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Experimental Production of Carcinoma with Cigarette Tar*

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The increasing frequency of primary cancer of the lung in many parts of the world has aroused great interest in this condition and has stimulated a search for an explanation. In 1950, Wynder and Graham (40), on the basis of a clinical and statistical investigation, presented evidence of a real association between lung cancer and smoking, especially of cigarettes. These data have been well substantiated by a large-scale British study by Doll and Hill (9, 10). Both studies showed that the risk of developing cancer of the lung increases in direct proportion to the amount of smoking. Ten other recent studies reached similar conclusions (3, 11, 15, 20, 21, 24, 26, 33, 34, 42). In 1952, The Council of International Organizations of Medical Sciences convened a symposium on the endemiology of lung cancer and agreed that the present evidence points to a relationship between lung cancer and cigarette smoking (12).

Tobacco is also thought to play some role in the production of cancer of the larynx, oral cavity, and esophagus. Although the studies of those relationships are not so complete as the studies on lung cancer, the collected data are suggestive (33, 41).

The increasing incidence of bronchiogenic carcinoma and the available evidence relating smoking to it and possibly to cancer of other sites led us to undertake the experimental work reported here. This investigation is directed toward determining in laboratory animals whether there are carcinogenic factors in cigarette smoke.

PREVIOUS INVESTIGATIONS

Many attempts have been made with tobacco products to induce cancers in a variety of experimental animals. The first was reported by Brosch in 1900 (4). He painted guinea pigs with tobacco "juice" for an unknown period of time and described epithelial proliferation. Subsequently, many different approaches to the problem were undertaken with various types of tobacco, different methods of tar preparation, and different species of animals. Many of those studies were carried on for too brief a period of time or with too few animals to be regarded as significant. Hoffmann and his associates (17), for instance, painted animals for only 14 days, at which time they noted hair loss. Wacker and Schmincke (37) observed proliferation of epithelium in rabbits' ears 21 days after a subcutaneous injection of pipe tar.

The first recorded experiment with mice and with tobacco tars as the suspected carcinogen was the one just cited by Hoffmann and co-workers. The more detailed of the subsequent studies are listed in Table 1. This table attempts to summarize the methods used in the various studies and the results obtained. In many instances the method of study was not described in sufficient detail to give all the information considered essential.

From this survey of the literature it is found that, before our study, all the previous attempts to produce experimental cancer in mice with tobacco products were successful in the production of only seven epidermoid cancers of the skin.

Several investigators attempted to induce pulmonary tumors in mice with tobacco smoke. Lorenz and co-workers (22) obtained negative results in this manner. Campbell (5), and especially Essenberg (13), however, claim to have found a significantly higher percentage of pulmonary adenomas in the experimental than in the control group. It is doubtful that such a finding is important. At any rate, so far these methods have not induced true bronchiogenic carcinomas.

The majority of the investigators working with tobacco tars used rabbits as the experimental animals (14, 23, 27-32, 36). In view of the fact that the present work deals with mice we shall only briefly list some of the studies with rabbits. Roffo reported the production of carcinomas in rabbit ears after painting the ears with a distillate of tobacco (28, 30, 32). Sugiura (36), in attempting

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TABLE 1. SUMMARY OF METHODOLOGY OF SOME STUDIES ON TOBACCO TAR AS A POSSIBLE CARCINOGEN FOR MICE

Author	Type of Tobacco	Method of Tar Collection	Temperature	Portion of Tar Used	Number of Mice	Solvent
Helwig (16), 1928	Not given	Tarry extract from bowls of burned briar pipes	Unknown	Denicotinized tar	50	Ether with olive oil
	Not given	Distillate produced at temperatures of 400°–500° C. and tarry product extracted with ether or chloroform	400°–500° C.	Extracts	50	Chloroform or ether
Bogen and Loomis (2), 1932	Kentucky Burley tobacco	Destructive distillation and extraction with H_2SO_4, alcohol, and ether	Not given	50 per cent whole tar solution in glycerine	12	Glycerine
Cooper (7), 1932	Not given	Intermittently puffed and exhaled pipe tobacco; the resulting tar condensate was then distilled	370°–390° C.; 490°–700° C.	I Tobacco tar of 400°–500° C.—wooden pipes		Alcohol, benzene, and glycerol
				A. 10 per cent in alcohol solution	4	
				B. 30 per cent solution in glycerol	6	
				C. Neutral fraction freed from bases or phenols using either		
				1. 50 per cent solution in benzene	13	
				2. 10 per cent solution in glycerol		
				D. Original tar dissolved in glycerol and applied hot at 45° C.	6	
				II Tobacco tar of 700°–800° C.—wooden pipes; 25 per cent solution in alcohol	9	
				III Tobacco tar of 400°–500° C.—clay pipes; 50 per cent solution in alcohol	12	
Schuterch and Winterstein (35), 1935	Cigars	Smoke of cigars collected in cotton which was extracted with chloroform. The solution was denicotinized with 2 per cent HCl	Not given	I 25 per cent tar in alcohol solution	100	Alcohol
				II Tar with nicotine applied for 3 months then used denicotinized tar	100	
				III Denicotinized tar extracted with chloroform	100	Chloroform
				IV Denicotinized tar extract heated to 130° C. prior to painting	100	
				V Denicotinized tar	100	
				VI Tar extract distilled at 100° C.	100	
				VII Tar extract distilled at 160°–170° C.	100	
				VIII Tar extract distilled at 190° C.	100	
				IX Tar extract distilled at 300° C.	100	
Taki (19), 1937	Unknown	Extract from tobacco pipes dissolved in ether and filtered, then denicotinized with HCl	Not given	Denicotinized tar	104	Ether
Campbell (6), 1939	Not given	Tar condensate (partial) of artificially smoked cigarettes	Not given	Tar condensate	23	None
Sugiura (36), 1940	Mixture of American and Turkish leaf tobaccos	Distillation at 100°–500° C. and redistilled at 500°–900° C.	100°–900° C.	I A. Distillates applied for 90 days	186	None
				B. After 90 days		
				1. Watery fluid of first distillate	44	
				2. Oily liquid of first distillate	57	
				3. Second distillate, 500°–900° C.	67	
				C. Cholesterol diet during painting	40	
				II Oily liquid of first and second distillates	30 C57 30 DBA	
				III Control animals using coal tar	40	
Flory (14), 1941	Entire leaf and stem of cured Kentucky tobacco	Destructive distillation at 700° C. for 6–8 hours yielding 3 fractions	130°–700° C.	I 350°–700° C. destructive distillate	30	None
				II Denicotinized 350°–700° C. destructive distillate	46	
				III Denicotinized 100°–330° C. destructive distillate	76	
				IV Mixture of watery parts of distillates	30	
				V Control group; coal tar and benzene in equal parts	15	
	Coarsely ground tobacco	Continuous suction applied to clay pipes at 450°–750° C.	450°–750° C.	Denicotinized whole pipe tar	60	None
Shubik,† 1950	Cigarettes	Tar extracted with acetone from smoked cigarette stubs	Not given	I Tar extract	30	Acetone
				II Tar extract applied for a limited period followed by applications of croton oil for duration of experiment	30	
Wynder, Graham, and Croninger, 1953	Cigarettes	Whole tarry condensate of intermittently puffed cigarettes	Burning tip—316°–699° C.; smoke—28.7°–60.0° C.; combustion—966° C. (max.), 682° C. (av.)	I 30 per cent whole tar in acetone		U.S.P. acetone; white mineral oil (used with croton oil)
				1. Tar	81	
				2. Tar/croton	31	
				II Acetone controls		
				1. Acetone	30	
				2. Acetone/croton	14	
				III 0.3 per cent methylcholanthrene	23	

