## CARCINOGENIC ACTIVITY OF SOOT FROM AIRPLANE ENGINES IN EXPERIMENTS ON ANIMALS

A. B. Linnik, G. A. Smirnov, and L. M. Shabad\*

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The carcinogenic activity of soot from airplane engines, of both piston and turbojet types, was demonstrated in experiments on  $C57 \times CBA$  hybrid mice.

The carcinogenic properties of soot have been known for a long time [10, 11]. Investigations [2, 3, 6, 8, 12] have shown that products of incomplete combustion of automobile fuel, when liberated into the human external environment, contain carcinogenic polycyclic aromatic hydrocarbons, notably benzpyrene (BP), and can induce tumors in experiments on animals.

A study of BP in soot discharged by airplane engines [5] showed that these engines expel between 2 and 4 mg BP per minute of work. The highest content of BP was found in soot from piston engines, and rather less in that from turbojet engines.

This is the first study of airplane engines as a source of carcinogenic pollution of the environment. Remembering that soot from various types of airplane engines can contain not only BP, but also other carcinogenic and cocarcinogenic substances, as well as substances inhibiting carcinogenesis, it was decided to investigate the carcinogenic activity of several samples of airplane soot in experiments on animals.

## EXPERIMENTAL METHOD

Several kilograms of soot were obtained from piston and turbojet airplane engines. The soot was extracted with benzene in Soxhlet apparatuses for 12 h. The resulting extract was concentrated by evaporation, and the BP concentration was determined by a fluorescence-spectral method [4]. The BP concentration in soot from piston engines was 30 mg/kg and in soot from turbojet engines 27 mg/kg.

Benzene solutions with a BP concentration of 0.1% were prepared for the experiments on animals.

The tests were carried out on first generation C57 × CBA hybrid mice. BP solution was applied to the shaved skin of the interscapular region for 4 months (twice a week for the first month, 3 times a week thereafter). The animals of all groups received 50 applications each.

The experimental animals were divided into 4 groups: 1) receiving extract of soot from turbojet engines (33 mice), 2) extract of soot from piston engines (33 mice), 3) 0.1% solution of BP in benzene (technical control) (34 mice), and 4) pure benzene (control) (20 mice).

Development of hyperkeratosis, flaking of the skin, and loss of hair were observed in animals of the first 3 groups soon after the beginning of treatment.

The first papillomas appeared in the animals of group 2 in the 11th week after the beginning of application; papillomas were found in groups 1 and 3 in the 13th week. The tumors soon became malignant (Fig. 1).

\*Academician of the Academy of Medical Sciences of the USSR.

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Fig. 1. Large carcinoma of the skin developing in a mouse following application of soot from an airplane engine to the skin. Small papilloma visible above.

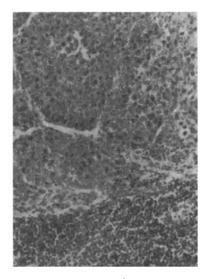


Fig. 3. Metastasis of squamous-cell carcinoma in a lymph gland. Mitoses. Hematoxylineosin,  $300 \times$ .

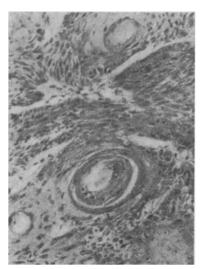


Fig. 2. Squamous-cell keratinizing carcinoma of the skin. Hematoxylin-eosin,  $300 \times$ .

Of the 33 animals (Table 1) in group 1, 4 died at the beginning of the experiment and the remainder had skin tumors by the end of the experiment.

At the time of appearance of the first papillomas, group 2 contained 29 mice, and by the end of the experiment all of these animals also had tumors.

Four of the 34 mice in group 3 died before the appearance of papillomas, and of the 29 mice surviving to the end of the experiment, 96.5% had developed tumors of the skin.

Irritation of the skin was more severe in the mice treated with soot extracts than in the animals of the control groups. The animals of these groups also weighed less than the control animals. Extracts of soot evidently had a marked toxic action. The most severe toxic action (irritation of the skin, retardation in weight) was observed in mice treated with extract of soot from a piston engine.

## EXPERIMENTAL RESULTS

The experimental results are shown in Tables 1 and 2.

