Sozial- und Präventivmedizin Médecine sociale et préventive 19, 55–58 (1974)

The interaction of cigarette smoke with thiol groups, a model study

Hans P. Stauffer

Numerous research groups are studying the basic key mechanisms which are involved when smoke is interacting with living tissues. This is a review of the literature on a specific mechanism – the SH – reactivity of smoke.

1. Introduction

Studying a list of the chemical compounds found in cigarette smoke as the one compiled by *Elmenhorst* and *Schultz* [7], reveals a host of chemically reactive substances. Many of them are rather volatile and their determination is usually performed by means of gas chromatography. In fact, the methodology developed originally by *Grob* [11] using capillary columns opened the way for systematic investigations on the predominantly volatile smoke constituents.

Based on an extensive literature, there is one particular line of thinking we would like to elucidate and to discuss in more detail.

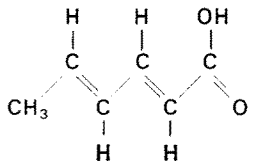
Until now, suspected unfavourable biological effects of cigarette smoke were mainly attributed to some components of the smoke condensate viz., the less volatile droplets of the aerosol. However, during the last few years new aspects were investigated regarding the importance of the gas phase of cigarette smoke. It may be worth it to mention, that the technology of smoke filtration depends very much on the hypothesis chosen as to which part of the aerosol is considered troublesome.

Lange [21] and *Sato* et al. [28] published findings that cigarette smoke inhibits SH-depending enzyme systems. Similar observations were made by *Benedict* and *Stedman* in 1968 [3]. In both cases the protective action of cysteine on the enzyme system was reported. Even more importance could be attached to the findings of *Powell* and *Green* [25]. In their paper of 1972 on the metabolic lesions in alveolar macrophages caused in vitro by cigarette smoke, they state that smoke is a potent inhibitor of glyceraldehyde-3-phosphate dehydrogenase (SH-enzyme) and that protection from inhibition by smoke is afforded by cysteine. It is also suggested by the authors that a relationship exists between loss of alveolar macrophage phagocytic competence and inhibition of glyceraldehyde-3-phosphate dehydrogenase. But for the tobacco chemist interested in smoke composition, the fact of greatest interest may be that the effects were observed by exposing the macrophages to the vapour phase only of the aerosol and not to whole smoke.

Dickens and *Cooke* [5] studied the rates of the interaction of lactonic alkylating agents with cysteine and demonstrated that, with the exception of aflatoxin and N-ethyl maleimide, the reaction rates could be correlated with the carcinogenic activity of the substance. Furthermore, *Dickens* et al. [6] had found that certain simple chemical compounds could act as complete carcinogens when administered via a certain route to experimental animals. Sorbic acid is such a substance. It reacts with cysteine and has the structural formul as shown below.

Subcutaneous injection of sorbic acid suspended in oil gave rise to appreciable numbers of tumours in rats, including such which showed marked proliferation and which contained giant multinucleate cells, so that they could be considered as being malignant.

Structural formula of sorbic acid (trans, trans-2,4-hexadienoic acid)



Referring to these findings it might be worth to compile in a more systematic manner investigations that centered about the interaction between thiol groups and specific components of cigarette smoke.

2. SH-reactive compounds in cigarette smoke

Unsaturated compounds are not the only smoke components that react with free SH-groups. *Tonge* [30] showed by using a specific experimental set-up that some substances of whole smoke react with the free SH-group of cysteine in aqueous solution. He assumed that the free radicals which are trapped in the condensate are responsible for the phenomenon, as it is known that the aerial oxidation of cysteine to cystine is mediated by free radicals. However, free radicals are not only present in the particulate phase of cigarette smoke but also in the vapour phase. This was shown by *Tully* et al. [31] in experiments where EPR measurements were performed on smoke after a passage through a Cambridge filter. The signals which were observed proved the presence of free radicals in the vapour phase. *Tonge* had speculated already 1962 as to the possible biological effect SH-reactive smoke constituents could have.

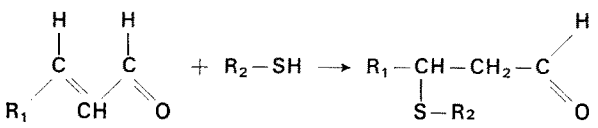
Leuchtenberger and *Leuchtenberger* performed a very differentiated set of experiments exposing alveolar macrophages in lung cultures to the different phases of smoke [22, 23]. The authors quote, for example, that cytotoxic effects of smoke were absent after it had passed through a filter of activated carbon which removes vapour phase constituents such as acrolein.

