PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 672-676 (1978)

Cigarette Smoking Baboon Model: Demonstration of Feasibility (40119)

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To investigate the mechanisms by which cigarette smoking affects atherosclerosis, chronic obstructive pulmonary disease, and fetal development, an animal model is required that meets several criteria. Ideally, the animal model should simulate human cigarette smoking in puff frequency, duration, and volume because puff characteristics affect smoke composition. The animal also should inhale through the mouth because nasal passages modify the smoke. The exposure system should avoid surgical manipulation or stressful restraint during smoke exposure because these procedures may affect physiological endpoints. The animal also should be susceptible to diet-induced atherosclerosis, and its respiratory and reproductive systems should be similar to those of man.

Most animal models in current use for cigarette smoke inhalation studies do not meet all of these criteria. These include small rodents exposed to cigarette smoke in chambers (1), rabbits exposed in chambers (2) or through face masks (3), dogs exposed through tracheostomata (4) or face masks (5), chickens exposed through face masks (6), and nonhuman primates exposed through face masks (7, 8). Preliminary experience with a few baboons (9) and the reported experiences of other investigators in training nonhuman primates to smoke (10–13) led us to believe we could train a substantial number of baboons to smoke and could control their smoking behavior. The present report describes a system which has accomplished this objective.

Methods and materials. Training methods. Seven baboons (*Papio cynocephalus*), ranging from 1 to 4 years in age, were given water from a handheld water bottle until they learned to suck rather than to lick water. Then a Smoking Inhalation Response Indicator and Conditioner (SIRIC) (14), a device delivering a measured water reward when the animal's puff exceeded investigator-controlled criteria, was mounted on each animal's cage. Smoke from the burning cigarette passed to the animal through a 6 cm tube which served as a cigarette holder and as a mouthpiece. To teach the animal to suck on the cigarette holder, the trainer squirted water through the cigarette holder of the SIRIC and blocked the end of the tube with his finger. When the animal sucked water from the tube with a negative pressure greater than 1.24 cm H_2O for a minimum of 0.25 sec, a water reward was delivered through a separate drinking tube.

After the animal had learned to activate the SIRIC and consume the water reward, the opening was left blocked with an unlighted cigarette so that the animal could activate the device at will. Within 25 days all animals learned to earn their entire water ration by activating the SIRIC. Then lighted cigarettes were presented, and the animals readily learned to make 0.25 sec puffs of cigarette smoke in order to earn their water. An attendant then presented lighted cigarettes at regular intervals (10, 15, 20, or 30 minutes) during 8 hr per day, 7 days per week. The minimum puff duration was increased gradually from 0.25 to 2.50 sec over 1–9 months (mean, 121 days).

Criterion for smoke inhalation. Carbon monoxide (CO) concentration in the blood was used as the criterion for smoke inhalation. CO is absorbed only slightly in the buccal cavity, but is readily absorbed in the lungs (15).

Venous blood samples were drawn under ketamine (Ketaset, Bristol Laboratories, Syracuse, NY 13201) sedation within 10–20 min after the animals had smoked the last of a series of cigarettes over a 3-hr period. The samples were analyzed for CO by the method of Ayres *et al.* (16) with a modified extraction

0037-9727/78/1574-0672$01.00/0

chamber; CO is extracted from whole blood and then measured by gas chromatography using a thermal conductivity detector.

As the criterion for smoking, we selected a concentration of CO in blood of 0.30 ml/dl, three times the mean (0.10 ml/dl) and twice the maximum (0.15 ml/dl) of the values observed in the seven animals prior to cigarette smoke exposure (range 0.05–0.15 ml/dl). We estimated the maximum amount of CO that baboon blood can carry by saturating 12 blood samples from smoking and nonsmoking animals with CO and measuring the CO concentrations. The four samples with the highest concentrations averaged 14.68 ml CO/dl.

Experimental cigarettes. We conducted most of the training using University of Kentucky Reference (UKR) cigarettes (17, 18); tests also were conducted with Code 5 and Code 14 experimental cigarettes (Series 1) of the Smoking and Health Program of the National Cancer Institute (19). Selected characteristics of these cigarettes are given in Table I.

Results. Training. The average time required to train the seven baboons to smoke cigarettes was 96 days (Table II); the minimum was 32 days and the maximum, 178 days. These durations were measured from the first day a trainer worked with the animal to the first day on which the blood CO concentration equaled or exceeded 0.30 ml/dl. Figure 1 illustrates acquisition of the cigarette smoking response and inhalation of smoke as measured by mean blood CO concentration. CO levels in week 8 were significantly higher than in week 2, and were significantly higher in week 14 than in week 8.

