Carcinogenicity Studies of Estonian Oil Shale Soots

by A. I. Võsamäe*

Several series of chronic experiments in white mice and white rats were carried out in order to determine the carcinogenicity of Estonian oil shale soot as well as the soot from oil shale fuel oil. All the investigated samples of soot showed a relatively low (from 14 to 1200 ppm) benzo(a)pyrene content.

The benzene extract of oil shale soot, painted on the skin of white mice, proved to be strongly carcinogenic: in most of the animals skin tumors developed. The benzene extract of shale fuel oil exerted a considerably weaker carcinogenic action than the extract of soot of solid oil shale.

The effect of oil shale soots as well as of tars extracted from oil shale soot on the bronchial mucosa and lung respiratory tissue was studied in white rats. Lung tumors were induced in a considerable number of cases in a series of experiments, where the tarry material from solid fuel soot, containing 1070 ppm benzo(a)pyrene, as a suspension in an aqueous solution of Tween 40, was administered by repeated intratracheal instillation. Lung tumor incidence among various control and experimental groups of rats is presented.

The peculiarities of oil shale combustion are described.

In the power economy of the Estonian S.S.R. and of the northwestern districts of the U.S.S.R., local fuels, i.e., oil shale (solid fuel) as well as fuel oil obtained from the thermal processing of oil shale, have found a wide utilization.

Because of the peculiarities of the content of kukersite oil shale (abundance of mineral substances and a high content, about 82%, of volatile substances in its combustible part) a great quantity (45–75% of the weight of shale oil) of ash results from combustion of oil shale. Especially in domestic stoves and furnaces, ash hinders the complete combustion of oil shale and, as a result, the products of incomplete combustion—soot and tars—develop and smoke, thus generating contaminants of the ambient air. Such considerable contamination of ambient air with the products of incomplete combustion of solid oil shale fuel has existed for a long time in several districts of the Estonian S.S.R., until the distant heating plants were introduced about 15 years ago.

Fuel oil obtained from the processing of oil shale, as all furnace oils, results in considerably less evolution of incomplete combustion products into the ambient air than does the solid oil shale fuel.

At present, the rising incidence of lung cancer in many countries is shown to be connected with the contamination with smoke wastes of the atmosphere. Our first studies were carried out to establish the carcinogenicity of samples of soot obtained from combustion of kukersite oil shale (solid fuel) or from combustion of oil shale liquid fuel (mazut).

In carrying out the experiments the customary methodology of chronic experiments in mice was employed: the skin was painted with benzene extracts of the investigated samples of soot. In the first series a benzene extract of solid oil shale fuel soot was painted on the skin of 100 white mice in the interscapular region twice a week, 50 times altogether, over a period of 5 months and 20 days. The second series of experiments was carried out on 188 mice with applications of the benzene extracts of soot from oil shale liquid fuel (mazut). The results of these experiments showed that the soot of solid fuel exerted a marked carcinogenic action.

Of 100 mice, 74 survived the development of the first skin papilloma and the majority of them (58 mice, that is, 78% of cases) showed skin tumors (Fig. 1). Most were malignant skin neoplasms (carcinomas in 36 cases, 9 of them with metastases to the lymph nodes, Fig. 2). The results of the second series of experiments with benzene extracts of soot from oil shale fuel oil soot demonstrated a moderate

* Laboratory of Morphology, Institute of Experimental and Clinical Medicine, Tallinn, Est. S. S. R.
carcinogenic action. This action was considerably less marked than that of the soot from the solid oil shale fuel. Skin tumors developing locally after painting on soot from oil shale mazut were chiefly benign, and most of them underwent regression after application of the benzene-soot solutions ceased. Of 141 mice surviving the 5-month period from the beginning of experiment, only 9 mice showed skin tumors at autopsy (1, 2).