A most interesting work was described by *Comber* in 1972 [4]. It concerned short term tests for comparing the biological activity of smoke or smoke fractions. Various cell cultures were used as model system. Attempts to determine which smoke constituents are toxic in respect to abnormal cell growth have indicated

that vapour phase rather than particulate phase constituents were responsible. The same author attempted to achieve a balance of all compounds which are responsible for the toxicity of fresh smoke. He concluded that acetaldehyde, acrolein or hydrogen cyanide could not account for the total toxic effect. Nevertheless, it seems that carbon filters reduce the toxic effects considerably. In 1963, *Kensler* and *Battista* [17] reported that smoke vapour phase depresses the ciliary movements. These authors have shown that adsorption of the vapour phase by activated carbon detoxifies the smoke in this respect. Among others, *Walker* and *Kiefer* [32] investigated which of the vapour phase constituents do inhibit ciliary activity. They found hydrogen cyanide, acetaldehyde, acrolein to be effective. Finally it was *Izard* [14] who showed 1967 in a biological model that the vapour phase of smoke inhibited cell multiplication and that cysteine had an undeniable protective action.

All these single pieces of evidence put together may serve as a base to appreciate a very interesting model study published as recently as 1972 by *Feron* [9] who links furfural with tumour promotion in the respiratory tract of hamsters. In his experiments the author induced tumours in the respiratory system of hamsters by instilling benzo-a-pyrene. He could show that simultaneous application of furfural accelerated tumour growth although, by itself, furfural does not possess carcinogenic properties of its own. Furfural is one of the aldehydes of the vapour phase of cigarette smoke which could react with thiol groups.

Following this line of thinking an analytical method for the determination of SH-reactive smoke constituents may be of particular importance. Consequently an interesting methodological development which singles out certain unsaturated smoke constituents was reported by *Testa* and *Joigny* [29]. It allows to determine compounds such as acrolein, crotonaldehyde, acrylonitrile which are vapour phase constituents. The principle of the method consists of a reaction between a thiol-group and a carbon double bond activated by a conjugated carbonyle, nitrile or carboxylic ester group:



In this type of analytical procedure, the polar fraction of the vapour phase of smoke is trapped in water-saturated Chromosorb G, the reactive compounds are washed out with water and butylmercaptan is added to the eluate. After completion of the reaction, the products are determined by gas chromatography. This approach to smoke analysis is a very interesting one as it opens new perspectives by determining certain groups of smoke constituents according to their chemical reactivity.

Lacharpagne and *Morée-Testa* [20] measured the interaction of different types of fresh smoke with the free SH-groups of a protein. Of great importance are their findings on the reaction kinetics. The vapour phase of smoke reacts very rapidly with the free SH-groups, reaching a plateau in minutes. The total reactivities of whole smoke or of charcoal filtered smoke are greater in total but the reaction rates are much slower. There is a measurable reaction rate even after 24 hours.

3. The role of the thiol group in the living cell

Having seen that certain components of cigarette smoke react with free SH-groups and that certain biological effects of smoke were associated by some authors with this reactivity, it is perhaps appropriate to review the biological function of the thiol group.

Jocelyn [15] compiled an outstanding book on this subject which appeared 1972. It deals with the occurrence, chemical properties, metabolism and biological function of thiols and disulfides. What is said in the following few paragraphs should only serve to highlight particular facts which seem to be especially relevant to the action of cigarette smoke.

There are four main areas where an SH-mediated mechanism could be involved in the interaction of cigarette smoke with cellular systems:

- cell division: interactions on the nuclear level
- energy generation: interactions on the mitochondrial level
- active transport: interactions on the level of the cell membrane
- protein synthesis: interactions on the level of the ribosomes

In chemical terms, the following mechanisms could be relevant for the interaction of smoke constituents with free SH-groups in the cell:

- Alkylation by free radicals
- Addition to $-C=C-$ groups (nucleophilic or free radical mechanisms)
- Addition to $>C=O$ (in fact, hemi-mercaptals are intermediates in many enzyme reactions)
- Addition to lactones
- Catalytic oxidation to $-S-S-$

Cell division is one of the most intriguing cellular processes. *Rapkine* [26] observed already in 1931 cyclic variations of thiol groups during cell division of the sea urchin egg. Similar fluctuations of SH-concentrations were found by *Kredova* [19] to occur in rat liver cells during mitosis.

