3.8 Effects of Tobacco Smoke on Biological Systems

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3.8.1 OBJECTIVES

The objective of this review is to assess the mechanisms of effects of cigarette smoke upon biological systems ranging from isolated enzymes to different cell systems. Such data, particularly dose-response relationships, would provide a basis for predicting the risks of exposure to environmental tobacco smoke. Biological systems will be compared for sensitivity and a hierarchy established of important effects. The effects of certain vapor phase compounds and nicotine have been well characterized in biological systems and these data will be applied to the problem.

Common to studies of many environmental agents is the difficulty in analysing effects. Acute and chronic effects may be unrelated, simultaneous exposure to other materials may be important, there may be synergism of effects, and disease effects may have latent periods of several decades. For example, the synergism with asbestos to produce lung cancer requires 20 years (Selikoff et al. 1968).

There are few observations on the effects of sidestream smoke exposure in man in which dose was measured. Cigarette tobacco is a complex mixture (Stedman 1968) and even its major chemical groups or the major fractions of tobacco smoke condensate are too complex (Scholtzhauer et al. 1972) (Table 1) to achieve the ideal of relating biological effects to single chemical components. Furthermore, pyrolysis of tobacco and its additives increases the complexity and yields over a thousand produces (Neurath 1972). Sidestream smoke contains greater quanties of chloroform, extractable tar, nicotine, pyrene and cadmium (Table 2). Concentrations depend upon the duration of puffing, condensation and volatilization plus filtration. Differences in mainstream to sidestream ratios for certain constituents of smoke are large as shown by Wakeham (1972) (Table 2).

Furthermore, chemicals may be introduced by bacterial or microbial action during storage or processing, whilst others such as sucker depressants and pesticides are deliberately introduced. Examples of the former group are aflatoxins B_1 and G_1 and penicillic acid by fungi during tobacco storage (Forgacs and Carll 1966, Smith and Louria 1974). That microbial action may operate to add toxic agents

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TABLE 1. *Chemical composition o flue-cured tobacco*

Leaf fraction	Constituents	% Dry leaf weight	
Ash	Inorganics	13.5 (21)	9.25 (22)
Crude fibers	Cellulose, lignin	11.2	7.34
Carbohydrates	Poly and monosaccharides, starch, dextrin	22.5	36.35
Pectins	Pectinic acids	8.0	8.48
Organic acids	Krebs cycle acids	12.2	9.96
Ether-solubles	Oils, waxes, resins	7.3	6.61
Tannins	Polyphenols	2.2	---
Nitrogen compounds	Proteins, amino acids, nitrates, alkaloids	15.2	---

It is noted that the leaf consists of a preponderance of carbohydrates, crude fibers, pectins, etc., with smaller amounts of nitrogenous materials, lipids, and polyphenols.

Source: Schlotzhauer et al.

TABLE 2. *Ratios of smoke constituents in sidestream and mainstream*

(Based on Yields per Gram of Tobacco consumed

Constituent	SS/MS
Acetic acid	0.42
Acetamide	0.68
HCN	0.03
Acetonitrile	2.3
Aldehydes (total	2.2
3,4-Benzo a pyrene	2.1
Benzene	1.9
Toluene	3.5
Phenol	2.1
Nicotine	2.5
Myosmine	7.0
Pyrrole	7.1
Pyridine	9.5
3-Vinylpyridine	16.0

Source: Wakeham

or detoxify those present was shown by Gibson (1968). He has studied bacteria which under the mildest conditions can completely degrade the benzene ring. Biologically potent materials are added to tobacco during growth either by nature or by man. These include from nature cadmium (Nandiet al. 1969; Lewis et al. 1972, Friber et al. 1973) and other metals (Stahly 1973) and from man arsenic and maleic hydrazide, a sucker control agent which has been shown to be mutagenic (Liu and Hoffmann 1973).

For epidemiologic studies index compounds are needed for cigarette smoke which contaminate the atmosphere, are absorbed by the non-smoker and excreted by him as an index of exposure. Suggested index compounds include carbon monoxide, hydrogen cyanide, nicotine, cotinine and cadmium and polonium-210 in tissue. The rate metabolism of phenacetin and lymphocyte levels of aryl hydrolase have also been suggested to measure exposure. Criteria for such a material are: 1) it should be specific for cigarette smoke; 2) obtainable by non-invasive technique; 3) be easy to analyze accurately; 4) related to toxicity; and 5) unaffected by genetic or nutritional differences.

