The need of experimental inhalation studies in animals for the problem of the influence of cigarette smoke on the induction of lung cancer

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Zusammenfassung

Dieser Bericht betont die große Notwendigkeit, experimentelle Modelsysteme zu entwickeln, die es erlauben, das Zigarettenrauchen des Menschen so ähnlich wie möglich im Tierexperiment nachzuahmen. Die Ausführungen beabsichtigen, Wissenschafter aus verschiedenen Disziplinen wie Chemie, Biologie, Pathologie und experimenteller Krebsforschung zu gemeinsamen Bemühungen und zur Zusammenarbeit anzuregen, auf breiter Basis *Inhalationsstudien mit frischem Zigarettenrauch* am Tier zu entwickeln und auszuarbeiten. Es steht zu hoffen, daß akute und chronische Inhalationsstudien in Tieren mittels frischem Zigarettenrauch und ihrer Kombination mit anderen Faktoren wie z.B. Luftverunreinigung, Viren, Strahlungen für die Abklärung der Etiologie und Pathogenese des Lungenkrebses beim Menschen sehr wertvoll sein würden.

Summary

This report stresses the great need for experimental model systems which permit duplication of the human habit of cigarette smoking as closely as possible in laboratory animals. The discussion intends to stimulate concentrated efforts of and collaboration between scientists from various disciplines of chemistry, biology, pathology and experimental cancer research in developing and pursuing large scale *inhalation work with fresh cigarette smoke.* It is hoped that acute and chronic inhalation studies in animals with fresh cigarette smoke and their combination with exposure to other factors, such as air pollutants, viruses, radiation, will be of great value for clarification of causes and pathogenesis of lung cancer in man.

Human lung cancer is one of the most serious public health problems today. Although epidemiological, clinical and histopathological studies carried out on non- smokers and cigarette smokers have been interpreted as implicating inhalation of cigarette smoke as one of the most important causative factors in the occurrence of human bronchogenic carcinoma [1], the problem is by no means solved, but requires additional research. The direct proof of a causal relationship between cigarette smoking and lung cancer in man faces the same difficulties that are inherent in any situation in which a large and unknown number of potentially carcinogenic and cocarcinogenic influences need to be

explored. It is necessary to determine and elucidate the possible role of other suspected endogenous and exogenous factors, such as genetic constitution, air pollution, radiation, hormones, preceding diseases, nutrition, host viruses, environment and living habits, as well as that of cigarette smoke. Experimental exploration of the possible biological effect of these factors, alone or in combination with others, on the human lung is also necessary because many other important questions remain unanswered today.

Only a few examples are mentioned here:

What is the pathogenesis of human lung cancer, i.e. what are the processes and mechanism by which it develops?

Does lung cancer development involve a sequential series of events?

Can this development be reproduced and assessed step by step in experimental animals?

If so, how and at what stage in the sequence do various factors affect the process of development? If cigarette smoke is involved, at what specific stage does it affect the process and in what manner?

In seeking answers to these important questions, well controlled experimental data are needed. Of course, direct experimentation on human beings is out of the question. Thus, for human data, we have only the possibility of additional observations through further epidemiological and clinical research. But the heterogeneity of the human population impedes standardization of endogenous and exogenous factors necessary for analyses and comparison of cigarette smokers and non-smokers. Assessment is further complicated by the long period of time over which these factors may operate and evoke tumors. Thus analyses and comparisons are extremely difficult, and it is impossible to come to definitive conclusions as to the question of a direct cause-effect relationship between cigarette smoking and human lung cancer.

It thus appears, that a concentrated effort of *experimental exploration using laboratory animals is urgently needed.* In other words, model systems are required where the biological effects of suspected factors such as those already mentioned, alone and in combination with each other, can be assessed at will under standardized conditions. However, experimentation with cigarette smoke in animals is a rather difficult task, mainly because cigarette smoking is an exclusive human habit which cannot be easily duplicated in animals. This difficulty probably explains the comparative scantiness of experimental data and also why among the experimental studies reported so far the *indirect approach* of painting tissues and of injections and instillation with *cigarette smoke condensates* has been widely used, while few efforts have been made to study *the direct effect of inhalation of cigarette smoke*, which *a priori* would be expected to imitate more closely conditions in human cigarette smokers. Although application of tobacco extracts, cigarette smoke condensates and other compounds to the skin and other sites of animals have produced cancer in some instances [1], *an*

extrapolation of these results to the question of *a causal relationship between cigarette smoking and human lung cancer appears open to criticism mainly for 2 reasons:*

1. These experiments have tested something else than cigarette smoke. All the compounds utilized are different in regard to their chemical and physical characteristics of cigarette smoke *per se.*

2. The anatomical structure and the function of the respiratory system are strikingly different from those of skin and other tissues. Therefore, the response of lung tissue to experimental agents would be expected to be different from that of other tissues.