The shift from SH to $-S-S-$ has been associated with the formation of the mitotic spindle. Disulfide bonds could be the structural elements of the protein

fibres which guide the movement of chromosomes. It seems also that a SH-mediated mechanism involving histones and derepression of DNA synthesis is a necessary step before mitosis can take place (*Hilton* and *Stockten* [12]).

The mitochondrial membrane and certain fractions of the endoplasmatic reticulum play a functional role in the energy generating process that enables the cell to perform the functions necessary for life.

Many of the enzymes which are associated with oxidative phosphorylation are part of the structure of the inner mitochondrial membranes. *Riley* and *Lehninger* [27] showed that they contain thiol groups.

A range of enzymes involved in glycolysis and the respiration chain contain free SH-groups. However, not all of these thiol groups are necessary for enzyme function. Some might have a dual function and might be involved in the enzymatic reaction as well as being part of a protein matrix. SH-reagents block cell respiration and oxidative phosphorylation in many places. But this is not the only effect. The thiol-depletion leads also to a blockage of protein synthesis, as *Zehavi-Wilner* et al. [33], *Furano* and *Harris* [10] and *Kosower* et al. [18] have shown 1971 in cell free systems.

Iccarino and *Berg* [13] demonstrated by substrate-thiol reagent competition that free thiol groups are necessary for protein synthesis.

Cell membranes contain free SH-groups, some of which are functionally engaged in mediating active transport. *Emmelot* et al. [8] showed that they can be directly titrated. However, the free SH-groups are also necessary to stabilise the matrix of the membrane and membrane rupture can occur on destruction of free SH-groups. All these vital mechanisms listed above are blocked when so called "SH-reagents" eliminate or reduce the available free thiol groups in the cell. But this is not all. Many investigations deal with the interaction of unsaturated carbonyles with specific SH-dependent enzymes. *Kapfer* and *Schauenstein* [16] studied the effects of 4-hydroxypentenal on succinic dehydrogenase in different tissues. Comparing hepatomas with normal liver, they found a differentiated response. At certain aldehyde levels only the enzymes of the tumour tissue are affected but not the ones of the normal tissue. The enzyme inhibition is due to the reaction of the unsaturated carbonyle with the functional SH-groups of the dehydrogenase.

Barry et al. [2] investigated 1972 three α-, β-unsaturated compounds in a short-term test which is regarded to be indicative of carcinogenic activity. O-chloro-benzylidenemalonitrile (CS-Gas), cinnamonitrile and cinnamaldehyde were compared in the sebaceous gland suppression test. The mechanism of this method is based on the inhibition of esterase activity in the treated skin. CS is known to react readily with thiol groups. As this reaction could account for the suppression of non-specific esterase activity, the related α-, β-unsaturated compounds were used for comparison. The three compounds react with thiol groups and their respective reaction rates with the SH-group of glutathion are CS $>$ cinnamaldehyde $>$ cinnamonitrile. On measuring the response, however, only CS showed a marked suppression of the sebaceous glands. This would mean that the reaction with thiols is not, by itself, entirely indicative for complete carcinogenic activity.

4. Discussion

Evidently there are different schools of thought on the magnitude of possible health hazards of cigarette smoking, and on the nature of mechanisms involved. This situation will essentially not change as long as epidemiological observations in human populations are linked with phenomenological observations in animal experiments, each involving a diversity of interacting factors and thus necessitating involved mathematical analyses. Such analyses, in turn, have to be based on certain assumptions which throw them open to different interpretations.

In order to see the problem of Smoking and Health in a fair scientific perspective, increased research ought to be carried out on the basic key mechanisms which are involved when smoke is interacting with living tissues.

Reviewing the information available on the subject "smoke and free SH-groups" shows that it is very likely that the exposure of living tissues to smoke results in a depletion of the cellular SH-pool. This fact could be of fundamental importance. It is known that a number of environmental burdens on the body are counteracted by free SH-groups of the body tissues. *Bacq* and *Alexander* [1] reviewed 1964 thiol protection against both alkylating agents and ionizing irradiation, and a modern review on the subject is presented in the "Biochemistry of the SH-group" in the chapter on radioprotection by thiols and disulfides [15]. Considering the SH-reactivity of smoke, the thought springs to the mind that this might be the common factor that links smoking with other environmental impacts.

Free thiol groups are interacting rapidly with a range of smoke components. Their functions in the cell are manifold and important. They are essentially involved in

- mitosis [12, 19, 26]
- glycosis, cell respiration, oxidative phosphorylation [27]
- active transport across membranes [8, 24]
- protein synthesis [10, 18, 33]
- many individual enzyme reactions [15, 16]

Studying their role with regard to smoke exposure could provide the scientist with a new base for investigating the problems of biological interactions of smoke and could provide the technologist with a guidance for future developments.

