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The effect of filtrate fractions of tobacco smoke on swimming endurance in the rat[1]

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Summary

20 minutes after the termination of exposure to filtered and unfiltered smoke the swimming endurance of 21 rats was measured. In experiment I (with only short pretraining of the rats) 10 and 20 puffs resulted in minor reductions of the swimming endurance, which did not surpass the threshold of significance. In the second experiment with the same rats (20 puffs only), the exposure sessions resulted in the following reductions of swimming endurance as compared to the control: Cambridge-filtered smoke 11 % (not significant), Cambridge-charcoal-filtered smoke 18 % (marginally significant), charcoal-filtered smoke 29 % ($p < 0.01$) and full smoke 32 % ($p < 0.01$). The differences between Cambridge-filtered smoke on one hand and charcoal-filtered and full smoke on the other hand also exceeded the threshold of significance.

A previous study (*Hrubes* and *Bättig*, 1970) showed that the inhalation of cigarette smoke had a detrimental effect on the swimming endurance of rats, whereas another study (*Bättig*, 1968) showed that nicotine injections enhanced the performance of rats in the same test.

The most likely reason under consideration for this difference in results (among other possibilities), was that the performance-stimulating effect of the nicotine in tobacco smoke could be antagonized by the CO or some other toxicologically active substances of tobacco smoke.

This experiment consisted of comparing the swimming endurance of rats after exposure to different filtrate fractions of cigarette smoke (particulate phase, volatile-vapor phase, and gas phase).

Method

Animals: 21 male albino rats with an average weight of 300 grams were used. During the entire experimental period the animals received water and food ad libitum.

Testing device and measurement: A water tub of 55 cm diameter and 85 cm depth was filled up to a height of 70 cm with water of 20 °. A load of lead of 7 % of their own weight was attached to the rats' tails. The animals were then put into the water and only rescued after they had submerged under the water surface for 8 seconds without emerging. The swimming time from the start of swimming until rescue from the water was measured by stop watch. One experiment per day per animal was made.

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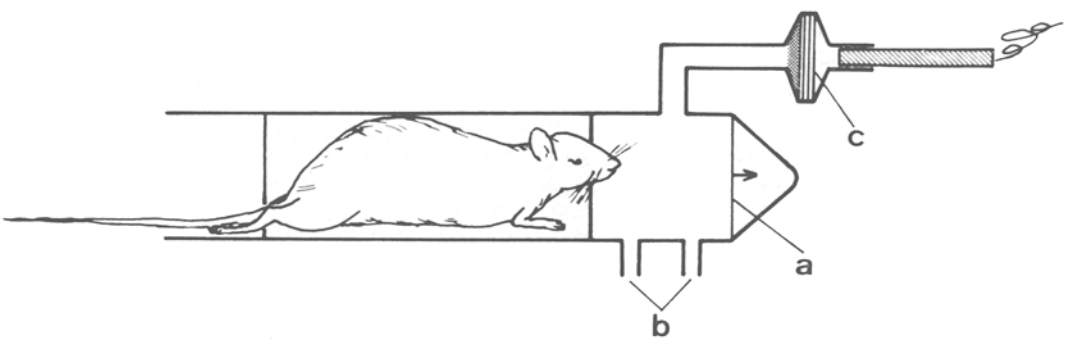

Fig. 1 Smoking: A. Smoke cycle. With the valves at *b* closed the membrane *a* moves forward, thereby creating a vacuum and aspirating smoke from the cigarette through the filter *c*. B. Fresh air cycle. With the membrane *a* in normal position, fresh air enters the smoke cylinder through one of the valves *b* and leaves through the other one.

Smoking machine: The device developed by *Davis* et al. (1970) was used (fig. 1). It consisted essentially of a cylinder in which the animal was kept immobilized, its head protruding from an opening in the front, and a smoke cylinder. The nose of the animal was introduced into the back of the smoke cylinder, where a membranous rubber collar formed an air-tight sealing. For 15 seconds during each minute an air-cigarette mixture was sucked into the smoke cylinder in the proportion of 2 : 3. During the remaining 45 seconds the cylinder was ventilated with fresh air. Between the cigarette and the smoke cylinder a filterholder was placed. The *gas phase* of smoke was introduced into the smoke cylinder by fitting into the filterholder a "Cambridge" filter (AP 250 Millipore Corp.) and behind this adding one gram of activated charcoal (Norit I). By inserting the Cambridge filter only, the combined *gas and vapor phase* of the cigarette smoke was obtained. By putting in the activated carbon alone, the *particulate and the gas phase* were obtained. For *full smoke* exposure the cigarette smoke was also sucked in through the filterholder, which contained no filters. Cigarettes of the American blend type of 85 cm length, without filter tip, were used. 10 puffs per cigarette were smoked and the filter was changed after each cigarette. In the control experiments the same procedure was used, without lighting the cigarette and without putting filters into the filterholder.