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is ideal for the purpose. Thiocyanate and cotinine in urine have been used and might become more satisfactory with further validation. Carbon monoxide is less specific; it requires alveolar air sampling or blood specimens and the amounts bound to hemoglobin may not be related to toxicity of other materials. The rates of detoxification of phenacetin are probably related to induction by smoke of enzymes which convert phenacetin to N-acetyl, p-amino phenol which has been shown to be inducible in animals by polycyclic hydrocarbons (Panturk et al. 1972). Because the urine of patients with bladder cancer contains no amino-phenol oxidation products with a phenoxyazone structure the urine of smoke exposed people could be analyzed for phenoxyazones as a measure of exposure. These are intermediate metabolies of tryptophane. The reactive agent may be methylnicotinamide and the possible mechanims the inhibition of the detoxifying enzyme by cigarette smoke (Wynder and Hoffmann 1968).

3.8.2 PRINCIPAL EFFECTS OF KNOWN COMPONENTS OF SMOKE

3.8.2.1 *Gases*

3.8.2.1.1 *Carbon monoxide* is reviewed in section 3.7.

3.8.2.1.2 *Hydrogen cyanide* poisons respiratory enzymes and is probably the cause of tobacco amblyopia (Heaton 1962). In mainstream smoke it may be responsible for rapid cessation of ciliary beating in the repiratory tract of smokers during smoking (Dalhamn 1966), but in sidestream smoke the cyanide levels are too low to anticipate any effect. Cyanide may be responsible for inhibitory effects on oral leukocytes (Eichel et al. 1969). Detoxification is by combination with sulphur (as sulfite or sulfhydryl) to form a thiocyanate ion which is excreted in urine and saliva. Such detoxification appears to depend on thioamino acids such as methionine and cysteine. The oxidized ferric form of cytochrome oxidase combines readily with cyanide. Cytochrome oxidase is also reactive with carbon monoxide. However, the hepatic enzyme rhodanese promotes formation of thiocyanate ion from cyanide ion and sulphite (White et al. 1958).

3.8.2.1.3 *Nitric oxide* and *nitrogen dioxide*. Oxides of nitrogen damage cell membranes in the lung, particularly cells of the respiratory bronchioles and capillaries (Dowell et al. 1971, Parkinson and Stephens 1973). They also combine with amines to produce nitrosamines which are oxidized to alkylating agents (HN_2). These are both potent carcinogens and sensitizing agents.

3.8.2.1.4 *Aldehydes*. Acrolein and formaldehyde are the most important examples of agents which cross-link proteins. They also stimulate mucus secretion in the airways. Both are excellent tissue fixatives, perhaps substituting for the alpha-amino adipic acid, semialdehyde, cross-linked collagen by forming Schiff bases with the amino groups or by aldol condensations (White et al. 1963). Acrolein inhibits the DNA polymerase in regenerating rat liver at levels between 2×10^{-5} and 8×10^{-4} M. It acts on the SH group of the enzyme (Munsch et al. 1973).

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3.8.2.2 *Water soluble agents*

3.8.2.2.1 *Alkaloids.* Nicotine is the most studied and most clearly important. It has many of the properties of acetylcholine. It is a neuromuscular transmitter, stimulates secretions, releases catecholamines, enhances intracellular cyclic AMP, and by these and or other mechanisms produces cerebral stimulation followed by depression. Furthermore, heart rate and blood pressure are enhanced. The addictive effect of tobacco is probably related to the nicotine and its metabolites. These include cotinine and myosmine (Stedman 1968).

3.8.2.3 *Fat soluble and insoluble particles or "tar"*

Tar materials, including the "brown" pigments in the particle phase, have defied analysis. Recently, however, sequential solvent extractions and analyses using gas-liquid chromatography and spectrophotometry, have identified polycyclic hydrocarbons, polyphenols derived from lignins and sugars, carbohydrates including cellulose, pectin, starches, brown pigments and metals and metal compounds (Table 3) (Chortyk 1972, Schlotzhauer et al. 1972).

3.8.2.3.1 *Polycyclic hydrocarbons.* These include benz(a)pyrene, benzanthracene and other potent carcinogens.

3.8.2.3.2 *Polyphenols.* These are derived from lignins and sugars by pyrolysis which induces either cyclization or depolymerization and cyclization. These materials are strong irritants and may serve as co-carcinogens serving the function of promoters to initiators such as benz(a)pyrene (Friedwald and Rous 1944).