Summary

Some constituents of cigarette smoke react easily with thiol groups. In view of the important role which is played by thiol groups in cell metabolism, this effect of smoke could be of importance.

Regarding the world-wide attention that is focussed on the development of a "low biological activity" cigarette a review of existing literature, as presented here, may be of particular interest.

Zusammenfassung

Einige Bestandteile des Zigarettenrauches reagieren leicht mit Thiol-Gruppen. Wegen der Wichtigkeit der Thiol-Gruppen im Zell-Metabolismus, könnte diese Eigenschaft des Rauches von besonderem Interesse sein.

Im Hinblick auf die weltweite Bedeutung, die gegenwärtig der Entwicklung einer «Zigarette mit geringer biologischer Aktivität» zugemessen wird, will der hier präsentierte Überblick über die neuere Literatur einige weniger beachtete Zusammenhänge aufzeigen.

Résumé

Certains composants de la fumée de cigarette réagissent facilement avec des groupes thiol. Du fait du rôle important joué par les groupes thiol dans le métabolisme cellulaire, cet effet sur la fumée pourrait avoir son importance.

Comme l'attention mondiale converge vers le développement d'une cigarette ayant une «activité biologique peu importante», un résumé de la littérature existante, comme présenté ici, pourrait être d'un intérêt particulier.

5. References

[1] *Bacq Z. M.* and *Alexander P.:* Importance for Radio-Protection of the Reaction of Cells to Sulphydril and Disulphide Compounds. Nature *203*, 162–164 (1964).

[2] *Barry D. H., Chasseaud L. F., Hunter B.* and *Robinson W. E.:* The Suppression of Non-specific Esterase Activity in Mouse Skin Sebaceous Gland by "CS" Gas. Nature *240*, 560–561 (1972).

[3] *Benedict R. C.* and *Stedman R. L.:* Complexity of Enzymatic Inhibition by Cigarette Smoke. Experientia *24*, 1205–1206 (1968).

[4] *Comber R.:* Research and Development at Southampton. Part. II. Biological Activity of Tobacco Smoke. Chem. Ind. *3*, 64–66 (1972).

[5] *Dickens F.* and *Cooke J.:* Rates of Hydrolysis and Interaction with Cysteine of Some Carcinogenic Lactones and Related Substances. Br. J. Cancer *19*, 404–410 (1965).

[6] *Dickens F., Jones H. E. H.* and *Waynforth H. B.:* Oral, Subcutaneous and Intratracheal Administration of Carcinogenic Lactones and Related Substances: The Intratracheal Administration of Cigarette Tar in the Rat. Brit. J. Cancer *20*, 134–144 (1966).

[7] *Elmenhorst H.* und *Schultz Ch.:* Flüchtige Inhaltsstoffe des Tabakrauches. Beitr. Tabakforschung *4*, 90–123 (1968).

[8] *Emmelot P., Bos C. J., Benedetti E. L.* and *Rümke Ph.:* Studies on Plasma Membranes. I. Chemical Composition and Enzyme Content of Plasma Membranes Isolated from Rat Liver. Biochim. Biophys. Acta *90*, 126–145 (1964).

[9] *Feron V. J.:* Respiratory Tract Tumors in Hamsters after Intratracheal Instillations of Benzo(a)pyrene alone and with Furfural. Cancer Res. *32*, 28–36 (1972).

[10] *Furano A. V.* and *Harris M. I.:* The Activity of Ribosomes whose RNA has been Degraded by Incubation in the Presence or Absence of Oxidized Glutathione. Biochim. Biophys. Acta *247*, 291–303 (1971).

[11] *Grob K.:* Zur Gaschromatographie des Cigarettenrauches 2. Teil. Beitr. Tabakforsch. *1*, 315–323 (1962).

[12] *Hilton J.* and *Stocken L. A.:* The Role of Thiol Groups in the Modification of the Template Activity of Histone-Deoxyribonucleic Acid Complexes. Biochem. J. *100*, 21c–23c (1966).

[13] *Iaccarino M.* and *Berg P.:* Requirement of Sulfhydryl Groups for the Catalytic and tRNA Recognition Functions of Isoleucyl-tRNA Synthetase. J. Mol. Biol. *42*, 151–169 (1969).

[14] *Izard C.:* On the Multiplication of Dunaliella Bioculata in the Presence of a Gaseous Phase of Cigarette Smoke and on Obtaining Mutations in the Presence of Acrolein. Compt. Rend. Hebd. Séances Acad. Sci. D, Sci. Nat. *265*, 1799–1802 (1967).

