The experimental exploration of health damaging factors in cigarette smoke[1, 2]

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Numerous components have been detected in cigarette smoke. The authors review the experimental research conducted to elucidate the cancer producing effects of the more important of these.

Introduction

Although epidemiological and statistical studies undertaken during the last 20 years have clearly shown that smoking of cigarettes is a health hazard, and plays particularly an important etiological role in the development of human lung cancer [22], the question what specific components in cigarette smoke are responsible for the lung cancer cannot be answered conclusively at present. This may appear surprising, but there are two main reasons which may perhaps explain this gap in our knowledge.

1. Cigarette smoke contains a very large number of components, about whose biological activities we know very little, especially when they are in combination. Although the analyses of cigarettes smoke are by no means completed, already over 1200 components have been detected [19, 22].

2. Experimental exploration of the biological effects of inhalation of cigarette smoke and its components on the respiratory, and other systems of laboratory animals is extremely difficult, because only man inhales voluntarily cigarette smoke, and thus an exact duplication of this human habit in animals poses great problems.

Within the last two decades, some progress has nevertheless been made in our knowledge concerning experimental assessment of biological effects of cigarette smoke. These advances, as incomplete as they may be, do permit at least some elucidation of the important question concerning type, biological effect, and mechanism of action of health damaging factors in cigarette smoke.

In this brief report, which of necessity is limited, only a few of the pertinent analytical and experimental biological data obtained on cigarette smoke can be presented. Main attention will be drawn to the finding that health damaging factors, especially those contributing to carcinogenesis, occur not only in the particulate phase but also in the gas vapour phase of cigarette smoke, and that further work in this direction is urgently needed. For detailed information on health damaging factors, see especially *Wynder* and *Hoffmann* [24], *Stedman* [19] and the *US Surgeon General reports on the health consequences of smoking* [22].

Analytical and experimental biological data

Table 1, based on data published in the *US Surgeon General reports on the health consequences of smoking* (1972), presents some of the important compounds of cigarette smoke, classified as contributors to health hazards. It should be mentioned that these data were gathered by a special committee already in 1970, and that the division in 3 groups of "most likely", "probable", and "suspected" health hazards has not been accepted by every member of the committee concerned with this question. However, regardless of the degree of classification, the data in this table clearly demonstrate that health damaging factors are present in both phases of cigarette smoke, that is in the particulate phase and in the gas vapour phase. It can also be seen that in the particulate phase "tar" and nicotine are judged to be the most likely contributors to health hazards, while in the gas vapour phase it is the carbon monoxide. Although cresol and phenol in particulate matter, and acrolein, HCN, NO, and NO_2 in the gas vapour phase are classified here as only "probable" health damaging factors, further biological work on these compounds appears necessary. This especially holds true for acrolein and for NO and NO_2. The latter two compounds are considered to be potential precursors in the formation of N-nitrosamine, a known carcinogenic substance found in cigarette smoke [2]. The recent observation that the gas vapour phase of cigarette smoke is a potent inhibitor of glyceraldehyde-3-phosphate dehydrogenase (SH-enzyme) and that protection from inhibition by the gas vapour phase is afforded by cysteine [18], would also necessitate the exploration of the biological significance of such agents as contributors to abnormal growth [17]. Further systematic studies on the biological effects of the factors in the "suspected" category appear also of importance, particularly to compare components of the particulate

Table 1
Compounds in cigarette smoke judged as contributors to health hazards[1]

CATEGORY	PHASE CLASSIFICATION	
	PARTICULATE	GAS VAPOR
MOST LIKELY	NICOTINE "TAR"	CARBON MONOXIDE
PROBABLE	CRESOL PHENOL	ACROLEIN HCN, NO NO_2
SUSPECTED	BUTYLAMINE DIMETHYLAMINE DDT ENDRIN HYDROQUINONE NICKEL COMPOUNDS PYRIDINE	ACETALDEHYDE ACETONE ACETONITRILE ACRYLONITRILE AMMONIA BENZENE 2,3-BUTADIONE CO_2 CROTONONITRILE ETHYLAMINE FORMALDEHYDE FURFURAL HYDROGEN SULPHIDE METHACROLEIN METHYL ALCOHOL METHYLAMINE

[1] Data from Surgeon General Report P.H.S. U.S.A. 1972. "Tar" = total particulate matter without nicotine.

