.2
EB	4.0	2.8	3.5	2.9	2.4	2.4	2.7	3.6	2.3	3.1	3.4	3.4
JM	12.5	14.7	11.8	12.9	19.1	12.6	7.5	15.2	9.5	9.5	18.6	10.9
JD	8.7	10.3	10.0	7.9	15.5	7.9	7.5	14.6	7.7	7.4	13.2	7.7
HH	1.6	1.6	1.2	1.6	3.3	2.0	1.3	2.2	1.3	1.3	3.5	1.6
FB	18.8	21.3	19.5	15.5	21.7	18.6	16.4	20.6	20.0	18.8	24.1	23.1
FO*	11.1	12.7	10.1	8.8	15.1	9.0	9.3	18.6	10.2	9.6	17.1	9.7
SM	8.3	8.0	5.9	6.5	14.3	9.2	4.3	12.5	7.5	6.5	10.8	7.2
S*	0.5	3.6	0.9	1.0	8.1	1.1	0.0	9.0	1.2	0.7	10.0	1.1
SS	6.4	7.7	7.6	5.1	8.7	8.3	4.3	7.9	8.1	6.2	9.4	7.4
Sum	76.1	92.9	78.2	89.6	133.0	80.1	59.5	128.3	74.0	70.4	134.3	81.7
Aver.	6.9	8.4	7.1	6.3	12.1	7.4	5.4	11.7	6.7	6.4	12.2	7.4
					Light Smokers (1-5 cigarettes or equivalent daily), first 3 subjects. Moderate Smokers (6-11 cigarettes or equivalent daily), next 5 subjects.							
PB	2.0	3.5	3.4	2.5	14.3	4.9	1.4	13.4	3.6	2.1	13.2	3.7
HMc	8.0	12.6	7.7	8.7	22.2	11.5	9.9	29.2	12.9	10.2	26.6	14.6
RS	6.8	10.6	8.6	7.5	16.9	9.4	7.5	19.3	9.1	8.1	17.7	10.0
HS	14.5	15.5	13.0	15.3	28.7	12.6	13.1	28.7	13.3	13.8	27.7	11.9
NC	5.6	9.2	5.1	5.9	11.9	5.5	4.7	9.4	5.4	5.2	8.9	4.3
JP	10.0	12.9	9.6	9.0	17.5	12.0	14.0	20.8	16.2	10.5	19.7	13.4
NM	17.0	22.9	20.7	19.0	28.0	24.3	17.3	23.8	22.1	12.8	20.3	17.8
AM	1.8	4.2	3.0	2.9	11.7	4.4	2.6	13.5	4.1	1.8	12.1	3.8
Sum	65.6	91.4	71.1	70.8	151.2	84.6	70.5	158.1	86.7	64.5	146.2	79.5
Aver.	8.2	11.4	8.9	8.9	19.9	10.6	8.8	19.8	10.8	8.1	18.3	9.9

	Heavy Smokers (12 or more cigarettes daily or equivalent)											
DM	8.1	9.2	5.5	7.3	17.8	7.2	4.4	14.3	6.1	6.6	19.8	10.1
ST	4.7	6.6	4.2	4.2	7.0	6.3	6.0	8.9	4.3	5.9	8.2	4.5
AK	12.5	18.2	14.8	12.7	22.0	15.4	12.0	21.3	16.4	12.6	23.4	16.0
CS	5.4	7.8	5.4	2.4	9.3	5.8	4.7	9.8	5.4	5.2	8.9	4.3
WmS	4.7	5.4	4.1	2.5	5.0	1.7	5.2	6.5	3.6	1.7	2.0	0.6
EE	4.2	12.3	7.6	5.3	10.3	4.1	8.3	14.1	10.4	5.5	11.4	6.0
ChR	4.3	4.7	5.6	5.2	11.9	5.9	5.3	12.0	4.9	5.0	10.7	5.1
HB	1.0	1.7	0.8	0.6	8.1	1.6	0.4	7.6	1.3	0.7	7.5	1.7
LM	4.2	8.3	5.0	3.1	10.5	4.4	2.0	8.8	3.8	3.1	11.1	5.1
Sum	49.1	74.2	53.0	43.3	101.9	52.4	48.3	103.3	56.2	46.3	103.0	53.4
Aver.	5.5	8.2	5.9	4.8	11.3	5.8	5.4	11.5	6.2	5.1	11.4	5.9
Total Sum	190.8	258.5	202.3	183.7	386.1	217.1	178.3	389.7	216.9	181.2	383.5	214.6
Total Aver.	6.8	9.2	7.2	6.6	13.8	7.8	6.4	13.9	7.7	6.4	13.7	7.7

* Women.