Taking both points together, if cigarette smoke condensates or extracts are applied to skin, the interaction and response obviously cannot be compared with those of cigarette smoke reaching the lung. While work with cigarette smoke condensates and other compounds is of scientific importance for the problem of experimental carcinogenesis, it is evident that no definite conclusions can be drawn from such results for the question of a causal relationship between cigarette smoking and human lung cancer.

Thus, there exists a great need for model systems which permit the duplication of the human habit of cigarette smoking as closely as possible in laboratory animals. Smoking machines should be developed which will enable exposure of large groups of animals, separately but chronically during their entire life span, to inhalation of puffs of fresh cigarette smoke alternating with fresh air, utilizing the same intervals as human smokers. To assure that the cigarettes are "smoked" similarly to cigarettes smoked by humans, such factors as volume of puff, duration of puff, interval between puffs on individual cigarettes and number of puffs per cigarette must be carefully controlled. Furthermore, the time lapse between the puff and the introduction of the smoke into the lungs of animals should approximate that of human smokers. Indeed, since people smoke in various ways, such as different volumes, duration, numbers and intervals of puffs, it is important that the model systems permit testing of variations of these smoking characteristics. Extremes as well as averages must be assayed, since valuable information may be obtained by varying smoking characteristics from those approximating human smoking to those far removed from it.

Because of the technical difficulties of developing such systems and the long time required for such experimentation, it is not surprising that only a few investigators have employed this important direct biological approach. A few early experimental studies were characterized by their incompleteness, lack of standardization or inadequacy of inhalation techniques. In recent years reports have been published from only a small number of investigators who have attempted systematic experimental studies in animals of one or more aspects

related to the possible biological effects of *inhalation of fresh cigarette smoke*. The authors began systematic chronic inhalation studies with cigarette smoke in mice in the U.S.A. in 1956 and published their first results between 1958 to 1960 [2, 3, 4].

Reports of other groups have appeared only within the last six years [5, 6, 7, 8, 9]. The studies of the authors and of these other investigators, as scanty and preliminary as they are, have nevertheless already provided some pertinent information and some background experience necessary for the development of model systems:

1. Available techniques have now progressed beyond the use of smoke-filled chambers in which the animals stay. For example, the authors have developed a machine which permits individual animals to inhale over a long period of time fresh smoke directly from a cigarette which can be controlled as to volume, duration, interval and number of puffs [10, 11].

2. It can be shown that mice and hamsters inhaling whole cigarette smoke do get the smoke into their lungs. They disclose nicotine in their lungs [6, 7, 10] and show an increase of carbon monoxide in blood after inhalation of whole cigarette smoke or its gaseous phase [10, 11].

3. Chronic exposure to inhalation of whole cigarette smoke produced in mice various changes in the respiratory tract such as bronchitis and atypical proliferation of bronchial epithelium resembling those observed by *Auerbach et al.* [12] in human cigarette smokers. However, in mice these lesions did not progress to bronchogenic carcinoma [2, 3, 4, 11] and were reversible on cessation of smoking [3].

4. While chronic inhalation of cigarette smoke has also not produced bronchogenic carcinoma in other strains of mice [7], rats [8] and hamsters [6], a different lung tumor, of an alveologenic type, has been observed in some mice [7, 11] and rats [8]. In order to see whether or under what experimental conditions cigarette smoke *per se* is directly related to the causation of these tumors, further exploration of this problem is necessary.

5. Some of the findings of the authors strongly suggest that infections with respiratory viruses, such as influenza virus, may contribute to the development of lung cancer both in cigarette smokers and non-smokers. In 1963, the authors reported that in mice the frequency of atypical proliferation was lowest after inhalation of cigarette smoke alone, higher after influenza virus and highest after a combination of the two [13]. The suggestion that *influenza virus per se* may be implicated in malignant transformation of cells is not only supported by *in vitro* and *in vivo* studies by the authors [14, 15], but by the recent results of *Harris* and *Negroni* 1967 [16], who produced alveologenic cancers of lungs in C 57 Black mice with 4 different strains of influenza virus alone.