Analysis of the smoke: In all filter conditions the HCN content in the smoke cylinder was measured by means of the *Artho* (1969) method. For purposes of comparison, the same measurements were also performed through manual smoking by means of a syringe. Furthermore, the smoke after Cambridge-filtering was gaschromatographically compared with that after combined Cambridge-charcoal-filtering. Smoking was carried out manually. The column used was a glass capillary 100 mm × 0.5 mm, coated with Polypropylene-Glycol 10 %. Probe 0.5 ml, split 1 : 10. Program: Isothermic at 10 °C, then 4 °C/min up to 100 °C.

Test plan: In experiment I each animal first underwent three training sessions in the swim tub after immobilization in the smoking machine without smoking. Subsequently, the following exposures were carried through in random sequence, each terminating 20 minutes before the swim tub experiment: 10 and 20 puffs of full smoke; 10 and 20 puffs of Cambridge and charcoal-filtered smoke, 10 and 20 puffs of Cambridge-filtered smoke, 10 and 20 puffs of charcoal-filtered smoke, 10 and 20 puffs control (without lighting of the cigarette). In experiment II the same rats were treated in the same way, but only receiving 20 puffs of each type of exposure.

Statistical analysis: All individual swimming times were subjected to a variance analysis. The differences between the average values of the individual treatments were subsequently examined by means of the Duncan test as to their significance.

Results

The results of the HCN-analyses from the smoke cylinder of the smoking machine, and with manual smoking are shown in tab. 1.

Tab. 1 HCN-content of the different filtrate fractions of cigarette smoke (N = number of cigarettes).

	Manual smoking			Smoking machine		
Filter	N	μg/cig.	ppm	N	μg/cig.	ppm
none	3	183	614	7	130 ±	96
Cambridge	2	91	299	4	27 ±	82
Charcoal	2	5.0	17	2	5.0 ±	15
Cambridge + Charcoal	2	4.0	13	2	5,0 ±	15

The values of this table were indicative as to the filter efficiency and the tightness of the smoking machine.
With manual smoking, the HCN content of the unfiltered smoke of a cigarette was about twice as high as after passing through a Cambridge filter. The Cambridge filter, therefore, absorbed about 50 % of the HCN. The charcoal filter was far more active, permitting only traces of HCN to remain.
With the smoking machine, conditions were different. Without a filter only about 130 instead of 180 μg HCN, as with manual smoking, were measured. This indicated that the smoking machine used gave a smoke yield of about 30 % less, presumably due to leakage. With a Cambridge filter the quantity of HCN in the smoking machine was only about 25 % of the yield of the unfiltered smoke as compared to about 50 % with the manual smoking. This finding was attributed to the increased draft-resistance of the Cambridge filter. Changes in draft-resistance are important with the smoking machine, as the smoke is aspired by means of a vacuum membrane pump with a fixed suction pressure.
The two gaschromatograms of fig. 2 (Cambridge, and Cambridge + charcoal filter) show the efficiency of the activated charcoal in relation to the substances of the vapor phase of cigarette smoke. Charcoal absorbed all of these substances to the trace level, allowing only the pure gas phase to pass.
Observation of the animals revealed a marked stress on the animals and a reduced breathing frequency during the 15" exposure to smoke. These symptoms were very marked with exposure to unfiltered smoke, and not so noticeable with the various filter conditions.
The state of stress manifested itself during the immobilization by increased defacation. After liberation from the immobilization cylinder a marked sluggishness and lack of motor activity often occurred, which rapidly

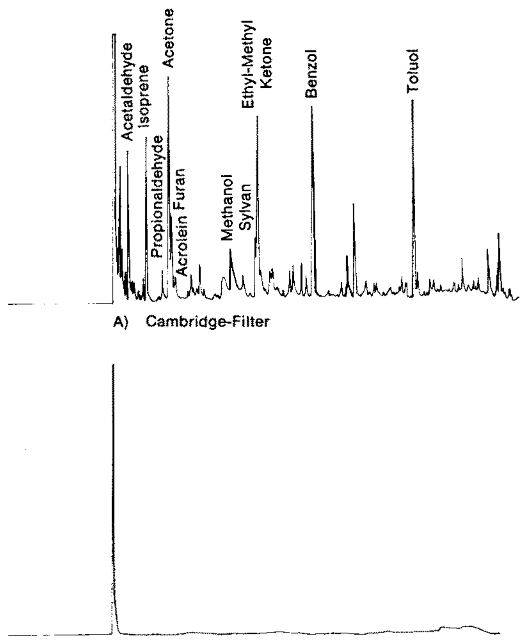

Fig. 2 Gaschromatogram of Cambridge-filtered (=gas and vapor phase) and Cambridge-charcoal-filtered cigarette smoke (gas phase).