In 8 of these people the glycerine-treated cigarettes evoked the greatest average response (stimulated saliva minus pre-control saliva), 12 times the cigarettes containing tobacco alone caused the most salivation, and in 8 persons the highest response followed the smoking of diethylene glycol-treated cigarettes. Here again there is no evidence that either of the treated cigarettes was more irritating than the other.

In order to determine to what extent these cigarettes are capable of classification as to irritating qualities in ordinary smoking we distributed among 29 students and research workers the 3 kinds of cigarettes previously described. Every subject received 3 envelopes containing 3 of the same kind of cigarette in each and was told to classify the smokes according to the 5 headings given in Table II.

TABLE II.

Classification of cigarettes based on the opinions of 29 subjects These persons tried each kind of cigarettes thrice but had no knowledge of the contents of the cigarettes. G—glycerine treated cigarettes. D—diethylene glycol treated cigarettes. T—cigarettes with tobacco only.

Name	Very Irritating	Irritating	Medium	Mild	Very Mild
JD		G T	G T	D D D	G T
AM		T T D	G G G T D D		
HS		G G T D D	G T T D		
YO		G G T D D D	G T T		
WmS		G G T	G T T D	D D	
DM		T D	G T D	G G T D	
JM		T T	G G T D D D	G	
MrsM		G T D	T T D D	G G	
NC	D D	D	T	G G T T	G
SS	G G	G T D D	T T D		
HMc		G T T D D	D	G G T	
HBr	T T D D	G G T D	G		
ChR		G G T	G T T D	D D	
SSm	G G G D	D D	T T T		
Fi		G	G G	D D D	T T T
MrsS	G G T T D D T	G D			
FS		G T	G G T D	T D D	
DeC	T	G T T	G D D	G D	
NM		G T D	G G T T D D		
EE		D D	G T T D	G G T	
WmL		G T T T	G G	D D	D
MissO	D D D	G T T T	G G		
Sch	G	T T T		G G D D D	
OL	D	D D		G T T	G G T
RW		G G T D G	D D	T T	
AM		G G G D D	T T T D		
LB		G	G G T D	T T D	D
CaH		G T D	G T	G T D	D
JSch		G G G D D	T T T D		
Summary:	%	%	%	%	%
Glyc.	8 (9)	32 (37)	27 (31)	16 (18)	4 (5)
Tob.	6 (7)	30 (34)	33 (38)	13 (15)	5 (6)
Dieth. glycol.	11 (13)	28 (32)	24 (28)	21 (24)	3 (4)

They were to be smoked in the manner customary to each individual and the subject wrote his opinion on the envelope immediately following the completion of the smoking. He or she did not look at the previous classification on the envelope before smoking, so that the opinion on every cigarette was quite uninfluenced. In these experiments also, neither subjects nor distributors knew which cigarette contained glycerine and which contained diethylene glycol; as in the preceding experiments all the results were at hand before the experimenter knew the composition of the cigarettes. The data are summarized in Table II.

Our data give no indication that cigarettes can be classified consistently as to the irritating quality of the smoke by supposedly normal humans, although Flinn's report suggests patients with various afflictions due to smoking are able to judge differences in cigarettes similar in nature to ours. In many cases the same kind of cigarette was at one time called mild and at a subsequent period pronounced irritating by the same person. We believe, therefore, that a method for determining the irritating properties of cigarettes which relies solely upon the opinions of ordinary smokers cannot be considered reliable.

The smoke of these 3 types of cigarettes increases the acidity of water to an equal extent, as determined by exact pH tests.

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Response of Anterior Pituitary of Immature Castrated Rat to Testosterone and Related Compounds.*

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It has been reported that injections of extracts containing the male sex hormone prevented the post-castration increase in the size and number of the basophilic cells in the anterior hypophysis of the rat.[1, 2] In later studies, Nelson and Gallagher[3] studied the action

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1 Reese, J. D., and McQueen-Williams, M., *Am. J. Physiol.*, 1932, **101**, 239.

2 Nelson, W. O., and Gallagher, T. F., *Anat. Rec.*, 1935, **64**, 129.

3 Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.