adrenalin. To date we have been able to demonstrate sphincter relaxation with so small a dose as 1-400,000,000 and a sphincter contracture with the minute dose of 1-1,000,000,000 of histamine. We suggest the study of the iris sphincter strip as a probable tissue for biological assay as well as its adoption in pharmacological laboratory teaching.

7630 C

Pharmacology of Inflammation: III. Influence of Hygroscopic Agents on Irritation from Cigarette Smoke.*

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We herewith report a successful attempt to measure objectively the irritant properties of cigarette smoke. We used the conjunctival sac of rabbits according to the technic of Hirschhorn and Mulinos.[1] In Fig. 1, smoke from the burning cigarette which is protected from drafts by a jacket *h,* is passed by way of the ammonia tube *g,* through 3 cc. of water, saline or Ringer solution, at room temperature (21 to 30°C.), and at 37.5°C. maintained by artificial means, by immersion of cylinder *f,* in a water bath. A few experiments were performed using light mineral oil as a solvent for the smoke. By means of a filter pump, tube *e* sucks air through the cigarette and through the funnel-stopcock system *b.* The tipping bucket *a* empties water into the funnel, temporarily preventing ingress of air. This sucks air first through the cigarette and then the solution. The water drops to the bottom of the cylinder and leaves through tube *d,* by gravity. When the tube *b,* becomes free from water, all the air sucked through the system goes through this tube, and none through the cigarette. This cycle yields a puff, 100 of which averaged 24.8 cc. with the limits of 21 to 28 cc., and which requires 15 seconds to complete, 4 seconds of which are taken up by suction through the cigarette. It requires 15 minutes or 60 puffs to smoke one cigarette. Through each 3 cc. of solution is drawn the smoke from 5 cigarettes in order to insure saturation.

* This research was made possible through a grant by Philip Morris & Co., Ltd., Inc.

[1] Hirschhorn and Mulinos, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 168.

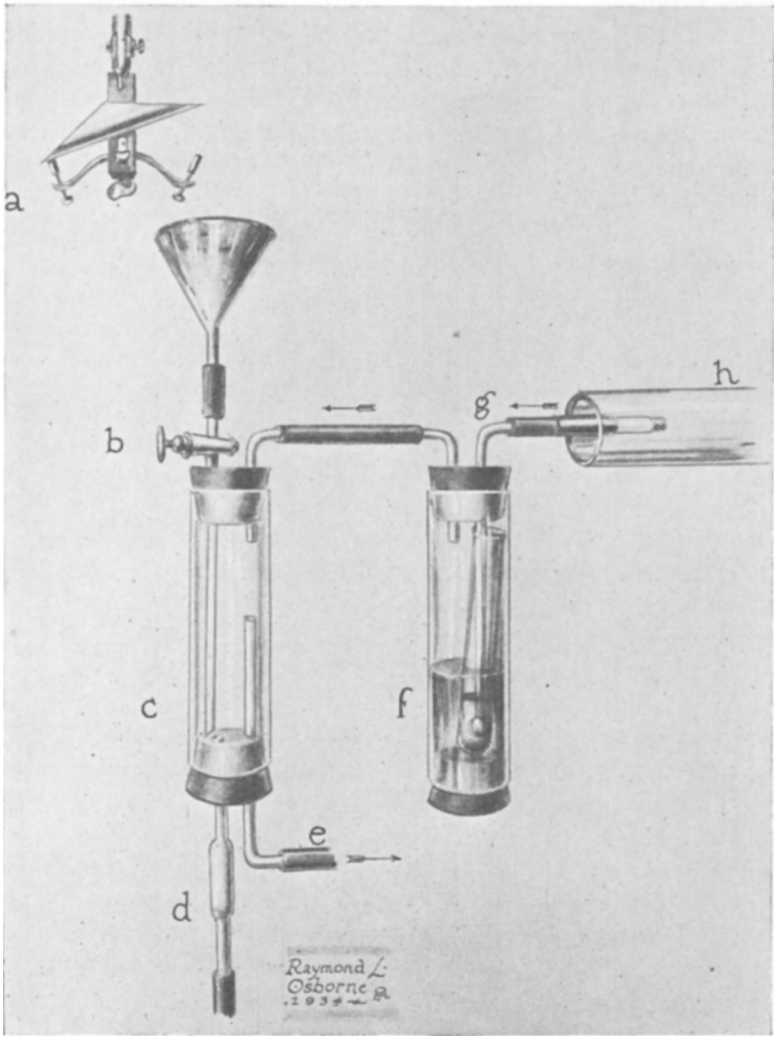

FIG. 1.
Apparatus used to puff smoke through the 3 cc. of fluid in the test tube in jacket *f*.