returned to normal. 20 minutes later, when the animals were put into the swim tub, the motor activity of their swimming behavior could not be visually distinguished from that of control animals which had not been immobilized or exposed to smoke previously.
Breathing frequency appeared to be about 50 % of the normal rate when the animals were exposed to full smoke. As a rule, the animals seemed to hold their breath as long as possible at the beginning of a 15" smoking period. The first, generally deep aspiration was frequently followed by reflexive head movements after which the animals displayed an irregular breathing pattern. In the subsequent 45" fresh air phases a compensatory increase in breathing frequency seemed

to occur. The swimming times measured in experiment I are shown in tab. 2.

Tab. 2 Average swimming endurance.

Exposure	10 puffs	20 puffs
Full smoke	4.72	4.28
Charcoal-filtered smoke	5.10	4.34
Cambridge-filtered smoke	4.81	4.96
Cambridge and charcoal-filtered smoke	4.73	4.83
Control	5.33	5.10

These results showed a tendency for deterioration of performance by smoke-treated rats as compared to the control experiments. This deterioration was more marked with 20 puffs than with 10 puffs, and stronger with full smoke and charcoal-filtered smoke than with the other types of exposure. However, as expected, the differences did not reach the threshold of significance. In this first experiment, the intraindividual variation of performance was considerably high, presumably due to the insufficient amount of previous training and adaptation. Therefore, a subsequent experiment (II) was carried through replicating the same experimental conditions as in experiment I, but using only the dosage of 20 puffs and leaving out the dosage of 10 puffs.

The result of experiment II is shown in fig. 3. Here, the same differences which were already observed in the first experiment were more marked. Consequently, there occurred several significant differences between the average values ($p < 0.05$). The deterioration of performance was significant as compared to the control performance after exposure to full smoke, charcoal-filtered smoke, and Cambridge-charcoal-filtered smoke. However, it was not significant after exposure to Cambridge-filtered smoke. The deterioration of performance as compared to Cambridge-filtered smoke was significant after exposure to full smoke and charcoal-filtered smoke.

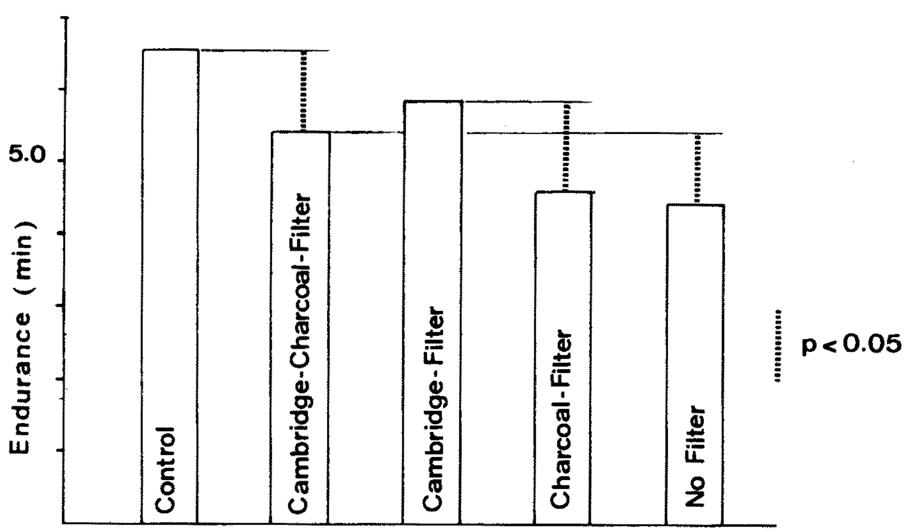

Fig. 3 Swimming endurance of the rats in experiment II after exposure to filtered and unfiltered cigarette smoke.

