

# Experimental Production of Carcinoma with Cigarette Tar

## II. Tests with Different Mouse Strains\*

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In an earlier communication we reported the production of skin cancers in C<sub>3</sub>H mice painted with condensed cigarette tar obtained by the smoking of cigarettes in a machine in a manner similar to human smoking (7). Forty-four per cent of 81 mice painted 3 times a week with this tar developed histologically proved epidermoid cancers. This represented a higher production of skin cancer than had been obtained by other workers applying tobacco tar to mouse skin. We previously outlined possible reasons for the variation in these results, which included different methods of tar preparation, amount and duration of tar painting, and strain of mice used. The present study was undertaken to determine whether the results of our former study were due to the use of a particularly susceptible strain of mice or to the methods employed.

### MATERIALS AND METHODS

Tobacco tar was prepared in the same manner as that used in the previous study. The tar as used, however, was different in one respect. Whereas in our first study it was freshly prepared every 1-3 months, the present study was begun with tobacco tar which had been prepared 6 months previously. Fresh tar was subsequently prepared, but on the average the tobacco tar was about 6 months old at the time of painting. The tobacco tar was stored in acetone in bottles in a refrigerator cooled to 4° C. All other phases of the experiment were as identical as possible to those described previously. The mice were painted 3 times a week with about 40 mg of tar/painting. Tar application continued throughout the life span of the animals.

An attempt was made to evaluate the role of

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croton oil as a cocarcinogen. Applications were made from the very outset, the animals being painted with a 5 per cent solution of croton oil in mineral oil. The mice were painted 3 times a week with tobacco tar (Monday, Wednesday, and Friday) and once a week with the croton oil solution (Saturday).

C57BL mice obtained from the Jackson Memorial Laboratory and Swiss mice obtained from the Rockland Farms, Long Island, New York, were painted concurrently with the same tobacco tar.

### RESULTS

*General observations.*—The Swiss mice tolerated the effect of the tobacco tar better than did the C57, which showed a more significant weight loss after the application of tobacco tar. As the experiment progressed, many of the C57 mice developed benign ulcerations of the skin. The survival rates of the two strains show no essential difference except for the 18-month period, at which time the death rate among the C57 was greater (Table 1).

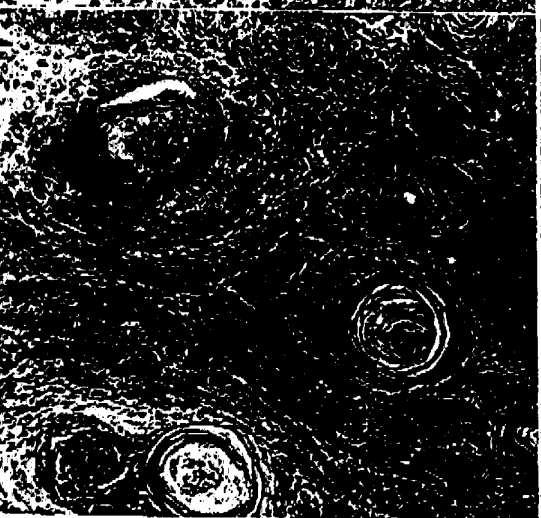
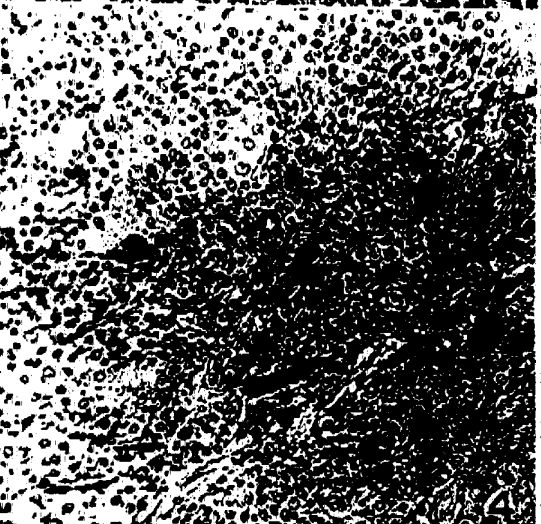
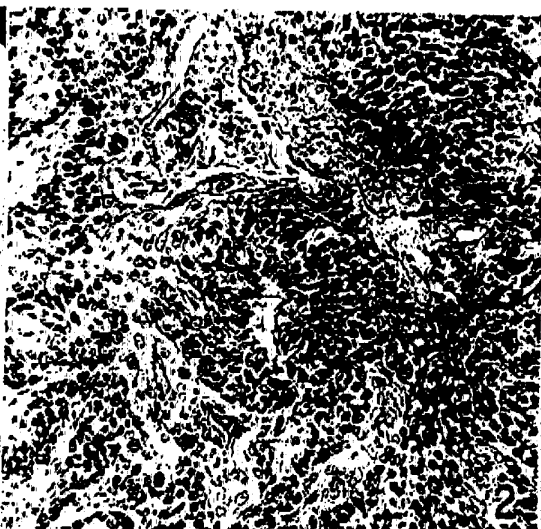
*Papilloma formation.*—Papillomas in the tar group began to appear at the same time in Swiss and C57 mice (8th month) (Table 1, Charts 1 and 2); 26.5 per cent papillomas were observed among Swiss mice and 11.2 per cent in C57 mice—percentages based upon the number of mice with which the experiment was originally started.