In order to avoid the interference of too many factors which are of necessity inherent in the problem, we have limited this first investigation to the influence of the 2 hygroscopic agents usually employed in the manufacture of cigarettes. These are glycerine and di-ethylene-glycol. These hygroscopic agents are deemed necessary to maintain the proper moisture content of the cigarette. It is possible that these chemicals may offer some interference with the combustion of the tobacco; or through their own combustion add quali-

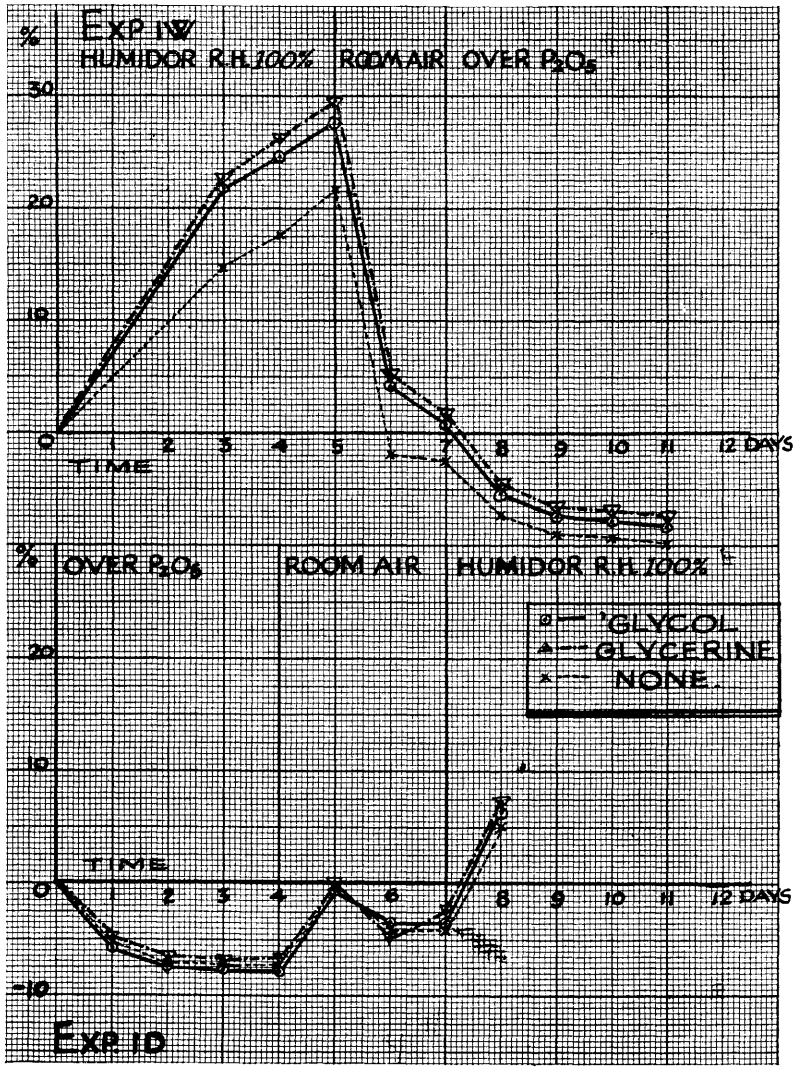

FIG. 2.

At *o*, 20 cigarettes of each variety were placed under the conditions noted on the chart.

ties to the smoke which are not present when the tobacco itself is burned.