As Table 1 shows, in all 3 groups of experimental mice all animals developed tumors. Nearly all the tumors were malignant (96.5% in group 1, 100% in group 2, and 93% in the technical control group). It can be expected that if the ani-

mals which died early had survived longer, they would also have developed malignant tumors. Metastases were found in groups 1 and 2 in 6 of the 52 mice (i.e., 11.5%, within the usual range of incidence of meta-tasization of experimental skin cancer).

Microscopic examination frequently revealed diffuse and focal hyperplasia of the epithelium and adenomatosis of the sebaceous glands in the skin in those parts which were treated with soot extracts and BP solution. In these cases the epithelium became stratified and the dermis also was frequently thickened.

At the beginning of the process, solitary and multiple papillomas with the characteristic microscopic structure were formed, and these subsequently changed into malignant tumors. In some places the beginning of infiltration could be observed, and the epithelium began to invade the deeper layers of the dermis.

TABLE 1. General Results of Experiments

Group	Extract	Number of animals at beginning of experiment	Time of appearance of first tumor (weeks)	Number of animals at time of ap- perance of first tumor	Number of animals with tumors	
					total	malignant
1	Soot from turbojet engines	33	13	29 (100%)	29 (100%)	28 (96.5%)
2	Soot from piston engines	33	11	29 (100%)	29 (100%)	29 (100%)
(Technical control)	BP solution 0.1%	34	13	29 (100%)	28 ( <b>96.5%)</b>	26 (93.0%)
(Control)	Benzene	20		20	0	0

TABLE 2. Distribution of Tumors by Types

		Mal			
Group		squamous-cell carcinoma of the skin	sarcoma	carcinoma and sarcoma	Metastases of carcinoma in lymph glands
1	1	26	0	2	4
2	$egin{pmatrix} 0 \ 1 \ \end{matrix}$	26 52	1 1	$rac{2}{4}$	$\frac{2}{6}$
3 (Technical control)	2	23	0	3	1

Malignant tumors of the skin were most commonly squamous-cell carcinomas with a varying degree of keratinization (Fig. 2). Sometimes separate epithelial pearls were found in the layer of epithelial cells, while at other times an apparently amorphous mass of keratin was present in the middle of the layer of epithelial cells. Less frequently, a squamous-cell carcinoma with very slight keratinization occurred. The cells progressively lost their differentiation and mitoses appeared more and more frequently. They were particularly numerous in one case (with metastases).

In many cases the tumor grew through the dermis and invaded the subjacent muscular layer, destroying the muscles. In a few cases, besides the primary focus of the tumor, metastases also were found in the regional lymph glands (Fig. 3). Sometimes mitoses also were frequent in these metastases, and sometimes they were highly keratinized.

Besides the squamous-cell carcinoma of the skin, in some cases (Table 2) a sarcoma was found. Sometimes groups of cancer cells were found among the tissues of the sarcoma. These cases were classified as carcinosarcoma.

The microscopic investigation thus confirmed physicochemical data indicating that soot from airplane engines contains carcinogenic substances. It is clear from Table 2 that the principal lesion obtained was a squamous-cell carcinoma with keratinization (52 of 58 cases). In 1 case a sarcoma developed in an area of skin to which soot extract had been applied, and in 4 cases the animal developed a carcinosarcoma. In 6 cases, metastases of carcinoma of the skin were found in the lymph glands.

In the control group, receiving a pure solution of BP, similar results were obtained: carcinoma of the skin in 23 of the 28 cases and carcinosarcoma in 3 cases. No skin tumors appeared in the "pure control" group.

The first conclusion to be drawn from this investigation is that the animal experiments reliably demonstrated the carcinogenicity of the samples of soot tested from airplane engines of the two main types.

The slight variations in the times of appearance of the first papillomas, in the writers' opinion, are of no significance. On the other hand, soot extracts were found to be more toxic than the pure BP solution; this was particularly true of soot from the piston engine, and it was manifested by a decrease in the body weight of the animals.

The results of these experiments clearly demonstrated yet again that the BP concentration is a reliable indicator of the carcinogenicity of a given product or mixture. If the presence of BP is established in such substances by the fluorescence-spectral method, a conclusion regarding their carcinogenicity can be drawn.

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