TABLE 3. *A comparison of smoke and leaf pigments*

	LP	SP
Chlorogenic acid	+	+
Glucose, rhamnose	+	−
Iron, silicon	+	+
Amino acids	+	+
Alkaloids	+	+ +
Yield	3–4 %	5 %
Hydrolysis resistance	±	+ −
Molecular weight, major	30,000	100,000

Source: Chortyk

3.8.2.3.3 *Carbohydrates.* These are long chained polymers of high molecular weight which are soluble in ethanol. They have a slow biological decay after phagocytosis and may serve to enhance the effects of other agents by making them persist (Warren 1972).

3.8.2.3.4 *Brown pigments.* These are compounds of carbon, hydrogen, nitrogen, sulfur and iron. Typically, they can be shown to contain iron, protein, chlorogenic acid and rutin in complexes. They are formed either directly by condensation or through the action of polyphenol oxidase in the typical browning reaction. They range in molecular weight from 2,000 to over 100,000 and when phagocytized may serve as intracellular sources of epoxides and hydroxides (Chortyk 1972).

3.8.2.3.5 *Metals.* Alveolar damage has been found after heavy occupational exposure to nickel, chromium, cadmium, and arsenic. Although these metals are contained in tobacco smoke and the low levels make it unlikely that they produce toxic effects, there is insufficient data to exonerate them. A possibility for chronic

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toxic effects is provided by cadmium content of lung, liver and kidney of smokers which has a mean of 15.8 mg compared to 6.6 mg in non-smokers (Friberg et al. 1973). Cadmium and arsenic may be implicated in emphysema (Nandi et al. 1969). Furthermore, organified metallic compounds such as nickel carbonyl are carcinogens in low concentrations.

3.8.3 TARGETS AND MECHANISMS FOR TOXIC EFFECTS

Targets include cells, lung cells and lung, platelets and thrombi, fetal development and cerebral function. Bioassays should have high sensitivity, moderately reliable dose-response relations, intra-laboratory reproducibility and be relevant to human responses.

3.8.3.1 *Cells, organelles and isolated enzymes* have been used as targets to assay cigarette smoke condensate and pure chemicals found in smoke (Rylander 1971, Bresch et al. 1972). There is evidence of stimulation, inhibition, cytotoxicity and neoplastic transformation. Condensates from tobacco smoke caused hyperplasia with pleomorphism in the newly formed cells in the bronchial epithelium of human fetal lung as noted by Lasnitzki (1958, 1968) and carcinogens including benz(a)pyrene, methylcolanthrene, and di-methylbenzanthracene induced similar changes in neonatal rat tracheal epithelium (Crocker et al. 1965). Fibroblasts from hamster lung were transformed in culture by exposure for 3 hours to crude cigarette tar (Inui and Takayama 1971). Signs of toxicity, such as pyknosis in nuclei, vacuolated cytoplasm, cell necrosis, and giant cells were found between 2 and 48 hours after treatment and transformation occurred over 100 days after treatment. Skin painting of mice with crude cigarette smoke condensate has been used to determine the most potent fractions. Bock et al. (1969) found the soluble weak acids and the nitromethane soluble neutral fractions had the greatest tumor stimulating activity while Wynder and Hoffman (1968) reported that in addition, strong acid fractions were potent.

Rhim and Huebner (1973), using the same fractions isolated by Bock et al. (1969), found *in vitro* cell transformation in rat and mouse cells infected with C-type RNA tumor viruses using the insoluble bases fraction 3, ether soluble weak acids, fraction 8, and nitromethane soluble neutrals, fraction 14. This C-RNA tumor virus assay was worked out by Freeman et al. (1971) in the Syrian hamster embryonic cell lines. They showed that after treatment with 3-methylcholanthrene or methylbenzanthracene, cigarette smoke condensate produced transplantable tumors which, although negative for infectious virus before innoculation, contained hamster specific C-type RNA viruses afterward. Thus, chemical treatment was associated temporally with activation of the virus.