Through the splendid cooperation of Philip Morris & Co., we obtained the necessary limitation of factors to make the investigation worth while. Under our personal supervision they prepared a batch of tobacco which was treated with the usual casing minus any hygroscopic agent. This was divided into 3 parts; to one was added

2.74% di-ethylene-glycol, to another 3.65% glycerine, and to the third nothing. From each of these, cigarettes were made in the usual manner. The above amounts of hygroscopic agents have approximately equivalent water holding powers, as is shown in Fig. 2, and are those ordinarily used in the manufacture of cigarettes. In addition we obtained some of the same tobacco to which we added subsequently 1, 3, and 5% of each hygroscopic agent.

The cigarettes were smoked under controlled conditions of humidity, and also with many variations in the water content of the cigarettes from very low (dried over phosphorus pentoxide), to very wet from standing over water for various periods of time. (Fig. 2.) However, these variations in the water content of the cigarettes had no demonstrable effect upon the irritating property of the cigarette smoke.

Two to 3 drops of the fluid through which had been sucked the smoke from 5 cigarettes were instilled into the conjunctival sac of each of 3 rabbits. The degree of blepharospasm and the amount of objection by the animal were noted. At 2 minute intervals the condition of the conjunctival mucous membrane was noted, and the edema and redness compared with that of the opposite untreated eye. In Fig. 3, the edema is reported as from plus to 4 plus.

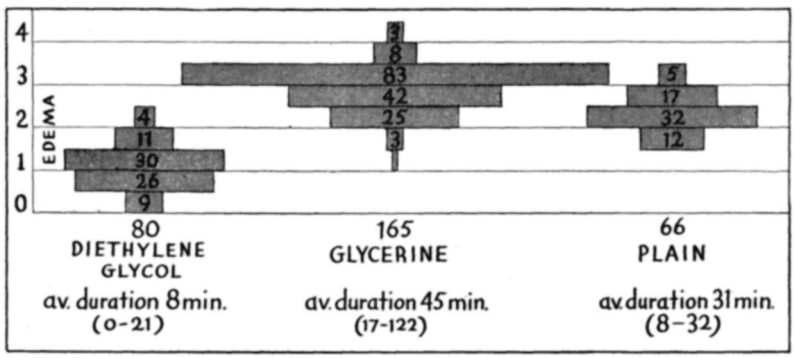

FIG. 3.

Frequency curve showing the distribution of the degree of edema in each experiment.

Results. Fig. 3 is a distribution curve of all the experiments performed. It is obvious that the cigarettes which had been made with di-ethylene-glycol as hygroscopic agent proved to be less irritating than those with no hygroscopic agent, and much less irritating than those with glycerine.

The solutions of the cigarette smoke were all acid to litmus, but no attempt was made to determine whether the acidity could account

for the inflammation produced. However, the following observation is of interest. Nicotine alkaloid in 0.005% solution which is alkaline to litmus, produced irritation about equal to that of the smoke from the di-ethylene-glycol treated cigarette. However, if the nicotine solution is made acid to litmus with an organic acid, or with carbon dioxide gas, the solution is irritating no longer, even if the concentration of nicotine is increased 10 fold. This shows that the irritating property of the cigarette smoke cannot be due to the amount of nicotine which is carried into the solution by the smoke. This conclusion is supported by the experiments of Dixon,[2] who found that the nicotine content of tobacco smoke was greater when the tobacco was moist. In our experiments, the moisture content of the cigarettes did not influence the irritating properties of the smoke, showing once more the independence between irritation and nicotine concentration in tobacco smoke.

Fig. 3 shows the maximum irritation, irrespective of time of onset or duration. The edema produced by the smoke solution from the untreated cigarette lasted an average of 31 minutes (8 to 82); that from the di-ethylene-glycol lasted 8 minutes (0 to 21); and that with the glycerine lasted 45 minutes (17 to 122).

Cigarettes made with 1, 3, and 5% glycerine respectively show a slight increase in irritation as the percent of glycerine increases. When di-ethylene-glycol is used, there is a slight but readable reduction in irritation as the percent increases. When the cigarette smoke is passed through mineral oil, the results are essentially the same as when water is used.

7631 C

Proliferation of Epithelium of Nipple of the Rat and Guinea-pig During the Oestrus Cycle.*

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Nineteen rats and 30 guinea pigs having regular cycles were used. Daily smears were taken in the rats for 3 weeks and in the guinea

2 Dixon, *Brit. Med. J.*, October, 1927.

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