* Applied by painting the skin

† P. Shubik, personal communication.

TABLE 1—*Continued*

Strain and Initial Age of Application	Frequency and Dosage of Application*	Duration of Applications	Results	Author
Not given	3 times a week; dosage not given	1 yr.	Ulceration	Helwig (16), 1928
Not given	Not given	8 mos.	Ulceration	
Not given	2 times a week; dosage not given	Over 1 yr.	Negative	Bogen and Loomis (2), 1932
Not given	3 day intervals; dosage not given	Over 16 mos.	One epithelioma from 10 per cent tar (produced at 400°–600° C.) in alcohol solution in 16 months	Cooper (7), 1932
			Negative	
			Negative	
Not given	2 times weekly, dosage not given	9 mos.	Atrophy of skin with slight hyperkeratosis and perivascular infiltration	Schwerch and Winterstein (35) 1935
	2 times a week	348 days	Same as I	
	2 times a week	265 days	Same as I	
	2 or 3 times a week	635 days	Atrophy of skin and chronic inflammation	
	2 times a week	270 days	Hair loss	
	Not given	2 mos.	Negative	
	3 times a week	190 days	Loss of hair	
	Not given	6 mos.	Loss of hair and atrophy of skin	
	Not given	480 days	Atrophy of skin and hair loss	
Not given	2 times a week; dosage not given	Not given	3 papillomas (1st observed at 85 days); 2 squamous cell epitheliomas	Taki (19), 1937
Not given	2 times a week; dosage not given	20 mos.	One typical epithelioma at 16–20 mos.	Campbell (6), 1939
Bagg albino mice, 2–3 months old	2 times a week; 0.02 gm/dose	90–600 days	A. Negative B. After 90 days 1. No results 2. No results 3. One squamous carcinoma C. No accelerating influence	Sugiura (36), 1940
C57 blacks and DBA mice, age of initial application not given		cholesterol diet—90–180 days C57 black 90–275 days; DBA died too early for results	No tumors	
Bagg albino		75–309 days	75 per cent developed progressively growing tumors with 51 squamous cell carcinomas	
White mice (not an inbred strain); age at initial application not given	5 times a week .015 gm/dose .018 " .015 " .20 "	18 mos.	Papillomas / Cancers 4 in 7½–11½ mos. / 0 8 in 7½–11 mos. / 1 in 8.5 mos. 1 in 14 mos. / 0 0 / 0 13 in 126–256 days	Flory (14), 1941
White mice (not inbred); age at initial application not given	5 times a week; 0.015 gm/painting	18 mos.	2 papillomas in 14 mos. 1 cancer in 17 mos.	
C strain; 3 months	Twice weekly	9 mos.	Negative	Shubik,† 1950

				Papillomas (no.)	Cancers (no.)	
CAF1, 8–12 weeks	Tar: 3 times weekly; 0.040 gm/painting Croton oil: 3 times weekly tar/acetone plus 1 painting/week of 5 per cent croton oil/mineral oil	24 mos. maximum	I Whole tar in acetone (1:1 ratio) 1. Tar 2. Tar/croton II Acetone controls 1. Acetone 2. Acetone/croton III 0.3 per cent methylcholanthrene	 48 13 0 0 25	 36 3 0 0 25	Wynder, Graham, and Croninger, 1953

to repeat Roffo's work, obtained negative results, whereas Flory (14) in a similar large-scale experiment obtained papillomas and several lesions which he designated as "carcinomatoids" in rabbit ears after application of a tobacco distillate (24, 39).