TABLE I. SELECTED CHARACTERISTICS OF SMOKE PRODUCED BY EXPERIMENTAL CIGARETTES.

Characteristics	Cigarette type		
	UKR	Code 5	Code 14
Total particulate matter (mg)	38.5	28.7	14.1
Tar (mg)	35.8	24.2	11.3
Nicotine (mg)	2.63	1.65	0.20
CO (ml)	17.6	14.5	11.9
Puffs[a]	10.9	10.2	5.9

[a] Number of puffs required to produce a butt of 23 mm under standard smoking machine conditions (16) of 35 ml puffs of 2 sec duration at a rate of one per minute.

Maintenance of smoking behavior. Once the animals were trained to smoke cigarettes for water rewards, no difficulty was encountered in maintaining or controlling the smoking behavior. Two animals performed without interruption for well over a year, earning all of their water by smoking cigarettes. The animals remained in good health throughout the period of training and testing of the system and gained weight at a rate normal for animals of their age. Hematocrits measured weekly for 1 month were within the normal range and showed no unusual fluctuations. No significant correlations were observed between hematocrit and total water delivered per day or ml water delivered per kg body weight.

In the six smoking animals examined weekly for 4 months, the average COHb was 5.9% (0.89 ml CO/dl); the maximum mean value was 16.0% COHb (2.40 ml/dl).

Variables affecting smoking behavior. After smoking behavior was established for six animals, various combinations of cigarettes, puff duration settings, cigarette frequency, ratio of reinforcement, and amount of water reward were tested for their effects on smoking behavior. The animals readily accepted Code 5 and Code 14 cigarettes. Puff duration

TABLE II. AGE, SEX, DAYS TO CRITERION, AND MONTHS SMOKING BEHAVIOR MAINTAINED FOR SEVEN BABOONS.

Baboon number	Age at start of training, months	Sex	Days to criterion	Months smoking maintained
B-734	32	Male	94	15
B-756	30	Male	134	14
B-941	9	Female	94	0.5[a]
B-944	9	Female	101	8
B-720	43	Male	178	2
B-778	40	Male	32	5
B-966	21	Male	40	3

[a] Animal died of unexplained causes.

required for a reward was advanced to as long as 3.0 sec.

Since the animals' primary incentive for smoking was the water reward, it was possible to control the number of puffs per day by adjusting the reinforcement contingency. For example, when the water reward per criterion puff was changed from 1.00 ml to 0.75 ml, a 25% reduction, the animals' mean number of puffs per day rose from 467 (SEM = 53) to 570 (SEM = 52), an increase of 22%. The mean water earned per day remained essentially constant, decreasing from 432 ml (SEM = 42) to 417 ml (SEM = 48), and puff duration requirements remained constant over the two test weeks. Other manipulations of basic operating conditions produced equally orderly changes in the animals' smoking behavior.

Variables affecting blood CO concentration. Although we did not perform controlled experiments to isolate the effects of single, investigator-controlled variables on blood CO during the feasibility testing period, it appeared that this measure of inhalation was affected by type of cigarette smoked as well as by other variables controlled by the investigator (Table III). There was a threefold difference between blood CO concentration in the five animals while smoking Code 14 cigarettes as compared to the blood CO while smoking UKR cigarettes. To make an equivalent number of puffs per 3 hr for each type, it was necessary to present more Code 14 cigarettes because they are loosely packed and burn rapidly.

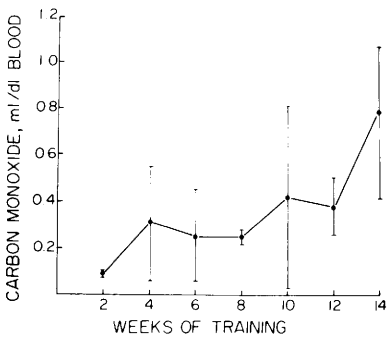

FIG. 1. Mean and 95% confidence intervals of concentration of carbon monoxide in blood of six baboons by week of training.

Use of model in controlled experiments. The feasibility, demonstrated here, of using the baboon as a model to study the effects of cigarette smoke inhalation was further confirmed when, subsequent to the developmental work, we trained an additional 44 baboons to smoke with the SIRIC and encountered no training failures. We also have extended the total daily smoking exposure to 12 hr and 48 cigarettes per day with no difficulty. One attendant can establish and maintain smoking behavior in approximately 20 baboons. Control animals can be subjected to identical training and maintenance procedures by using a filter with the approximate draw resistance of a cigarette as a sham.