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EFFECTS			PROCEDURE AND MATERIAL						
			SKINPAINTING INJECTION INSTILLATION EXPOSURE C	INHALATION EXPOSURE C					
				PURE CONSTITUENTS			CIGARETTE SMOKE		
			CONDENS. EXTR TAR	NICOTINE	CO	NO_2	ACROLEIN	FRESH WHOLE SMOKE	FRESH GAS VAPOR PHASE
ANIMALS	IMMUNOSUPPRESSION					+		+	+
	INCREASE OF CO Hb				+			+	+
	CYTOTOXICITY	CILIARY ACTIVITY		+		+	+	+	+
		ALVEOLAR MACROPHAGES				+		+	+
	BRONCHITIS					+		+	+
	EMPHYSEMA					+		+	+
	LUNG	ATYPICAL PROLIFERATION	+			+	+	+	+
		CANCER	(+)				+	+	+
	OTHER TUMORS (SKIN, TRACHEA, LARYNX)		+				+	+	
	VASCULAR LESIONS	LUNG		+	+			+	
		HEART		+	+			+	
EXPOSURE OF CELL CULTURES	CYTOTOXICITY		+	+		+	+	+	+
	ATYPICAL PROLIFERATION		+					+	+
	ABNORMAL DNA METABOLISM AND NUMBER OF CHROMOSOMES		+					+	+
	MALIGNANT TRANSFORMATION		+						

Table 2
Experimental evidence on animals and on animal and human cell (C) cultures implicating cigarette smoke as a health hazard

phase with those of the gas vapour phase and their interactions.

Turning now to existing experimental biological data on cigarette smoke, it should be mentioned that in view of the statistical and epidemiological results, implicating cigarette smoke as the most important causative agent of human lung cancer, most of the experimental biological work has been concerned with the interrelationship between cigarette smoke and carcinogenesis. For this reason, this report limits itself to discuss experimental evidence implicating cigarette smoke in carcinogenesis. Some of the essential model systems developed for the experimental exploration, and some of the main biological results regarding this particular problem are presented in table 2. For further information concerning other approaches and data, see *US Surgeon General reports on the health consequences of smoking* [22].

There are 3 main model systems which are utilized to assess carcinogenic effects of cigarette smoke:

1. *Painting of skin, injections or instillations of animals with condensates or extracts of cigarette smoke, that is with "tar"*, the main component of particulate matter.

2. *Inhalation experiments in laboratory animals with fresh cigarette smoke,* whereby the animals are either exposed to *whole fresh cigarette smoke* or to its *gas vapour phase alone,* or to some pure constituents of the gas vapour phase.

3. *Exposure of human or animal cell cultures, especially those of the lungs, to cigarette smoke condensates or to fresh cigarette smoke (whole smoke, gas vapour phase).*

Since many years, the indirect method of painting tissues, injections, or instillation of animals with cigarette smoke condensates, that is with "tar", has been the method most widely used. Relatively few efforts have been made to study the direct effect of chronic inhalation of fresh cigarette smoke in animals, an approach which a priori would be expected to imitate more closely conditions in human cigarette smokers. Skin painting in animals with cigarette smoke "tar" has become the method of choice mainly for two reasons:

1. Skin painting in animals with "tar" is a relatively easy technique, especially when compared with the painstaking efforts needed to carry out long term chronic inhalation experiments in animals with fresh cigarette smoke.

2. Skin painting experiments have shown that "tar" of cigarette smoke is carcinogenic in animals. Ever since *Wynder* et al. [23] first demonstrated in 1953 that skin cancer can be produced in animals after painting the skin with cigarette "tar", confirmation of the carcinogenic property of this "tar" has been brought forward [22]. Furthermore, application of "tar" and smoke condensates have also produced tumors of larynx and trachea in animals, and have been shown to enhance malignant transformation of hamster cell cultures [5].

It is therefore not surprising that these data have led to the widespread accepted concept, that it is mainly "tar" of cigarette smoke which is responsible for the development of lung cancer in the human cigarette smoker [22]. However, as pointed out by us previously [10], in spite of the importance of these findings, it must be taken into account that an extrapolation of experimental results with cigarette smoke "tar" to the question of a causal relationship between cigarette smoking and human lung cancer appears open to criticism already for two main reasons: 1. the physical and chemical composition of cigarette smoke "tar" or condensates is quite different from that of *fresh* cigarette smoke; 2. the anatomical structure and function of the respiratory system, especially of the lungs, are strikingly different from those of skin, larynx, or trachea. Consequently the response of these tissues to an agent,

such as cigarette "tar", cannot be considered to be equal to the response of bronchial system and lungs exposed to inhalation of puffs of fresh cigarette smoke. For this reason, we have since many years stressed the need of experimental inhalation studies in animals with cigarette smoke itself, and have ourselves carried out such experiments [6–10]. In our recent experimental studies, utilizing simultaneously inhalation experiments in mice, and exposure of cell cultures, we have employed exclusively puffs of fresh cigarette smoke itself, that is the type of cigarette smoke inhaled by man [13]. In the inhalation experiments with fresh cigarette smoke we have also tried to imitate as closely as possible in mice the human habit of cigarette smoking [12].