Roffo also used rats as experimental animals. He implanted pills of tobacco tar into rat bladders and noted cancer-like growths after 8 months (29). McNally (25) applied a water-soluble product of cigarette smoke to the mucous membrane of the oral cavity and to the tongue and skin of rats. All animals were lost within 4 months due to the toxicity of the material, and no significant changes were noted.

MATERIALS AND METHODS

The method of study employed in this investigation is presented in some detail because the varying results of different workers may be a reflection of different methods used. In this study the guiding principle in collecting the tobacco tars was to simulate human smoking habits as closely and as practically as possible. It was felt that any change in temperature or the removal of any fraction from the smoke might alter the effect of the resulting tars. Therefore, we used a smoke condensate obtained from smoking cigarettes intermittently, and, unlike most other investigators, we did not remove the nicotine from the tars in the initial phases of the experiment.

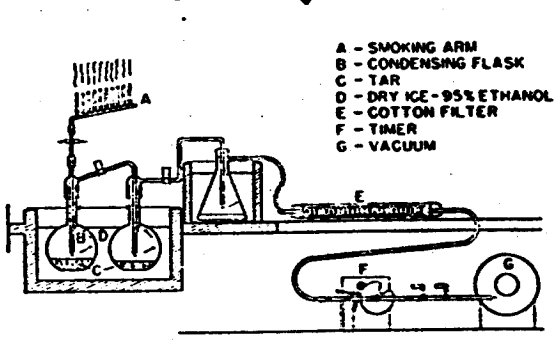

CHART 1.—Schematic drawing of smoking apparatus

Tobacco used.—A popular brand of domestic cigarettes was used.

Smoking apparatus.—The tobacco tars were collected in the following manner. Sixty cigarettes were placed in a battery of glass smoking arms (A) containing separate holders for ten cigarettes each and lighted with a multiple jet gas burner (Chart 1). The smoking arms were rotated and changed as the cigarettes burned down, keeping the apparatus filled with 60 cigarettes at all times after the original loading. The arms were placed in position on glass candelabra-like receivers on each of the two smoking units. These receivers were jointed and connected to a series of three 2,000 ml.-glass flasks (B) and connecting tubes on each unit. A vacuum was created by a small electrically driven air pump (G) (1.3 cu. ft. displacement of free air per minute at a vacuum of 27 in. of mercury). At 18-second intervals an open-shut valve, geared to a three r.p.m. ¼ horsepower motor (F), was automatically opened for a 2-second period, allowing the vacuum from the air pump to be applied to the condensing flasks and ultimately to the burning cigarettes. The condensing flasks were enclosed in insulated stainless steel tanks filled with 95 per cent ethanol and dry ice (D) to lower the temperature to approximately −60° C. The smoke that failed to condense inside the cooled tanks then passed on to a third flask outside each unit and insulated with an asbestos jacket. The two units met at a common source of vacuum, at which point a cotton filter (E) was placed in the line to protect the motors from tar corrosion. The smoking time per cigarette averaged from 7½ to 8 minutes or from 28 to 32 puffs per cigarette at a rate of three 2-second puffs per minute. The cigarettes were burned down to approximately a 2-2.5-cm. length.

As the smoke condensed, a dark brown viscous liquid formed and adhered to the glass apparatus with diminishing density to the third set of flasks. After 50 cartons were smoked, all of the tar contained in the glassware above the cotton filter was washed clean with approximately 300 cc. of acetone by dismantling and corking the separate glassware pieces. This tar-acetone mixture was then stored at −7° C. in glass-stoppered bottles until made up into solutions.

Preparation of tars for application.—Solutions were made up fresh monthly from 50 cartons of cigarettes. The choice of acetone as a solvent for these tars was based primarily on the fact that acetone was known to be noncarcinogenic and also because it was found to be the best dissolving medium for this substance.

The tar-acetone mixture was poured into 150-ml. porcelain evaporating dishes, which had been previously weighed; these were placed in an exhaust hood containing a rotating fan for 6 hours and were then left overnight. The average weight of the residual tar obtained in this manner was 9.7 gm/200 cigarettes. An equivalent volume of acetone was added to each gm. of residual tar and this mixture (1 cc. acetone to 1 gm. tar) was placed in 125-ml. Pyrex glass-stoppered bottles and stored at −7° C. until used. When used, small amounts of these solutions were repoured into other bottles to reduce the evaporation of the acetone as much as possible.

Experimental conditions.—Tests for the amount of vacuum applied on the smoking cigarette were made at three stages of burning, i.e., at the start of burning, at midpoint, and at butt stage. In the first stage the magnitude of the vacuum at the start of the puff was zero; it increased to a maximum value of −9 mm. of water and then fell to an average of −8 mm. This value was found to reach a maximum of −7 mm. and an average of −6 mm. in the second stage, and a maximum of −5 mm. with an average of −4 mm. in the butt stage.

Temperatures of the burning cigarette:[1] All the temperature studies were made with iron constantan thermocouples with welded hot junctions. No. 26 gauge duplex glass-insulated wire with a combined resistance of 1.4 ohms/ft at 21.1° C. and a No. 28 gauge duplex enamel insulated wire with a combined resistance of 2.2 ohms/ft at 21.1° C. were used. The temperatures were registered on a suspended galvanometer potentiometer-type null balance, calibrated from 0 to 2,000° F. The burning tip temperatures depended on the penetration of the thermocouple into the combustion zone. The results of 60 temperature readings ranged from 316 to 599° C. The average of the 60 temperature readings was 438° C. with a room temperature of 26.7° C.