Discussion. Comparison of human and baboon smokers. Studies of human smokers have shown mean COHb saturation values of 4.4% (20); 4.7% (21); and 6.7% (22). Only 2% of human smokers had COHb saturation equal to or greater than 14% saturation. Thus, blood CO levels of 5.9% COHb are similar to those shown by human smokers and can be maintained for long periods in the cigarette smoking baboon.

Moody and associates (27) found that the human cigarette smoker made, on the average, 9.5 puffs per cigarette on 21.6 cigarettes per day, giving an average "dose" of 204 puffs of cigarette smoke per day. Over the final 17 week period of this study the 6 ba-

TABLE III. CIGARETTES PRESENTED, PUFF DURATION, NUMBER OF PUFFS, AND BLOOD CARBON MONOXIDE CONCENTRATION IN FIVE BABOONS AFTER 3 HR OF SMOKING TWO TYPES OF CIGARETTES, MEAN OF MEASUREMENTS 1 DAY PER WEEK FOR 5 WEEKS.

	Type of cigarette						
	UKR		Code 14				
Variable	Mean	SEM	Mean	SEM	*df*	*t*	*P*
Cigarettes presented, number	8.7	0.34	23.3	0.32	—	—	—
Minimum puff duration, sec	1.66	0.28	2.18	0.26	4	3.90	<0.02
Puffs, number/3 hr	238.0	44.2	260.0	37.5	4	1.60	NS
Carbon monoxide in blood, ml/dl	0.48	0.06	1.50	0.27	4	4.75	<0.01

boons averaged 535 puffs per day. The mean puff duration for humans (27) was 2.3 sec; the baboons' average puff duration was 2.0 sec. The system described here allows controlled simulation of the general features of human cigarette smoke exposure with the baboon. Furthermore, because cigarette smoke appears to lack strong appetitive or aversive properties in the baboon, the investigator can control any specified degree of cigarette smoke inhalation by manipulating appropriate variables.

Difference in blood CO between cigarettes. The CO yield from UKR cigarettes on standard smoking machines is 1.5 times that from Code 14 cigarettes (23). CO yields of commercial cigarettes vary by factors of two to four (24–26), but CO levels in smokers vary considerably more, probably because of differences in puffing characteristics such as puff volume and duration (25).

Code 14 cigarettes have a lower draw resistance than UKR cigarettes (5.05 cm H_2O pressure drop vs 7.07). Therefore, for the animals to achieve negative pressure sufficient to activate the SIRIC, they had to generate a larger puff volume. In addition, the longer mean puff duration requirement when the animals were smoking Code 14 cigarettes (2.18 sec vs 1.66 sec) also contributed to larger puff volume and deeper inhalation. CO is thought to be responsible for some of the deleterious effects of cigarette smoking. The variables affecting CO absorption can be tested with this system, since the investigator can control each of the conditions affecting puffing characteristics.

Usefulness of the baboon smoking model. The baboon is suitable for the investigation of cigarette smoking effects on experimental atherosclerosis (28, 29) and reproductive physiology (30). Its large size facilitates performing tests of pulmonary function. The combined instrumentation and operant techniques permit controlled experiments on the effects of cigarette smoke inhalation with adequate numbers of subjects. Since we have encountered no training failures, it is possible to assign animals randomly to cigarettes or to shams. Investigator control of smoking performance reduces potential confounding by either habituation or avoidance by the animal. The short smoke delivery path minimizes changes in smoke composition due either to removal of smoke components or to particle agglomeration. The demonstration of elevated blood CO concentrations confirms delivery of cigarette smoke to the lower respiratory tract.

Summary. Using operant conditioning techniques and a specially designed apparatus, and without surgical manipulation or obvious stress, we trained seven baboons to puff on lighted cigarettes and to inhale cigarette smoke. Smoking behavior was maintained for up to 15 months and could be controlled by manipulating minimum puff duration, water reward, and frequency of presenting cigarettes. Concentrations of blood carbon monoxide after 3 hr of smoking equaled or exceeded those reported for heavy human cigarette smokers. The cigarette smoking baboon models human smoking performance and is a useful animal model for studying the mechanism of the effects of cigarette smoke inhalation on the cardiovascular, pulmonary, and reproductive systems.

The dedicated efforts of Russell E. Stevens, III, in the early stages of this project are gratefully acknowledged. Supported by Grant No. HL-15914 from the National Heart, Lung, and Blood Institute of the National Institutes of Health and Internal Research Project 13-9114 from the Southwest Research Institute.

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Received November 9, 1977. P.S.E.B.M. 1978, Vol. 157.