Discussion

The exposure to full smoke confirmed the performance deterioration already found previously in a similar swimming experiment (*Hrubes* and *Bättig*, 1970). Differences between the results of these experiments did not exist in a qualitative but in a quantitative way. In the previous study the swimming endurance amounted to about 66 % after 10 puffs and about 55 % after 20 puffs as compared to the control performance. In the present study the performance in experiment I amounted to about 88 % after 10 puffs and decreased to 82 % after 20 puffs, as compared to the control performance, thus not reaching the threshold of significance. In experiment II 20 puffs resulted, similar to former studies, in a significant performance reduction to about 66 %, as compared to the control performance.

This quantitative difference probably resulted from the differing degree of training of the animals between the two studies. In the former study the smoke exposures were preceded by 8 training sessions, 4 of which included habituation to immobilization in the smoking machine. In the present study, experiment I was preceded by only 3 training sessions with habituation to immobilization. Experiment II was preceded in addition by the 10 sessions of experiment I. These results suggest that the preferred test for determination of swimming endurance after inhalation of cigarette smoke would call for longer pretraining periods. Habituation to smoking and its pharmacological effects may have been an additional factor responsible for the difference between experiment I and experiment II of this study.

The performance reductions in experiment II must be considered from the standpoint of the specific effects of the various filters and filter combinations.

The exposure to the gas phase of smoke (Cambridge-charcoal filter) resulted in a significant performance reduction whereas the exposure to combined gas and vapor phase (Cambridge filter alone) resulted in a somewhat smaller, and not significant, performance reduction. Since in the vapor phase considerable amounts of HCN and various irritants were detectable, whereas these substances were virtually lacking in the gas phase, these results appeared to be paradoxical.

This could, however, be explained by observations of the breathing frequency under different filtration conditions, which have been verified by later studies in this laboratory (*Driscoll* and *Bättig*, 1970). In exposures with combined Cambridge-charcoal filter the breathing frequency has been observed to be more pronounced than with the Cambridge filter alone, this being assumed due to the lack of irritants present. It could therefore be supposed that with the combined Cambridge-charcoal filter a stronger gas phase exposure compensated the lack of the vapor phase. Thus, a performance-reducing effect for the gas phase could be accepted, whereas conclusions as to the effect of the vapor phase would only have been possible if in the same experiments the dosage could be measured quantitatively in relation to the actual inhaled smoke volume, by determining exactly the number of respirations for the conditions involved.

As to the cause of the performance-reducing effect of the gas phase, the CO content should first of all be taken into consideration. In a similar method of passive smoke inhalation (but with continous instead of intermittent exposure) *Reckzeh* and *Dontenwill* (1970) found a uniformly similar lowering of the body temperature of hamsters after exposure to the gas phase of cigarettes and after exposure to 4 % CO in air.

The exposures to charcoal-filtered and unfiltered smoke resulted in reductions of performance which were not only significant as compared to the control, but also as compared to the Cambridge-filtered smoke conditions. There was no significant difference between unfiltered and charcoal-filtered smoke, and also between Cambridge- and Cambridge-charcoal-filtered smoke.

The modest performance reduction with these last two filter combinations might be explained by the finding that the Cambridge filter reduced the smoke yield by about 50 %, as measured in the HCN content investigation. However, this was in part compensated by the observation that exposure to unfiltered smoke also reduced the exposure by reducing the number of respirations.

The use of a charcoal filter or no filter were the only conditions of this experiment which exposed the animals to the particulate phase of cigarette smoke. Pharmacologically, the most important fractions of the particulate phase are nicotine and the secondary alkaloids of the smoke. In small but adequate dosage, nicotine has led in various behavioral situations not to performance deteriorations, but mainly to performance improvements (*Larson* and *Silvette*, 1968). This was the case when measuring swimming endurance with nicotine injections of 0.1 mg/kg and 0.2 mg/kg (*Bättig*, 1968).

The performance reduction displayed by the particulate phase in this present experiment was unlikely a consequence of too large a dose of nicotine or merely a manifestation of stress, as 10 minutes of full smoke (using the same dosage and smoking method) improved performance significantly under extinction of avoidance response conditions in another experiment (*Driscoll* and *Bättig*, 1971). In contrast, 10 minutes of full smoke significantly reduced performance in a previous swimming endurance test similar to this present study (*Hrubes* and *Bättig*, 1970). The substances of the gas and vapor phase alone could also not produce these results, as their combined effect (Cambridge filter) also had only a small performance-reducing effect. Thus the question arises as to whether the particulate phase of cigarette smoke contains substances which, alone or together with the performance-reducing substances of the gas and vapor phase, surpass the performance-stimulating effect of nicotine.

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