The concept that there are not only the "tar" components of cigarette smoke, but that components of the gas vapour phase must also be considered as contributing to carcinogenesis, has been especially supported by comparative studies of recent years, assessing simultaneously biological effects of fresh whole cigarette smoke or its gas vapour phase on animals and on cell cultures [3, 4, 11, 12, 15, 16, 17].

As can be seen from the data in table 2, exposure of animals to inhalation of fresh whole cigarette smoke or of the gas vapour phase alone gives essentially the same results. Cell cultures, including human lung explants, also are affected in essentially the same manner after exposure to either whole fresh cigarette smoke or to its gas vapour phase alone. This holds not only true for alterations, such as immunosuppression [1, 3, 16], a feature closely related to carcinogenesis [20, 21], or damage to alveolar macrophages [4, 14] and other effects, but also when studying the carcinogenic effect on the lung. We observed enhancement of lung carcinogenesis in mice, that is earlier occurrence and higher frequency of lung cancers than in controls, not only after chronic inhalation of whole fresh cigarette smoke, but also after chronic inhalation of the gas vapour phase alone [12, 16]. Furthermore in cell cultures, including human lung explants, essentially the same sequential alterations, such as cytotoxicity, followed by stimulation of DNA, RNA, and protein synthesis, abnormal cell division, and atypical proliferation, have been observed after exposure to either whole fresh cigarette smoke or to the gas vapour phase alone [4, 15]. The significance of the gas vapour phase of cigarette smoke for cytotoxic and atypical proliferative effects on lung cultures is further supported by our recent findings. Using two types of cigarettes, having the same content of nicotine and "tar", but differing in content of gas vapour phase constituents and SH reactivity produced strikingly different effects on hamster lung cultures. Exposure of hamster lung cultures to a cigarette smoke with *reduced* gas vapour phase constituents and low SH reactivity evoked not only less cytotoxicity, but also less abnormal growth than smoke with higher amounts of gas vapour phase constituents and higher SH reactivity [17].

On the basis of these experimental data obtained with fresh cigarette smoke in laboratory animals or in lung cultures, it thus would appear that not only constituents of the particulate phase, such as "tar", but that also constituents of the gas vapour phase from cigarette smoke are implicated in carcinogenesis. A further exploration of the type of constituents and their mechanism of action in relation to lung carcinogenesis and other alterations seems urgently needed.

Conclusions

Although, as pointed out in the beginning of this brief report, our knowledge regarding type and biological activity of health damaging factors in cigarette smoke is still scanty, the present experimental data justify the following conclusions:

1. Health damaging factors, such as those contributing to carcinogenesis, are found in the particulate and in the gas vapour phase of cigarette smoke.
2. Painting experiments in animals and exposure of cultures to particulate phase, that is to "tar" of cigarette smoke, have shown the carcinogenic properties of cigarette "tar" for various tissues, such as skin, trachea, larynx.
3. Inhalation experiments in animals and exposure of animal and human lung cultures to fresh cigarette smoke have shown enhancement of lung carcinogenesis, not only after exposure to whole cigarette smoke, that is to smoke which contains both, particulate and gas vapour phase, but also after exposure to the gas vapour phase alone.
4. Further work is urgently needed to characterize the responsible components in particulate and gas vapour phase, and their mechanism of action. However, the important observation that health damaging factors are not only present in the particulate phase, but also in the gas vapour phase of cigarette smoke, should already be taken into account, when warning the public against the health hazards of cigarette smoking. The public should be informed that, on the basis of our present knowledge, cigarettes with either reduced content of particulate matter, such as "tar", or reduced content of gas vapour phase constituents, cannot be considered as safe cigarettes, that is smoking of such cigarettes does not eliminate damage to health, such as the risk of lung cancer.

Summary

Although our knowledge regarding types, biological effects, and mechanism of action of health damaging factors in cigarette smoke is still scanty, experimental biological studies have already led to the following results:

1. Painting of skin and other tissues of animals with cigarette "tar" results in tumors, thus implicating the particulate phase of cigarette smoke as containing carcinogenic agents.

2. Inhalation experiments in animals or exposure of animal and human lung cell cultures with fresh cigarette smoke have shown that not only whole fresh cigarette smoke (particulate phase + gas

vapour phase), but that also the gas vapour phase alone enhances lung carcinogenesis.

3. The public should be informed, when warned against the health hazards of cigarette smoke, that neither reduction of particulate matter, such as "tar", nor reduction of gas vapour phase constituents implies that smoking of such cigarettes is harmless to health.