Combustion temperatures of the cigarette: Combustion temperatures were obtained by inserting 28 gauge thermocouples up through the mouth end approximately three-quarters of the length of the cigarette and by then smoking the cigarette to the point where the hot junction of the thermocouple would pass through the combustion zone. Tempera-

[1] Temperature studies were carried out by the Fillo Sales & Engineering Co., St. Louis, Mo.

tures up to 966° C. were obtained by this method. The average reading, however, was 682° C.

Temperatures of the smoke as distinguished from that of the burning cigarette: Twenty-eight gauge iron constantan thermocouples were inserted in the receiving end of the cigarette arm, and the cigarettes were smoked down to the short butt stage. No appreciable rise in the temperature of the smoke was noticed until the cigarettes were consumed to butt length. The temperatures of the smoke ranged from 26.7 to 60° C.

Animals used.—One hundred fifty-six CAF_1 mice (genetically homogeneous F_1 hybrids from the cross A/Cloudman inbred strain males by BALB/C inbred strain females obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine), with an equal distribution of males and females, were started at 8–12 weeks of age.

All the mice were housed in wire-mesh cages containing 12 animals each, and were fed a diet of Rockland mouse pellets, a rolled oat supplement, and water ad libitum. Toward the end of the experiment, animals developing lesions were segregated and placed in individual glass jars.

Subgroups: The CAF_1 mice were originally divided into two groups, i.e., one group of 112 mice painted with tobacco tars in acetone and a second group of 44 mice receiving acetone alone. Preliminary studies on 60 Swiss mice showed that the animals were sensitive to tars containing nicotine, particularly in concentrated solutions. Being cognizant of the sensitivity of mice to tobacco tars, we began the CAF_1 group cautiously using dilute solutions, the concentrations of which were gradually increased. The tarred group was started with tri-weekly paintings of a residual tar/acetone solution containing 1 part of tar to 3 of acetone. The dosages were gradually increased to 1:2 and finally to 1:1 ratio in 2 months; the latter dosage averaged 40 mg of tar/acetone solution per painting.

Prior to each painting the entire dorsal area of each mouse was shaved of excess hair with an electric clipper, and one saturated brushful of the tar/acetone mixture was applied to the shaved area with a No. 5 camel's hair brush; several strokes were used, beginning at the nape of the neck and working backward to the tail region medially and laterally. The control animals were shaved and painted with acetone in the same manner.

Seven months after onset of painting, 31 of the 102 surviving tarred mice and 14 of the 42 surviving controls on acetone were segregated and given an additional painting per week of 1 per cent croton resin in acetone (Berenblum [1]) in order to determine the cocarcinogenic properties of croton resin in relation to tobacco tars. Each subgroup contained an equal number of males and females. The croton resin solution was found to be extremely irritating to the skin of mice and caused widespread ulceration. Dosages were reduced without relief of this condition. Two months later these animals were changed to a 5 per cent solution of croton oil (Croton Oil N. F. VII, Magnus, Mabee, and Reynard, Inc., New York City) in mineral oil (pure Superla No. 34, Standard Oil Co. of Ind.), which, although much less irritating to the skin, seemed to induce severe diarrhea.

In the 15th month of painting a few of the tar/acetone and tar/croton/acetone animals were placed on a denicotinized 1:1 tar/acetone solution, prepared by extracting the nicotine with 1 per cent hydrochloric acid. This treatment was used only when the animal had an open ulcerating lesion. When the lesion scabbed over, the whole tar/acetone solution was reapplied. Whereas most of the animals with lesions were kept on the whole tar/acetone solution throughout the experiment, it was occasionally necessary to resort to this less toxic treatment with denicotinized solutions in order to preserve the animal. Also, as the animals became weakened by old age or disease, the tar paintings were decreased by painting merely the lesion itself. When a lesion had become a grossly positive carcinoma, the tar paintings were stopped completely. The animal was then allowed to live free of paintings and was sacrificed only when death appeared imminent.

RESULTS

General observations.—The whole tar/acetone solution promoted a general thickening of the skin, but rarely produced lasting areas of complete hair loss. The tar/croton group showed more hair loss, thickening, reddening, and proliferation of epithelium than the regular tar group. The acetone/croton group showed hair loss and thickening and scabbing of the skin, but not to so great a degree as those animals on tar/croton. The skin of the acetone controls remained soft, pliable, and normal throughout the experiment.

A difference in the rate of hair regrowth was noted between the experimental and control groups. Tarred animals regrew hair rapidly between paintings and required regular shaving. The tar/croton and acetone/croton animals remained denuded for longer periods, showing spotty and sometimes complete hair loss. The acetone controls always kept a short stubble of hair; yet they regrew hair at a much slower rate than the animals on tar or on croton oil. Histologic sections on a separate group of CAF_1 mice at 10 days showed hyperplasia, and hyperkeratosis in 75 per cent and disappearance of sebaceous glands in 100 per cent of the animals painted with the tar/acetone solution.[2] None of these changes were observed in animals painted with acetone alone.

Little difficulty was encountered because of drug intoxication, since, as described above, the animals' tolerance to nicotine was increased. In one instance, however, unintentional evaporation of a solution apparently led to an increased concentration of nicotine and brought about a number of deaths in the tar/croton oil group. At the onset of the painting procedure, the mice appeared to be in a partially drugged state and exhibited occasional fine tremors following paintings. These tremors and stuporous states were rarely observed after about 2 months of application, but frequently reappeared in old age until the dosages were decreased.

Papilloma formation.—Of 81 tarred CAF_1 mice, 59 per cent—26 females and 22 males—developed papillomas. The earliest appearance was observed during the 33d week, with a mean time of 56 weeks. Of the papillomas, 8.6 per cent regressed. The lesions were uniformly distributed over the painted area. Twenty-three animals had more than one papilloma (Table 2; Chart 2).