The content of benzo(a)pyrene (BP) in both samples of soot used in our present experiments was confirmed by fluorescence-spectrography analysis. The soot of solid fuel was found to contain 14 ppm of BP, and the benzene extract of this soot contained 0.04 mg BP/ml extract. In the soot from the oil shale liquid fuel the BP content was 1200 ppm, far more than in the sample of soot from solid fuel.

The results of the above experiments in mice proved the carcinogenicity of oil shale soot. Hence, the question arises whether it is possible to induce tumor formation in the lungs of rats by applications of oil shale soot or tar substances extracted from oil shale soot.

From the literature (3–5), we know that to eluci-date the role of carcinogenic contaminants of the ambient air in lung tumorigenesis in man and to combat such air contamination it is of importance to produce an adequate experimental lung cancer model. Such a model has been produced in rats and in hamsters by using applications of potent pure carcinogens, such as BP, 7,12-dimethylbenz(a)anthracene, and 3-methylcholanthrene.

In our investigations we first studied the possibility of inducing lung tumors in albino rats by intratracheal application of oil shale soot and tars extracted from it.

In lung tumor induction experiments randomly bred albino rats were used. The rats were obtained from the Rappolowo Laboratory Animal Production Farm of the Medical Academy of Sciences of the U.S.S.R. According to the literature data (6) as well as data from our control studies, the rats from the farm do not develop spontaneous epithelial tumors of the lung.

Oil shale soot and tars extracted from this soot are to be estimated by their PB content as weak carcinogenic agents. Therefore, in order to discover any carcinogenic effect of these substances in rat
lungs, we decided to combine their action with some chemical or physical factors, capable of inducing lung tumors in rats (ethyurethane, ionizing radiation) or known to have carcinogenic activity (using Tween 40 solution).

In the first series of experiments on 597 white rats, repeated intratracheal instillations of several samples of oil shale soot or tars isolated from them were combined with long-term oral administration of urethane. In the control experiment, urethane alone was administered orally to the rats (in a dose of 50 mg, 3 times a week, over a 16-month period from the beginning of the experiment). Epithelial lung tumors developed only in 9 rats of 145 animals surviving the period of experimentation. In seven of these cases there were alveolar adenomas, and in two cases keratinizing lung epitheliomas were seen: a benign one, and the other an epidermoid carcinoma, which invaded the lobar branch of the pulmonary artery.

In the experimental groups of rats, in addition to urethane, the following substances were administered: (a) oil shale mazut soot with a BP content of 200 ppm in a dose of 10 mg, twice, suspended in 0.5 ml of physiological saline solution; (b) oil shale solid fuel soot with admixture of fly ash and with a BP content of 4.1 ppm, in a dose of 20 mg twice, suspended in physiological saline solution; (c) oil shale solid fuel soot with a BP content of 107 ppm in a dose of 2 mg in 0.2 ml of peach oil, three times.

In none of these experimental groups could we notice any significant rise in the frequency of lung tumors as compared with that in animals receiving only urethane. The administration into the lungs of tars extracted from soot in peach oil did not induce any tumors in the lungs.

The results of the present study are in agreement with the data of several authors, who attempted to produce lung tumors using intratracheal applications of varying samples of soot, coal tar, and non-radioactive dusts and received negative results (3, 7).

In further studies our attention was mainly to producing conditions for penetration and deposition of carcinogenic substances in rat lung tissue. Literature data (8–11) have shown that detergents may be physical modifying factors that may favor the penetration of carcinogens into tissues and thus increase the effects of small doses of carcinogens. Guided by these data, in the second series of experimental studies we instilled tars extracted from a sample of oil solid fuel soot (the BP content 107 ppm) in two different suspension media intratracheally into rats.

Tars in a dose of 100 mg in 12.5% aqueous solution of Tween 40 were administered intratracheally 10 times, at one week intervals in rats of group 1 (125 animals) and tars in peach oil were administered to rats of group 2 (100 animals). After the intratracheal instillations were stopped in the 5th or the 6th month of the experiment, some of the animals from both groups were exposed to a single total-body x-ray irradiation in a dose of 400 R.