*Cancer formation.*—Twelve out of 86 Swiss mice developed histologically proved cancer (14 per cent) compared with only two cancers among 89 C57 mice (2 per cent) by the 24th month (Table 1, Charts 1 and 2; see also Figures 1-6, gross lesions and photomicrographs of histologic sections of representative lesions). Tabulated as percentages of mice still alive at 1 year, these percentages are 18 for Swiss mice and 3.3 for C57.

*Croton oil and tar experiments.*—Twelve Swiss mice received both croton oil and tar applications throughout the experiment. The survival period of these mice was shorter than that for mice

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painted only with cigarette tar (Table 1). Thus, only three mice were alive at 12 months. Four of the original twelve mice had developed papillomas, the first as early as the 5th month, and one developed histologically proved cancer which ap-

sions can be drawn because of the small number of animals used.

Twenty-four C57 mice were used in the cigarette tar and croton oil experiment. By the end of 1 year, nineteen mice were still alive, a number which is comparable to the survival rates of animals painted only with tobacco tar. At the end of the experiment four of the animals had developed papillomas, the first appearing in the 6th month, and one animal developed cancer, appearing first in the 18th month. These data suggest that the additional painting of croton oil may also

TABLE 1  
SUMMARY SHOWING TIMES OF APPEARANCE OF HISTOLOGICALLY PROVED PAPILOMAS AND CARCINOMAS IN C57BL AND SWISS MICE\*

APPLI- CATION:	Month	C57BL mice		Swiss mice		
		No. mice	Pap. Ca.	No. mice	Pap.	Ca.
Tar	1	89		86		
Tar croton		24		12		
Acetone:		12		12		
Tar	2	87		84		
Tar croton		24		10		
Acetone:		12		10		
Tar	4	83		79		
Tar croton		24		6		
Acetone:		11		9		
Tar	6	75		76		
Tar croton		23		5	4	
Acetone:		11		8		
Tar	8	64	1	71	4	
Tar croton		23	1	4	4	1
Acetone:		10		9		
Tar	10	62	2	69	4	
Tar croton		23	2	4	4	1
Acetone:		10		7		
Tar	12	60	2	67	5	
Tar croton		19	2	3	4	1
Acetone:		10		7		
Tar	14	56	3	56	6	
Tar croton		14	2	2	4	1
Acetone:		10		6		
Tar	16	51	3	48	10	1
Tar croton		11	3	1	4	1
Acetone:		10		4		
Tar	18	30	3	44	18	7
Tar croton		10	3	0	4	1
Acetone:		9		3		
Tar	20	19	8	22	20	11
Tar croton		9	3	0	4	1
Acetone:		9		1		
Tar	22	6	8	5	22	12
Tar croton		5	3	0	4	1
Acetone:		8		0		
Tar	24	2	9	0	22	12
Tar croton		2	4	0	4	1
Acetone:		7		0		
Tar	26	0	10	0	22	12
Tar croton		0	4	0	4	1
Acetone:		3		0		

\* In the columns headed "Pap." and "Ca.," the total number of each of the tumors is given up to and including the stated month. The previous occurrences of the designated tumors are included in the totals given for each month.

peared first in the 7th month. These data would suggest that for Swiss mice the additional application of croton oil may accelerate cancer and papilloma formation, though no definite conclu-

CHART 1.—Percentage of papilloma formation in Swiss and C57 mice. The tar used in the painting for the most part was about 6 months old at the time of application and had been in acetone during that period.