A typical papilloma appeared as a firm, low,

[2] Dr. Victoria Suntzeff, personal observation.

TABLE 2

APPEARANCE OF LESIONS IN CAF$_1$ MICE AFTER PAINTING WITH CONDENSED CIGARETTE TAR, CROTON OIL, AND ACETONE*

Group*	No. alive	Mos. of painting	Papillomas† (Gross) No. of mice	Carcinomas‡ (Gross) No. of mice
T	81	1		
T/C§	51	1		
A	30	1		
A/C§	14	1		
T	69	8	3	
T/C	31	8	1	
A	28	8		
A/C	14	8		
T	69	10	8	
T/C	31	10	2	
A	28	10		
A/C	14	10		
T	62	12	15	2
T/C	18	12	3	
A	28	12		
A/C	12	12		
T	50	14	22	2
T/C	8	14	7	
A	23	14		
A/C	11	14		
T	47	16	39	20
T/C	8	16	10	1
A	22	16		
A/C	11	16		
T	41	18	43	25
T/C	7	18	10	1
A	19	18		
A/C	9	18		
T	26	20	48	31
T/C	4	20	11	2
A	18	20		
A/C	7	20		
T	8	22	48	36
T/C	3	22	13	3
A	16	22		
A/C	3	22		
T	0	24	48	36
T/C	0	24	13	3
A	9	24		
A/C	3	24		

* T = tar/acetone, T/C = tar/croton oil/acetone, A = acetone, A/C = acetone/croton oil.

† This column represents cumulative totals on the first gross appearance of papillomas.

‡ This column represents cumulative totals on the first gross appearance of carcinomas, all of which later were verified histologically. In two cases cancer was shown microscopically to be present in tumors that appeared grossly to be papillomas. In these two cases the month of histologic verification was taken as the date of first appearance.

§ Croton oil was started in the seventh month.

broad-based, clearly outlined wart, varying in size from 0.1 to 0.4 cm. in diameter. In most cases these warts were well-defined nodules, occasionally budding before sloughing and showing definite craters.

Cancer formation.—Of 81 tarred mice, 44.4 per cent developed epidermoid cancer of the skin. The first was noted grossly during the forty-second week; the mean time of appearance was 71 weeks. Of 62 mice still alive in the tar group at 12 months, the time at which the first gross cancer was observed, 58 per cent developed cancer.

Twenty-five of the carcinomas occurred in females and 11 in male mice. The females, although their average weight was about 4 grams less than that of the males, had a longer survival time in the tar group than the males. Of 41 male and 40 female mice started on experiment, at 12 months 34 female and only 28 male mice were alive. At 18 months the respective figures were 26 and 19. In the control group twice as many males lived for 18 months as females.

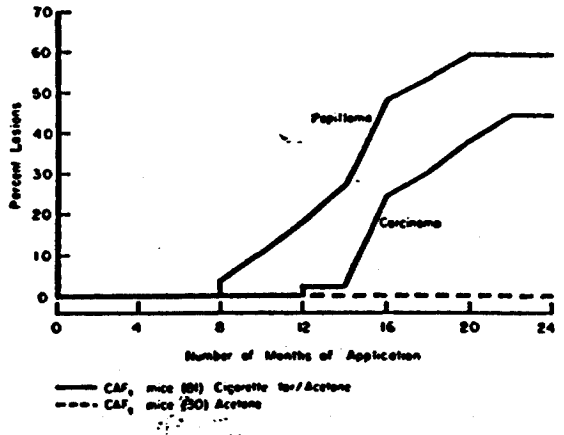

CHART 2.—Month of first gross appearance of papillomas and carcinomas in CAF$_1$ mice painted with a cigarette tar/acetone solution, and a group of CAF$_1$ mice painted with acetone alone. All of these lesions were subsequently proved histologically. In two instances, where a cancer was found in a tumor which appeared grossly to be a papilloma, the month of death was taken as the time of first appearance of the cancer.

Of the cancers, the majority—27 (75 per cent)—had previous papillomatous growths; however, a number arose from localized ulcerating sites. Two cancers, which had been classified as papillomas at autopsy, were discovered upon histological examination. The cancers were always found only on the painted area, and in advanced cases often spread laterally beyond the area of painting. Six mice had two separate skin cancers. Prior to the development of the typical cancer, a localized thickening and ridging of the epidermis surrounding the papilloma in the shape of a rolled, puckering rim was noted. During this process of initial invasion, the papilloma proper sloughed off and left a small ulcerating crater. These craters widened and continued to ulcerate. They frequently became necrotic and grossly infected. The rims and bases also became more fully cratered and hardened. In

the advanced stages the cancers appeared as grossly massive ulcers with definite rims (Figs. 1, 3, 5, 7, 8, 10, 11, 13, 14, 16, 18, 20). Histological examination of these lesions showed the usual characteristics of highly malignant epidermoid skin cancer—downward invasion of epithelial cells with extension through the basement membrane, lack of differentiation, and many typical mitotic figures (Figs. 2, 4, 9, 12, 15, 17).

Tar/croton oil.—The tar/croton oil group was begun in the 7th month after the onset of tar application. Of 31 mice segregated into this group, 42 per cent developed papillomas and 9.7 per cent carcinomas. The lesions did not appear earlier than in the tarred group. The first papilloma was noted in the 8th month, and the first cancer in the 16th month.

It is not possible to evaluate these data because 77 per cent of the animals died between the 12th and 14th months, subsequent to marked weight loss. On one occasion, one group of twelve animals was thought to have received an overdose of tar, resulting from excessive acetone evaporation. This group of animals died after having convulsions.