Zusammenfassung

Die experimentelle Erforschung gesundheitsschädlicher Komponenten im Zigarettenrauch

1. Obwohl epidemiologische Studien klar auf die gesundheitsschädliche Wirkung von Zigarettenrauch hinweisen, insbesondere auf die ätiologische Rolle des Zigarettenrauchs für den Lungenkrebs des Menschen, kann die Frage, welche spezifischen Komponenten dafür verantwortlich sind, heute noch nicht eindeutig beantwortet werden. Der Hauptgrund für diese Lücke in unserem Wissen liegt nicht nur in der enormen Anzahl von Komponenten, die in den beiden Hauptphasen des Zigarettenrauchs (Teilchenphase, Gas-Dampf-Phase) enthalten sind, sondern auch in der Schwierigkeit, die biologische Wirkung von *Inhalation* des Zigarettenrauchs am Laboratoriumstier experimentell zu erforschen.

2. Experimentelle Studien mit der Hauptkomponente der Teilchenphase des Rauchs, dem «Teer», ergaben nach Bepinselung der Haut und anderen Geweben mit solchem «Teer» Tumoren. Diese Befunde führten zu der weitverbreiteten Auffassung, dass hauptsächlich der Zigarettenteer für den Lungenkrebs des Zigarettenrauchers verantwortlich zu machen ist.

3. Experimentelle chronische *Inhalationsstudien* mit *frischem Zigarettenrauch* am Tier und Berauchung von Tier- und menschlichen Lungenzellkulturen mit *frischem* Zigarettenrauch zeigten, dass nicht nur der Gesamtzigarettenrauch (Teilchen- und Gas-Dampf-Phase), sondern auch die Gas-Dampf-Phase allein lungenkrebsfördernde Eigenschaften besitzt.

4. Der experimentelle Nachweis von krebsfördernden Komponenten in der Teilchenphase wie auch in der Gas-Dampf-Phase des Zigarettenrauchs muss berücksichtigt werden, wenn die Öffentlichkeit vor gesundheitsschädlichen Faktoren im Zigarettenrauch gewarnt wird. Weder Verminderung von Komponenten der Teilchenphase, wie zum Beispiel Teer, noch Verminderung von Komponenten der Gas-Dampf-Phase des Zigarettenrauchs gibt eine Garantie, dass der Rauch solcher Zigaretten unschädlich ist. Weitere intensive analytische und experimentell biologische Forschungen mit der Teilchen- und der Gas-Dampf-Phase des Zigarettenrauchs, insbesondere in ihrer Wechselbeziehung zueinander, sind notwendig, bevor sichere Aussagen über spezifisch gesundheitsschädliche Komponenten und deren Eliminierung gemacht werden können.

Résumé

Recherches expérimentales des facteurs nocifs à la santé dans la fumée de cigarette

1. Des études épidémiologiques ont montré que la fumée de cigarette est nocive pour la santé, en particulier que la fumée de cigarette est responsable du cancer du poumon des humains. Mais la question, quels facteurs spécifiques dans la fumée de cigarette sont responsables ne peut pas être répondue aujourd'hui avec sûreté. Il y a probablement deux raisons principales à cette incertitude: premièrement la fumée contient un nombre énorme de facteurs chimiques dans les deux phases principales (phase particulaire, phase vapeur-gaz). Deuxièmement les recherches expérimentales sur l'effet biologique de l'inhalation de la fumée de cigarette par l'animal de laboratoire sont extrêmement difficiles.

2. Le badigeonnage de la peau et d'autres tissus de l'animal avec du goudron de cigarettes a démontré que ce goudron provoque des tumeurs de ces tissus, et a résulté dans l'opinion que c'est le goudron de cigarettes qui est responsable du cancer du poumon chez les fumeurs de cigarettes.

3. Des études expérimentales concernant l'inhalation chronique par des souris de fumée *fraîche* de cigarettes, et l'exposition de cultures cellulaires de poumons d'animaux ou d'humains ont démontrés que ce n'est pas seulement la fumée totale de cigarettes (phase particulaire et phase vapeur-gaz), mais que la phase vapeur-gaz seule aussi contribue à la carcinogénèse des tumeurs du poumon.

4. La preuve expérimentale des facteurs cancérigènes dans la phase particulaire et la phase vapeur-gaz de la fumée de cigarettes doit être considérée, quand on veut mettre en garde le public contre les dangers pour la santé de la fumée de cigarette. Ni la réduction des facteurs de la phase particulaire, comme par exemple du goudron, ni la diminution des facteurs de la phase vapeur-gaz donnent la garantie que de telles cigarettes sont sans danger pour la santé. C'est pourquoi il est nécessaire de continuer les recherches sur les deux phases de la fumée de cigarettes avant de se prononcer avec sûreté sur les facteurs responsables.

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