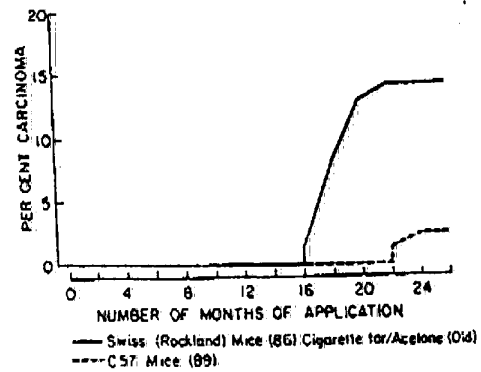


CHART 2.—Percentage of carcinoma formation in Swiss and C57 mice. See legend under Chart 1 for statement of how the tar was handled before use.

accelerate cancer and papilloma formation in the C57 strain.

The earlier experiments with tobacco tar and croton oil applications on CAF<sub>1</sub> mice had shown no significant increase in tumor formation; however, the croton oil applications had been begun only in the 8th month of tobacco tar painting.

Acetone.—None of the animals painted with acetone alone developed any lesions.

The cigarette tar and croton oil experiment. By the end of 1 year, nineteen mice were still alive, a number which is comparable to the survival rates of animals painted only with tobacco tar. At the end of the experiment four of the animals had developed papillomas, the first appearing in the 6th month, and one animal developed cancer, appearing first in the 18th month. These data suggest that the additional painting of croton oil may also

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## DISCUSSION

The present experiment demonstrated that cigarette tar prepared in a manner simulating human smoking habits can induce papillomas and cancers of the skin in strains of mice other than the CAF<sub>1</sub> strain. It has also shown that the number of tumors obtained may vary significantly among different mouse strains.

This study does not afford a reliable comparison with our previous experiment with CAF<sub>1</sub> mice, since the studies were done at different times and with cigarette tar which was not stored in the same manner, though the tar preparation and painting techniques were the same.

This report is presented to show differences in results obtained for Swiss and C57BL mice and to emphasize the importance of standardizing the factors of tar storage and methodology of tar application. As different investigators attempt to repeat our reported cigarette tar experiments, it seems of importance to standardize these points if one wants to obtain comparative results. Only if experimental techniques are as similar as possible can results from one experiment be compared with those of another.

*Storage of tar.*—The only difference, at least to our knowledge, between the CAF<sub>1</sub> and the present experiment is that the cigarette tar in the present study had been standing in acetone solution for about 5 months longer than that used in our first study. This difference, of course, could not affect the observed variations in the results between the Swiss and C57 strains, which were painted with the same "old" cigarette tar, but could conceivably be a factor in making the results of the present experiment different from those of the first (7). It is possible that the carcinogenic activity of the tar may be decreased by long standing. Of course, even the tar used in our first experiment had usually been standing in acetone solution from 1 to 3 months. Wright<sup>1</sup> has made the following comments in regard to this problem:

"Attention must be given to possible deterioration of tobacco tar as a result of aging. Condensation reactions may occur with acetone used as the solvent. The condensation reactions of Knoevenagel type may be enhanced by the presence of the alkaloidal bases. They might be minimized by maintenance at low temperature.

"It seems to be a characteristic of many carcinogens that they undergo quinone-type oxidation in the presence of oxygen. If the oxidation were to precede application before biologic testing, it is reasonable to assume a carcinogen might lose

<sup>1</sup>G. F. Wright, Professor of Chemistry, University of Toronto, Ontario, Canada.

activity. Because of ambient temperature and pressure changes, a glass-stoppered bottle will breathe and thus provide free oxygen for the degradation process. Oxidation could be prevented by maintenance in carbon dioxide atmosphere. Thus it may be advisable to store tobacco tar in an insulated container filled with dry ice."

Later studies, when perhaps the active carcinogen(s) in cigarette tar has been identified, may determine whether these substances lose potency upon standing in an acetone solution. As a precaution we now store cigarette tar in dry form in brown bottles in the refrigerator and prepare the tar/acetone solutions at biweekly intervals as they are needed for painting.