Acetone group.—Thirty animals comprised this group. Nine—30 per cent—were still living at the end of 24 months, in contrast to 0 per cent in the tarred group. No lesions were noted in this group of mice. The epidermis remained soft and pliable throughout the experiment. The average weight of animals in this group through most of the study was about 1 gm. more than in the tarred mice, although, at the onset of the experiment, the average weight of the mice in the tarred group was 2 gm. greater than in the acetone control group.

Acetone/croton oil group.—A group of fourteen mice originally treated only with acetone received an additional painting of croton oil per week starting at the 7th month. In this group three mice—21 per cent—were still living at 24 months. The epidermis became roughened and thickened following repeated applications of croton oil. No papillomas or carcinomas appeared in this group.

Methylcholanthrene group.—Twenty-five CAF_1 mice were painted with a solution of 0.3 per cent methylcholanthrene in acetone 3 times a week. They all developed carcinomas within 4½ months. The first papilloma was noted during the 6th week, and their average time of appearance was 7 weeks. The respective figures for carcinoma formation were 12 and 16 weeks.

Transplantation.—Two of the cancers in the tarred group have been successfully transplanted. One cancer was transplanted for 4 generations by Dr. V. Suntzeff before it became grossly infected. The method of transplantation was as follows: The tumor was excised from the living host, and small healthy pieces were cut and placed in either a penicillin-streptomycin solution or a saline solution before being transplanted subcutaneously with a sterile trocar to the lateral chest region. The other cancer was transplanted in our laboratory directly from the living animal to another CAF_1 mouse with a sterile trocar without first placing the tumor into a solution. The host and the mouse to receive the tumor were previously injected subcutaneously with 100 units of dihydrocillin;[3] this dose was repeated for 3 days after transplantation. For every third generation since the sixth generation, the tumor has been excised, minced, and soaked in either dihydrocillin or syncrobin[4] for 15–45 minutes prior to transplantation as a protective measure against infection; subcutaneous injections of antibiotic have been omitted. Preliminary data on the rate of growth of this tumor show that transplants without the intermediary soaking in antibiotic grow to approximately 0.5–1 cm. in 15–21 days before ulcerating and being retransplanted, whereas transplants previously soaked in antibiotic grow more slowly (¾–1 cm. in size in 29–43 days). This tumor is still an active epidermoid carcinoma in the 18th generation with 100 per cent takes (Figs. 6 and 19).

DISCUSSION

Evaluation of method of present study.—The basic principle in tar collection was to simulate human smoking habits as closely and as practically as possible. A popular brand of cigarettes was used. The temperature factors were found to be similar to those encountered in human smoking. The frequency of smoking (3 times a minute) is greater than that encountered in the average human smoking. In view of the fact that this increase involved no significant temperature changes, the more frequent puffing rate was chosen for the practical purpose of obtaining the required amount of tar more rapidly and economically.

Since we assumed that the carcinogenic effect of tobacco tars might be owing to a summation effect of subthreshold carcinogens or that such a factor might be in the alkaloid fraction of the tars, the whole condensate was used. Acetone, an established noncarcinogen, was found to be the best solvent for the tobacco tar and, therefore, the tar was administered as a tar/acetone solution.

[3] Combination of streptomycin and penicillin by Upjohn & Co.

[4] Combination of streptomycin and penicillin by Schenley, Inc.

We postulated that tobacco tars have only a weak carcinogenic activity and thus would exhibit a long latent period. Therefore, we started with 8–12-week old mice. Our results clearly indicate that if all the mice had died during the first year of painting or if we had stopped the experiment at that time, the data would have been much less significant. For the same reason, we used a higher dosage schedule than had been used by previous investigators. Subsequent studies may show that an additional increase in dosage may further accelerate tumor formation.

Preliminary studies indicated some difficulties with the tar solution that contained nicotine. This problem could be overcome, however, with the desensitization procedure employed.

The basic factors of the method, therefore, were to collect the tar in a manner simulating human smoking habits, to use the whole tar obtained, and to apply it in relatively large quantitites and for a long time. This approach may be in part responsible for the greater production of cancer in this investigation compared to that of previous studies. The possibility remains that the CAF_1 strain of mice may be particularly susceptible to the carcinogenic effect of cigarette tars.

Effect of croton oil.—Subgroups were painted with croton oil to determine whether tumor formation could be speeded up, as had been indicated by Berenblum (1). The CAF_1 mice received croton oil in mineral oil once a week starting with the 7th month after the painting with tobacco tars was begun. Because of a high mortality during the 12th and 14th months in this group, the croton-oil effect on tumor formation cannot be properly evaluated from this experiment, although within the period of observation no acceleration of cancer formation was noted.

Tobacco versus methylcholanthrene.—Although this study establishes condensed cigarette smoke as a carcinogen for mouse epidermis, its activity is less potent than that of methylcholanthrene. Methylcholanthrene, of course, is a very potent carcinogen, rather than a crude substance in which suspected carcinogens may be diluted.

Although crude tobacco tar is less potent as a carcinogen than methylcholanthrene for mouse epidermis on the basis of the number of cancers induced and the length of the latent period, the fact remains that over 40 per cent of the animals painted with tar from cigarette smoke developed carcinomas, whereas no lesions were observed in the control animals.

Animal versus human data.—Animal data do not necessarily confirm or deny human data, although historically much of our present understanding of carcinogenesis is based on corollary studies between clinical and laboratory research. The studies on coal tars from Pott to Yamagiwa and Ichikawa and to Cook, Hieger, and Kennaway serve as a classical example. In coal tar investigations experimental data confirmed the clinical data, and thus added import was given to both.