6. In work still under way the authors have used whole fresh cigarette smoke and only the gaseous phase, that is cigarette smoke from which particu-

late matter such as "tar" and nicotine have been removed by use of a Cambridge Filter.

Results indicate that mice develop tumors after inhalation of whole smoke as well as after the gaseous phase [10], and that the frequency and spectrum of tumors is significantly greater in mice inhaling whole fresh cigarette smoke than in those inhaling the gaseous phase [11].

However, many more experiments need to be done before any definite conclusions can be reached, particularly because in these experiments the intervals at which puffs were drawn from the cigarettes themselves were much shorter than the intervals at which puffs are drawn by human smokers. Nevertheless, 3 comments seem pertinent:

a) Further investigation of the biological effect of the gaseous phase in living animals and in tissue and organ culture [17a, 17b] will be of great importance, and will be helped by the growing knowledge obtained from analytical work regarding the gaseous compounds of cigarette smoke. Work in this area is under way in the authors' laboratory.

b) The fact that some tumors were obtained apart from the particulate phase imposes further caution regarding extrapolation of results obtained in animals with cigarette smoke condensates and other compounds.

c) The ultimate significance of present and future experimental cigarette smoke inhalation work for the problem of human lung cancer causation is not now known. Any evaluation has to take into consideration the observations that the anatomical structure of human and mouse lung are not identical [18] and that it is possible that there is also a difference in sensitivity between them at the cellular level [19].

At this stage, however, information obtained from experimental inhalation studies in animals with whole fresh cigarette smoke and its gaseous phase provides a fruitful basis upon which one would expect to build further research seeking a solution for this problem.

It is evident that the information outlined in the above 6 paragraphs by no means unravels the complex questions of what interrelationships may exist between cigarette smoking, other factors and human lung cancer. On the other hand efforts in which scientists from various disciplines such as of chemistry, biology, pathology and experimental cancer research, collaborate in developing and pursuing large scale inhalation work on animals with cigarette smoke *per se* and with other factors, on animals is necessary for further progress and clarification. Such a joint and concentrated program carefully planned and executed by qualified scientists would permit direct analyses of the biological effects of many suspected factors, including air pollutants, respiratory diseases, radiation and others as well as cigarette smoke. It would also permit assessment and exclu-

sion of those factors obscuring in each instance an exact evaluation of possible cause-effect relationships between particular factors alone or in combination and the occurrence of pathological alterations. A few specific and fruitful approaches are mentioned below:

1. Further improvement and standardization of many inhalation techniques including of course construction of smoking machines for laboratory animals, should be undertaken as a collaborative effort between biologists, engineers and physicists.

2. Large scale use by many investigators of standardized inhalation techniques, employing cigarettes of known and various composition on many species of animals alone and in combination with other factors, appear of great importance.

3. Extensive investigations of sequential cell and tissue alterations at many periods of cancer development are urgently needed, focusing particular attention on possible *early* changes and *interaction* between factors such as air pollutants, viruses, cigarette smoke, and important intracellular components such as genetic material DNA and messenger RNA. Utilizing a combination of various methods, such as cytochemistry, molecular biology, gas chromatography, electron microscopy, simultaneously on the same material and correlating them with those of cyto- and histopathology, would push forward frontiers regarding *early* events in carcinogenesis, of which we know so little and which are the basis for understanding the process of malignant transformation of cells. Such an assessment, in the perspective of other research that must be done, might also open new pathways for detection and elimination of cell damaging agents contained in any of the factors studied.

In view of the fact that it is not known how cancer evolves, only careful experimental exploration of all the steps in this long process is the path to reliable knowledge. Sweeping interpretation based upon partial knowledge can too readily obscure the data or distort its significance, with the result that misguided preventive or curative efforts may have effects opposite from those intended.

In closing, we wish to express the hope that this report may contribute to a reorientation of experimental investigations concerned with the influence of cigarette smoke on the induction of lung cancer. Facilities and means should be provided to stimulate investigators to explore this important health problem by the use of one of the most rational and logical experimental bioassays, namely inhalation studies in animals with cigarette smoke. There can be no doubt that a close collaboration and free exchange of scientific information between biological scientists, chemists and others engaged in this specific approach and those concerned with other aspects of lung cancer research would

be of immense value for clarification of causes and pathogenesis of lung cancer in man, and thus contribute to reduction and prevention of this serious public health problem.

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