3.8.3.2 *Mechanism of carcinogenesis*
The mechanism of these effects has been elucidated, at least in part, recently. It appears to depend upon a microsomal enzyme system, aryl hydrocarbon hydroxylase (AHH). The AHH enzyme system both activates and detoxifies. Although it converts polycyclic hydrocarbons to either weak or non-carcinogenic compounds (Gelboin et al. 1972, Wattenberg 1972),

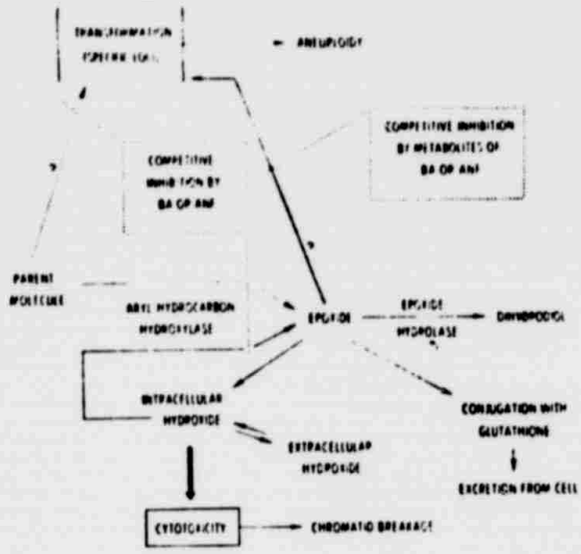


Figure 1. Diagram of polycyclic hydrocarbon metabolism in cell culture, ultimately leading to the two distinctly different processes caused by certain carcinogens: transformation or cytotoxicity. Source: Benedict et al. 1972.

it also activates them to induce malignant transformation. The toxicity of the polycyclic hydrocarbon to cells grown in culture is correlated with the presence of AHH activity and the inhibition of hydroxylase activity in the cells by 7-8 benyflavone as paralleled by an inhibition of the toxic effect. Activation and inhibition may be unique for each hydrocarbon as suggested by Gelboin et al. (1972). Further experimental studies in inbred mice have shown that aryl hydroxylase induction is most likely controlled by an autosomonal trait in skin, peritoneal lining, lung, liver, kidney and bowel. Apparently basal levels of hydroxylase activity are sufficient in these tumor prone mice to induce carcinoma as shown by Benedict et al. (1973). Although there is much species and tissue variation in the microsomal biotransformation of polycyclic hydrocarbons, the relation to epoxide formation, to intracellular hydroxides and to cytotoxicity has been most coherently formulated by Benedict et al. (1972) (Figure 1). Two additional generalities have emerged. One by Feuer et al. (1973) that methylation of 7-coumarin, BHT-alcohol, 7-pyrazole, amphetamine and nicotine induce the microsomal metabolizing enzymes and raise the level of microsomal phospholipid methytransferase. The other by Ames et al. (1972) showing in homogenates of rat hepatic cells that epoxides of benzanthracene, particularly epoxides, formed by the aryl hydroxylase are potent frameshift mutagens suggesting at least one pathway for a relationship between mutagenicity and carcinogenicity in rodents (Buerki et al. 1973).

The normal metabolism of polycyclic hydrocarbons is to an epoxide by the AHH system (Figure 1). The epoxide can be converted nonenzymatically to a monohydroxyl derivative, metabolized by an epoxide hydrolase to a dihydrol or

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conjugated. If specific binding to DNA is essential for transformation, it may be inhibited by benzanthracene or alpha-naphthoflavone. Direct binding of the parent molecule with DNA was thought unlikely in view of the fact that both the K region epoxide and the corresponding dihydroldiol (DHD) transform cells in culture and bind cellular constituents much more readily than the parent compound or the phenol. However, the latter conclusion is questionable since DHD did not bind or transform in subsequent experiments (Marquardt 1972). The monohydroxyl derivative may be further metabolized (Figure 1). Cytotoxicity would reflect the accumulation of phenolic derivatives in sufficient amounts to surpass the threshold depicted by the wide arrow resulting in disruption of the mitotic spindle, of transcription and of translation, as well as in euplodity with chromatid breakage. Using several different tissue culture cells, Diamond (1971), showed that the kinetics of hydrocarbon metabolism may be sequential, involving conversion to alkaline extractable derivatives and then to water soluble derivatives. When growth in cell cultures was inhibited, the water soluble derivatives of the hydrocarbon were found. Little or no ability to metabolize hydrocarbons was found in cells resistant to the cytotoxic effects. Pregnant rats treated with benz(a)pyrene showed a 15 fold increase in aryl hydroxylase activity in the liver compared to control and there was detectable enzyme activity in the fetus after 15 days of gestation which increased with age compared to no activity in the control fetuses (Schlede and Merker 1972).

Of most relevance to lung cancer are the observations of Kellermann et al. (1973) who studied the AHH system of lymphocytes from patients with lung cancer and tumor and non-tumor controls. The AHH was induced in the lymphocytes of healthy controls and tumor controls by 3-methylcholanthrene showed distributions of approximately 45 % low, 45 % intermediate, 10 % high inducibility, while patients with lung cancer were 4 % low, 66 % intermediate and 30 % high. All 50 patients with lung cancer had been heavy smokers for 20-60 years.