*Methods of tar application.*—The variations in results obtained by different investigators using tobacco tar may be accounted for by several points which will seem obvious to those experienced in techniques of skin painting. In observing different investigators, however, we have been impressed with the variations in techniques with which tobacco tar is applied to the mouse skin. Some workers paint only a small area of the back, some apply the tar with but one stroke of the brush or with a glass rod, while others do not shave the animals between paintings when hair regrowth occurs.

While the brush method of painting cannot be very accurate quantitatively, it can nevertheless be fairly well standardized from experiment to experiment. This seems of particular importance if an investigator attempts to repeat a previous experiment. We always shave the mice prior to each painting if hair has regrown. Using a No. 5 camel's-hair brush, we rub tar into the skin with five or six brush strokes ranging from the nape of the neck to the tail covering the whole width of the back. Thus, we attempt to bring the tar into close contact with a wide area of skin. Such results probably increase the chance of cancer development when one is dealing with a dilute carcinogen.

The results of different investigators, even if using the same tar, can only be compared when the same methods of tar application have been employed. It is for this reason that we call attention to this rather obvious but apparently frequently neglected factor.

*Strain differences.*—C57BL mice have long been regarded as a strain relatively resistant to skin cancer as summarized by Berenblum, though Poel recently found that it is more susceptible to low doses of benzpyrene than either the Swiss or CAF<sub>1</sub> strain (1, 4).<sup>2</sup> Of course, the skin may not only differ in its resistance to cancer formation but may also vary in respect to different carcinogens.

<sup>2</sup>Personal communication, 1955.

The Swiss mouse, being genetically impure, can be regarded as a less reliable index of comparison for results of different investigators.

The results of the Swiss and C57 mice painted with the identical tar clearly demonstrate that the C57 strain would be a poor strain to use for these studies. Our observation may account for the relatively negative findings of Sugimura, who used C57 mice in his tobacco tar experiments in 1940 (5).

*Significance of animal-cigarette research.*—The ultimate proof of whether or not a given agent causes cancer in man rests exclusively on clinical, statistical, and incidence data obtained from man. The studies relating lung cancer to cigarette smoking in all of these respects are now so extensive and well confirmed that there is no disagreement among workers who have actually investigated this subject that there is a causative relationship between cigarette smoking and lung cancer in man (2, 3, 6). An animal experiment cannot significantly add and certainly cannot detract from these human observations. The purpose of the laboratory experiment is chiefly to identify active carcinogenic components which must be in cigarette tar. Such a procedure can only be done through laboratory investigations. It can only be assumed that the carcinogen(s) isolated for the animal will also be the carcinogen active for man. The fact that in tobacco research the animal experiment is in line with the human experience emphasizes this possibility.

#### SUMMARY

1. Swiss and C57 mice were painted with condensed cigarette smoke in a manner similar to that previously reported for CAF<sub>1</sub> mice, with the exception that the tar had been standing in acetone for a longer period of time in the second experiment.

2. Among 86 Swiss mice 22 papillomas and twelve carcinomas were noted. The results for C57 mice were ten papillomas and two carcinomas among 80 mice.

3. The data show Swiss mice to be a more susceptible strain to cigarette tar painting than C57 mice.

4. Application of croton oil, in addition to cigarette tar, appears to accelerate the formation of lesions in Swiss and C57 mice.

5. The possibility is discussed that the higher percentage of lesions previously reported among CAF<sub>1</sub> mice could be on the basis that that experiment was conducted with tar that had been standing in acetone for a shorter period of time before use than that used in the second experiment.

6. It is suggested that in experiments using condensed cigarette tar as a possible carcinogen the tar be stored refrigerated in undiluted form, in brown bottles, and that the solutions to be used for the applications be prepared at biweekly intervals.

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FIG. 1.—No. 392 Swiss mouse, carcinoma, 591 days.

FIG. 2.—No. 392 Swiss mouse, photomicrograph of carcinoma.

FIG. 3.—No. 401 Swiss mouse, carcinoma, 201 days.

FIG. 4.—No. 401 Swiss mouse, photomicrograph of carcinoma.

FIG. 5.—No. 221 C57 mouse, carcinoma, 712 days.

FIG. 6.—No. 221 C57 mouse, photomicrograph of carcinoma.