

# EXPERIMENTAL INDUCTION OF LUNG CANCER IN RATS BY INTRATRACHEAL ADMINISTRATION OF 9, 10-DIMETHYL- 1,2-BENZANTHRAcene

L. N. Pylev

Laboratory for Research on Carcinogenic Substances (Head –  
Corresponding Member, Academy of Medical Sciences, USSR,  
Professor L. M. Shabad) Institute of Experimental and Clinical  
Oncology (Director – Active Member, Academy of Medical  
Sciences, USSR, Professor N. N. Blokhin), Academy of Medical  
Sciences, USSR, Moscow  
(Presented by Active Member, Academy of Medical Sciences, USSR,  
A. D. Timofeevskii)

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In the course of the last decades numerous reports have appeared in the literature concerning the increase in the incidence of lung cancer. For example, according to Doll [7] the lung cancer mortality increased in England in the period between 1900 and 1953 forty three times. In other countries the lung cancer mortality constitutes 1/7th and up to 2/3rds of the mortality observed in Great Britain. In the USSR, the lung cancer morbidity and mortality increased in the course of the last 50 years approximately ten times [2]. All these facts force the research worker to study again and again the causes for this phenomenon. At present, the majority of authors regard chemical substances, which enter the human body from the contaminated air and during the act of smoking, as the main etiological factor leading to lung cancer. In this context the endeavor to create an adequate experimental model for human lung cancer is fully understandable.

## The Development of Lung Tumors in Rats Dying Five Months after the Beginning of the Experiment

Number of DMBA injections	Total dose of the car- cinogenic substance (in mg)	Cortisone	Number of animals used for the experiment	Total num- ber of suc- cumbing animals	Among them animals dying after five months and later	Number of animals suffering from lung cancer
A single injection	2.5	Administered	75	14	7	—
		Not administered	80	2	2	—
Three injections	6	Administered	115	37	16	3
		Not administered	140	9	4	1
Five injections	10	Administered	63	43	30	8
		Not administered	25	18	10	1

A great number of papers have been devoted to this problem.

Painting of the skin of mice with coal tar, injection of tar and, later, of pure carcinogenic substances into the rectum or into the vagina of mice, led to the development of pulmonary adenomas in these animals [9]. Notwithstanding the prolonged duration of the experiments, no lung cancer developed in the mice. Experiments involving

the direct administration of the substances into the lungs were more successful. Andervont [4] introduced a thread drenched with 1,2,5,6-dibenzanthracene into the lungs of mice and produced, in addition to pulmonary adenoma, squamous cell carcinoma. Niskanen [10] induced squamous cell carcinoma in rats by operative introduction of 1,2,5,6-dibenzanthracene solution in olive oil through the wall of the trachea. Kuschner and co-workers [8] introduced into the trachea of rats a ball, drenched with 3-methylcholanthrene, and induced a squamous cell carcinoma in the lung. Blacklock [5] described lung cancer in rats after the administration of 3,4-benzpyrene, methylcholanthrene and concentrated cigarette smoke into the "exposed" tissue.

All these experiments with carcinogenic hydrocarbons however, devolved a more or less severe trauma to the animal and the method of administration was different from the natural entrance into the lung of substances, capable of inducing the development of a tumor. In recent years, altogether a single paper appeared, the authors of which made an attempt to approach as closely as possible the natural conditions under which the carcinogenic substance enters the lung almost permanently in small doses during the breathing [6]. The authors administered 9,10-dimethyl-1,2-benzanthracene (DMBA) and condensed tobacco tar by intratracheal intubation into Syrian golden hamsters; for 15-20 weeks they administered 50-100 mg DMBA and 500 mg tobacco tar once in a week. In experiments in which pure tobacco tar was used, no tumors developed.

Administration of DMBA into 102 animals was followed by the development of tumors in the larynx, the esophagus, the trachea and also by a case of adenocarcinoma in the main bronchus. In the experiments the importance of the dose in which the substance in question had been administered became apparent: the majority of tumors developed after the administration of the highest dose of the carcinogenic substance (2 mg).