[15] *Jocelyn P. C.:* Biochemistry of the SH-group. Academic Press. New York and London (1972).

[16] *Kapfer E.* und *Schauenstein E.:* Über die Hemmbarkeit der Succino-Dehydrogenase in tierischen Tumoren und Normalgeweben durch 4-Hydroxypentenal. Z. Krebsforschung *77*, 180–188 (1972).

[17] *Kensler C. J.* and *Battista S. P.:* Components of Cigarette Smoke with Ciliary-Depressant Activity: Their Selective Removal by Filters Containing Activated Charcoal Granules. New Engl. J. Med. *269*, 1161–1166 (1963).

[18] *Kosower N. S., Vanderhoff G. A., Benerofe B.* and *Hunt T.:* Inhibition of Protein Synthesis by Glutathione Disulfide in the Presence of Glutathione. Biochem. Biophys. Res. Commun. *45*, 816–821 (1971).

[19] *Kedrova E. M.* and *Rodionov W. M.:* The Effect of Ionizing Radiation on Nuclear Cell Proteins: Changes in SH-group Content in Proteins Synthesized in Late Interphase. Int. J. Radiat. Biol. *16*, 359–366 (1966).

[20] *Lacharpagne J.* et *Morée-Testa P.:* Action de la fumée de cigarette sur les thiols des protéines du cristallin. Ann. Seita *7*, 144 (1969).

[21] *Lange R.:* Inhibiting Effect of Tobacco Smoke on Some Crystalline Enzymes. Science *134*, 52–53 (1961).

[22] *Leuchtenberger C.* and *Leuchtenberger R.:* The Reticuloendothelial System and the Immune Response, Plenum-Press, New York, 1971.

[23] *Leuchtenberger C.* and *Leuchtenberger R.:* Cytologic and Cytochemical Effects on Primary Mouse Kidney Tissue and Lung Organ Cultures after Exposure to Whole, Fresh Smoke and its Gas Phase from Unfiltered, Charcoal-filtered, and Cigar Tobacco Cigarettes. Cancer Res. *29*, 862–872 (1969).

[24] *Meister A.:* On the Enzymology of Amino Acid Transport. Science *180*, 33–39 (1973).

[25] *Powell G. M.* and *Green G. M.:* Cigarette Smoke-A Proposed Metabolic Lesion in Alveolar Macrophages. Biochem. Pharmacol. *21*, 1785–1798 (1972).

[26] *Rapkine L.:* Sur les processus chimiques au cours de la division cellulaire. Ann. Physiol. Physico chim. Biol. *9*, 383 (1931).

[27] *Riley M. V.* and *Lehninger A. L.:* Changes in Sulfhydryl Groups of Rat Liver Mitochondria During Swelling and Contraction. J. Biol. Chem. *239*, 2083–2089 (1963).

[28] *Sato T., Suruki T.* and *Fukijama T.:* Cigarette Smoke: Mode of Adhesion and Haemolyzing and SH-Inhibiting Factors. Brit. J. Cancer *16*, 7–14 (1962).

[29] *Testa A.* and *Joigny C.:* Dosage par chromatographie en phase gazeuse (CPG) de l'acroléine et d'autres composés α-, β-insaturés de la phase gazeuse de la fumée de cigarette. Paper No. 86, Coresta/TCRC Joint Conf., Williamsburg, Va. USA, 22.–28. Oct. 1972.

[30] *Tonge B. L.:* Effect of Tobacco Smoke Condensate on the Aerial Oxidation of Cysteine. Nature *194*, 284–285 (1962).

[31] *Tully G. W., Briggs C. D.* and *Horsfield A.:* EPR Absorption by the Vapour Phase of Cigarette Smoke, Condensed and Examined at Low Temperatures. Chem. Ind. 201–203 (1969).

[32] *Walker T. R.* and *Kiefer J. E.:* Ciliastatic Components in the Gas Phase of Cigarette Smoke. Science *153*, 1248–1250 (1966).

[33] *Zehavi-Wilner T., Kosower E. M., Hunt T.* and *Kosower N. S.:* Glutathione. V. The Effects of the Thiol-Oxidizing Agent Diamide on Initiation and Translation in Rabbit Reticulocytes. Biochem. Biophys. Acta *228*, 245–251 (1971).

Adresse des Autors

Dr. *Hans P. Stauffer*, The Burgdorf College of Technology, Dept. of Chemistry, 3400 Burgdorf, Switzerland.