In the control groups, 29 rats received intratracheally Tween solution only, and 27 rats received peach oil. These control animals did not develop any epithelial lung neoplasms. The animals of the third control group were exposed only to x-ray irradiation in a dose of 400 R, and in two of these adenomatous lung tumors were found.

Statistical analysis of the results of the experiments in this present series showed that the total body irradiation of rats after preliminary intratracheal administration of tars from soot in Tween 40 or in peach oil did not exert any significant effect on the frequency and characteristics of lung tumors. The latter depended only on the character of the

![Figure 3. Proliferating keratinizing epithelioma ("inverted" papilloma) of uncertain malignancy in the lung from rat that died 19 months after the start of intratracheal instillations of tar substances from oil shale solid fuel soot in Tween 40: (a) peripheral area of the tumor (×240); (b) central area of the same tumor (×120).]
mals out of 57 developed epithelial lung tumors, all benign ones.

The results of our study confirm the possibility of the induction of lung tumors by the action of tars from oil shale soot, i.e., from material containing only a small amount of BP. On the other hand, the present experiments indicate that the carcinogenic action of repeated intratracheally administered tars from soot can be considerably varied by noncarcinogenic substances used as suspension media for the carcinogen.

REFERENCES
1. Võsamäe, A. On the carcinogenic action of combustible schist soot on mice. Vopr. Onkol. 4 (4): 408 (1958).
2. Võsamäe, A. On the blastomogenic action of the Estonian shale oil soot of liquid fuel obtained from the processing of shale oil. Acta Unio Internat. Contra Cancrum 19 (3/4): 739 (1963).
3. Shabad, L. M. Pathology and pathogenesis of experimental lung tumours with reference to human lung cancer prevention. In: Lung Tumours in Animals (Proc. 3rd Quadrennial Conference on Cancer, Perugia 1965), Univ. of Perugia, 1966, pp. 85-100.
4. Stewart, H. H. Comparison of histologic lung cancer types in captive wild mammals and birds and laboratory and domestic animals. In: Lung Tumours in Animals (Proc. 3rd Quadrennial Conference on Cancer, Perugia 1965), Univ. of Perugia, 1966, pp. 25-58.
5. Roe, F. J. C. The relevance and value of studies of lung tumours in laboratory animals in research on cancer of the human lung. In: Lung Tumours in Animals (Proc. 3rd Quadrennial Conference on Cancer, Perugia 1965), Univ. of Perugia, 1966, pp. 101-126.
6. Ird, E. A., and Konoplev, V. P. Spontaneous tumours in rats from Laboratory Animal Productive Farms of the Medical Academy of Sciences of the U.S.S.R. Vestn. Akad. Medits. Nauk. S.S.S.R., 1962 (11): 89 (1962).
7. Roe, F. J. C., and Walters, M. A. Some unsolved problems in lung cancer etiology. In: Progress in Experimental Tumor Research. Vol. VI., F. Homburger, Ed., Karger, Basel-New York, 1965, pp. 127-227.
8. Setalä, H. Tumor promoting and co-carcinogenic effects of some nonionic lipophilic hydrophilic (surface active) agents. An experimental study of skin tumours in mice. Acta Path. Microbiol. Scand. (Suppl.) 115: 1 (1956).
9. Setälä, K. Mechanism of experimental tumorigenesis. X. Problems of methods in studies of artificial carcinogenesis in mouse skin. Acta Pathol. Microbiol. Scand. (Suppl.) 155: 5 (1962).
10. Herrold, K. M., and Dunham, L. J. Induction of carcinoma and papilloma of the tracheobronchial mucosa of the Syrian hamster by intratracheal instillation of benzo(a)pyrene. J. Nat. Cancer Inst. 28: 467 (1962).
11. Rigdon, R. H.. Effect of methylcholanthrene on the respiratory tract of the white Peking duck. Arch. Pathol. 68: 578 (1959).
12. Shabad, L. M. Experimental cancer in the lung. J. Nat. Cancer Inst. 28: 1305 (1962).