In view of the fact that at present no data concerning the systematic development of lung cancer in animals after intratracheal administration of carcinogenic hydrocarbons are available [3] we set ourselves the task of studying the changes developing in the lungs of rats after insufflation of a carcinogenic substance, and also tried to produce tumors adequate to human lung cancer.

In recent years, reports have appeared in the literature according to which the hormone of the adrenal cortex: cortisone, inhibits the growth and the development of connective tissue and lymphoid tissue. After the administration of the carcinogenic substance into the tissue, first of all sarcomas and only later, tumors originating from the epithelial tissue develop.

In view of this fact, we decided to use cortisone for the induction of lung cancer. A similar successful attempt was made by T. Gurskii (1959) in the laboratory of L. M. Shabad to produce pancreas carcinoma in mice after direct administration of the carcinogenic hydrocarbon into the gland.

#### EXPERIMENTAL METHOD

Rats not belonging to a definite strain and rats of the "Wistar-strain" were used for the experiment. DMBA was used as carcinogenic substance. The experimental animals were divided into four groups: which were given 1, 3,

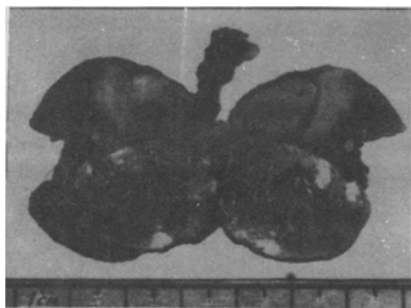


Fig. 1. Squamous cell carcinoma of the lung developing ten months after the beginning of the experiment after five injections of DMBA in a dose of 2 mg.

and 5 injections of the substance respectively; the first group served as control. The carcinogenic substance was administered in a dose of 2.5 mg, in the case of single insufflation and in a dose of 2 mg in the case of repeated insufflation once a month. Within each group the animals were divided into two subgroups, in one of which the animals were given 8-10 days after the beginning of the experiments cortisone in a dose of 5 mg twice a week by subcutaneous injection. The carcinogenic substance was administered into the lungs by intratracheal intubation in the shape of a suspension in colloidal infusin. The suspension was prepared *ex tempore*. 2 mg DMBA were weighed into a porcelain mortar and indian ink powder was added to mark the carcinogenic substance. 0.2 ml colloidal infusin was added to the mixture which was thoroughly triturated and sucked up into the syringe. Under a light hexenal narcosis (sol. hexenali 10% - 0.02 ml by subcutaneous injection) the rats were placed into a specially made box with an opening for the head. The head was fixed. An ear tube was introduced into the mouth through which the strip of light from a forehead reflector showed the epiglottis, the entrance to the larynx and the vocal cords. A needle, the point of which had been filed off, was introduced into the entrance of the trachea and through this needle the prepared suspension was administered with the aid of the syringe. This method secures full control of the administration and also enabled us to carry out repeated intubation.

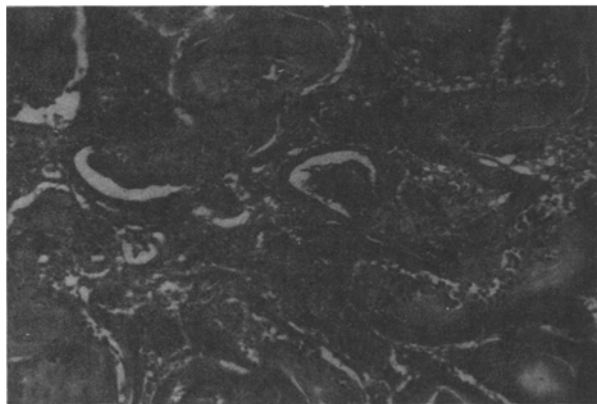


Fig. 2. Squamous cell carcinoma of the lung developing ten months after the beginning of the experiment after three injections of DMBA in a dose of 2 mg, hematoxylin-eosin stain, magnification  $\times 130$ .