A similar relationship now exists in the tobacco tar field. Here, too, a clinical association between smoking and cancer seems established. It has been shown that a condensate of this smoke may induce epidermoid cancer of the skin in the experimental animal. The suspected human carcinogen has thus been proved to be a carcinogen for a laboratory animal.

The most far-reaching effect of this observation, perhaps, does not lie in its immediate relationship to human carcinogenesis, but, rather, in that the proven susceptibility of animals furnishes us with a working tool which may enable us to identify and isolate the carcinogenic agent(s) within the tars.

It is an interesting and perhaps significant fact that with the CAF_1 mice it was necessary to have an exposure to the tar for approximately one-half of the life span of the animals (average 71 weeks). This agrees roughly with the previous finding of Wynder and Graham (40) that the maximum incidence of bronchiogenic carcinoma in the human occurs after 30–35 years of smoking—about one-half of the human life span.

Tobacco as a carcinogen.—It appears surprising that, in view of the detailed work carried on with coal tars and its derivatives, relatively little work has been done with tobacco tars. Because recent clinical data have placed increasing emphasis on tobacco as a human carcinogen, it is our hope that more investigators may approach the tobacco-cancer problem.

Although several hundred chemical and physical agents have been found to induce cancer in animals, each one of these agents seems to have a specific carcinogenic action. Chronic traumatic irritation as such does not induce cancer to any significant degree. Therefore, in view of the positive animal and human data, we suspect that tobacco contains specific carcinogen(s).

Which fraction of the tar is carcinogenic is not yet known. Roffo (31) claimed to have identified benzpyrene in tobacco tar, but this could not be confirmed by Hirst and his co-workers (7), and more recently that substance could not be detected by Waller (38). An examination by Eby[5] of the tobacco tar used in this study did not reveal any spectroscopic evidence of the known carcinogenic aromatic hydrocarbons. Arsenic, an accepted hu-

[5] J. Eby, personal communication.

man carcinogen, is present in tobacco, but recent studies by Daff and co-workers (8) based on the arsenic content of various types of European tobacco tends to place less emphasis on this inorganic element. Heat, cigarette paper, flavoring, and wetting agents have been suggested as etiologic factors in the production of cancer, but it must be noted that clinical evidence has also pointed to cigar-smoking, pipe-smoking, and tobacco-chewing as possible factors in the production of cancer of the respiratory and alimentary tract.

The actual carcinogenic agent or agents in tobacco remain to be identified. Studies combining chemical and biologic efforts leading to their identification are urgently needed. Should one be able to identify definite carcinogens and succeed in removing them, or at least in reducing their quantity in tobacco, proper preventive methods would be at hand. Such studies may further our understanding of human and animal carcinogenesis and may lead to the development of practical preventive measures against cancer.

SUMMARY AND CONCLUSIONS

1. A cigarette tar condensate was obtained with a smoking machine which simulated human smoking habits. The resulting tar was dissolved in acetone and applied to the backs of CAF_1 mice in a dosage of 40 mg. of tar/acetone solution 3 times a week. Control mice were painted with acetone.

2. Of 81 tarred mice, 59 per cent developed papillomas. The first lesion was noted in the 33d week, and the mean time of appearance was 56 weeks.

3. Of 81 tarred mice, 44 per cent developed histologically proved carcinomas. The first carcinoma was observed in the 42d week, and the average time of appearance was 71 weeks. Of 62 mice alive at 12 months, 58 per cent developed cancer. Seventy-one weeks constitutes approximately one-half of the life span of CAF_1 mice. This corresponds roughly with the fact already noted that in the human about 30–35 years of smoking, or approximately one-half the life span, are required for the production of bronchiogenic carcinoma.

4. One carcinoma was transplanted for 4 generations and another one is currently growing in the 13th generation.

5. Control mice painted with acetone alone showed no skin lesions. At the end of 20 months of painting, 53 per cent were still living, compared to 9.8 per cent in the group painted with tobacco tars.

6. The group of mice painted with croton oil in addition to the tar, starting in the 7th month, cannot be properly evaluated because of a greater number of deaths occurring during the 12th and 14th months, although within the period of observation no acceleration of cancer formation was noted.

7. The group of mice started with acetone and receiving croton oil beginning in the 7th month showed roughening and thickening of the epidermis, but no tumor formation was noted.

8. All CAF_1 mice painted with 0.3 per cent solution of methylcholanthrene in acetone developed cancer within 4½ months. The first papilloma appeared during the 6th week, with average appearance during the 7th seek. The first carcinoma was observed during the 12th week, with a mean time of appearance of 16 weeks.

9. The results obtained with CAF_1 mice establish condensed cigarette tar as a carcinogen for mouse epidermis. These studies provide a tool to determine and isolate the possible carcinogenic agent(s) within tobacco tar. At present it is not known which fraction or fractions in tobacco tars are carcinogenic. Combined chemical and biologic studies are now in progress to search for such agents. Such studies, in view of the corollary clinical data relating smoking to various types of cancer, appear urgent. They may result not only in furthering our knowledge of carcinogenesis, but in promoting some practical aspects of cancer prevention.

ACKNOWLEDGMENTS

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Fig. 1.—No. 87 CAF_1. 322 days. Beginning carcinoma.

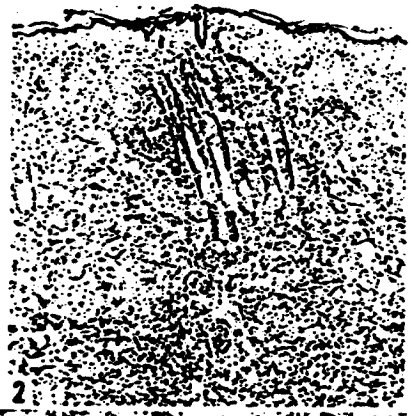
Fig. 2.—Photomicrograph of carcinoma. No. 87 CAF_1 at 371 days. ×52.

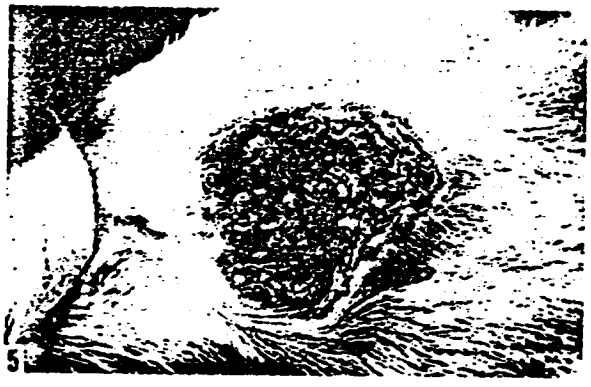
Fig. 3.—No. 87 CAF_1. 371 days. Advanced carcinoma.

Fig. 4.—Photomicrograph of carcinoma. No. 87 CAF_1 at 371 days. ×215.

Fig. 5.—No. 87 CAF_1. 371 days. Advanced carcinoma.

Fig. 6.—Photomicrograph of fourth generation transplant of tumor from No. 87 CAF_1. ×215.





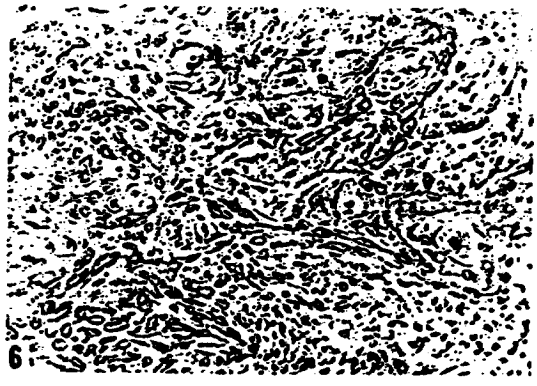


FIG. 7.—No. 37 CAF_1. Early carcinoma (2 separate lesions), 540 days.

FIG. 8.—No. 37 CAF_1. Advanced carcinoma, 590 days.

FIG. 9.—No. 37 CAF_1. Photomicrograph of lower lesion at 590 days. ×285.

FIG. 10.—No. 16 CAF_1. Early carcinoma, 494 days.

FIG. 11.—No. 16 CAF_1. Advanced carcinoma, 564 days.

FIG. 12.—No. 16 CAF_1. Photomicrograph of carcinoma at 564 days. ×200.

FIG. 13.—No. 15 CAF_1. Early carcinoma, 494 days.

FIG. 14.—No. 15 CAF_1. Advanced carcinoma, 605 days.

FIG. 15.—No. 15 CAF_1. Photomicrograph of carcinoma at 605 days. ×200.

FIG. 16.—No. 3 CAF_1. Beginning carcinoma, 451 days.

FIG. 17.—No. 3 CAF_1. Photomicrograph of carcinoma at 564 days. ×210.

FIG. 18.—No. 3 CAF_1. Advanced carcinoma, 494 days.

FIG. 19.—Photomicrograph of third generation transplant of tumor from No. 3 CAF_1. ×210.

FIG. 20.—No. 3 CAF_1. Advanced carcinoma, 564 days.

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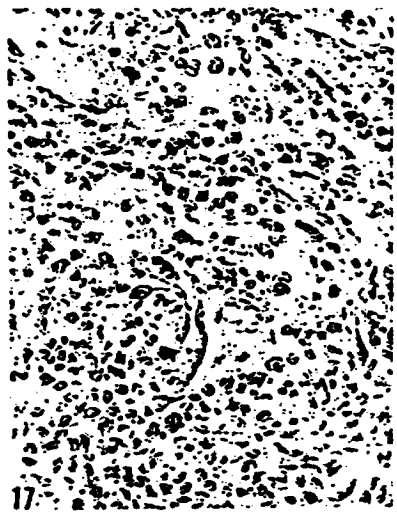

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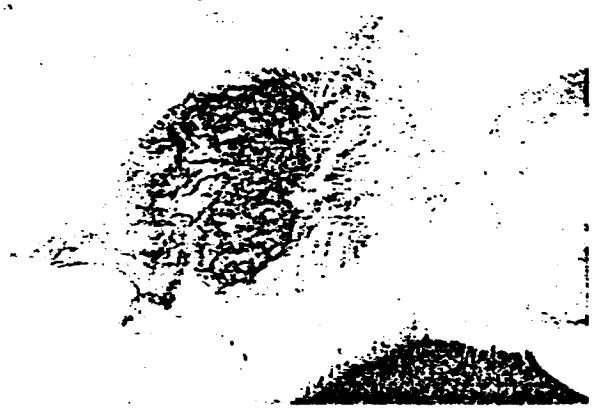

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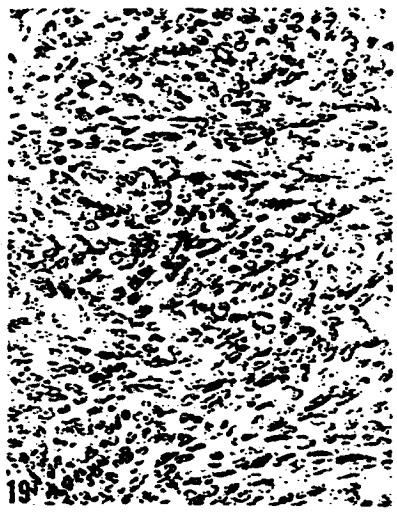

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