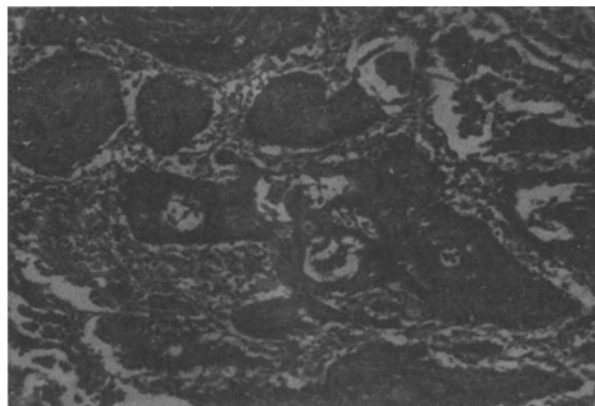


Fig. 3. Squamous cell carcinoma of the lung developing ten months after the beginning of the experiment after five injections of DMBA in a dose of 2 mg, hematoxylin-eosin stain, magnification  $\times 130$ .

#### EXPERIMENTAL RESULTS

Part of the animals is still alive today. The preliminary results obtained are set forth in the table.

As the first lung tumor was observed in a rat which succumbed five months after the beginning of the experiment, we investigated animals which perished after that period in the greatest detail. At present we have investigated 69 animals which died within five months after the beginning of the experiment and later (up to 11 months). In the lungs of 13 rats, tumors were found which, with the naked eye, represented nodes of different size varying from several mm to 1.5-2 cm in diameter. The middle and lower lobe of the right lung were most frequently affected. The small nodes were arranged along the periphery of the lobes in the thickness of the pulmonary tissue and could be discovered only by transection of the tissue. The greater nodes were elevated over the surface of the lungs. Usually they were of a greyish color, solid and in the center of the greater nodules; sometimes cavities containing detritus could be seen (Fig. 1).

Histological investigation of the lung showed proliferation of stratified keratinized squamous epithelium, which infiltrates the surrounding pulmonary tissue forming keratin beads. The tumor develops from the epithelium which constitutes the mucosa of the bronchi and bronchioli.

The cells of the tumor were large and of irregular shape. Figures of mitotic division could frequently be seen. The tumor infiltrates the pulmonary tissue and destroys the surrounding blood vessels; in consequence the tumor is surrounded by a zone of hemorrhages. In the depth of the tumor and in its surroundings, as a rule indian ink particles can be seen, which indicate the presence of the carcinogenic substance. The histological picture warrants the diagnosis of keratinized squamous cell carcinoma of the lung (Figs. 2, 3).

Autopsy of 69 animals which perished in later periods after the beginning of the experiment thus led to the discovery of 13 lung tumors which, on histological investigations, proved to be squamous cell carcinoma. The greater the dose of the substance administered the greater the number of tumors developing in the animals. In animals which had been given a dose of 2.5 mg DMBA, tumors did not develop until the moment of investigation whereas a dose of 6 or 10 mg was followed by tumors in 21.6% of cases. It seems that 6 mg is the most effective dose as a dose of 10 mg proved toxic for many rats (see table). For example, in the second series of experiments in which the animals were given 6 mg DMBA, 46 out of 255 animals had perished within 11 months ( $18 \pm 1.4\%$ ), whereas in the third series of experiments in which the animals were given 10 mg DMBA, 61 out of 88 rats perished ( $69 \pm 5\%$ ).

The influence of cortisone upon the development of lung tumors requires further study.

#### SUMMARY

9,10-Dimethyl-1,2-benzanthracene was introduced intratracheally to 3 groups of rats in a dose of 2 mg. — once, thrice and 5 times with one month interval. An examination was made of the animals which perished 5 months from the beginning of the experiment. Squamous cell cornifying cancer was revealed in 13 of the 69 dead rats. The possibility of inducing cancer of the lungs in experimental animals largely depends on the dose of the substance administered and the choice of the site of administration.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

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