This system may be important in human exposure to sidestream smoke. Cigarette smoke exposure of the non-smoker could constitute a heavier exposure to polycyclic hydrocarbon carcinogens than is derived from fossil fuel combution except under exceptional circumstances (Hoegg 1972). The levels of benz(a)pyrene, methycholanthrene or benzanthracene might be significant for those individuals with genetically high inducibility of of AHH and further examination of this relationship is imperative. Recently it was shown in rats that a single dose of nicotine reduced bilary excretion of radioactive metabolites of 3,4-benzopyrene and the nicotine reduced the AHH activity *in vitro*. Because such microsomal metabolism of these polycyclic hydrocarbons to epoxides appears to enhance their carcinogenic effect, nicotine *may* have a protective role (Weber et al. 1974).

3.8.3.3 *Effects on pulmonary cells*

It is doubtful whether lung cells have any advantage in the assay of cigarette smoke, cigarette smoke condensate or fractions thereof. Nicotine and water soluble fractions of cigarette smoke condensate have

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been found to suppress IgM and IgG responses in lymphoid cell cultures from rabbits to stimulation with sheep erythrocytes. The rabbits were sensitized to sheep erythrocytes before obtaining lymphoid cultures from the spleens (Roszman and Rogers 1973). Cigarette smoke exposure of lymphocytes inhibited phytohemaglutinin-stimulated thymidine uptake by lymphocyte cultures from 92 patients. However, in eight patients who were hypersensitive to cigarette smoke stimulation did occur (Savel 1970).

3.8.3.3.1 *Aryl hydrocarbon hydroxylase.* Mixed function oxidase activity in liver and lung was compared in rabbits and was found to be concentrated in the microsomal fractions of both organs, requiring NADPH and oxygen for activity and more inhibited by carbon monoxide-cytochrome C. Cytochrome P-450 levels and aniline hydroxylase activity alone were approximately 20% those of liver while NADPH cytochrome-C reductase, benzphetamine demethylase and biphenyl hydroxylase were nearly equal in lung and liver microsomes (Gram 1973). Matsubara and Tochino (1971) showed similar results in rabbit whole lung and liver microsome systems. This naturally led to the question of which cells in the lung have the mixed function oxidase activity. Two studies are pertinent in this regard. Hook et al. (1972) found that the microsomal fraction of alveolar macrophages had very low levels of biphenyl 4 hydroxylase, benz(a)pyrene hydroxylase, and d(+) benzphetamine-N-d-methylase while cytochrome P-450 was absent; but both NADPH cytochrome-C-reductase and NADH cytochrome-C-reductase activities were present, suggesting that the macrophages play a small role, if any, in detoxification of materials in the lung. Reid et al. (1972) found that alveolar macrophages alkylated para amino benzoic acid to the same extent as did the liver and lung parenchyma, but microsomes from macrophages lacked detectable aryl hydrocarbon hydroxylase activity, even in animals treated with 3-methylcholanthrene. Similarly, brombenzene was metabolized by microsomes prepared for liver and lung but not from alveolar macrophages. However, these macrophages may not have been induced because of their extra vascular (alveolar) location.

3.8.3.3.2 *Polymorphonuclear leukocytes (PMN's).* Oral PMN's cluster and fail to spread and migrate after exposure in the oral cavity to cigarette smoke. This effect is related to the gas phase in which acrolein and cyanide are present. Exclusion of the gas phase showed that particle phase did not produce the inhibition (Eichel et al. 1969). Exposure of guinea pigs to cigarette smoke significantly increased the numbers of polymorphonuclear leukocytes recoverable by pulmonary lavage over a 10 week period (Flint et al. 1971). Rylander (1971) has also shown that the number of polymorphonuclear leucocytes obtainable by lavage are increased after cigarette smoking. Recently Kilburn and McKenzie (1974) showed that whole smoke and water soluble extracts of cigarette tobacco recruit polymorphonuclear leukocytes into smaller airway in a manner similar to that for cotton dust (Kilburn et al. 1973). Maximum recruitment occurs in 4-6 hours. Exclusion of the gas phase by charcoal filtration does not alter the recruitment effect. However, removal of the particle

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phase by double Cambridge filters abolishes the recruitment and instead produces pyknosis of nuclei, karyorrhexis, karyolysis, ciliary loss, nuclear exfoliation, perinuclear vacuolation, cytoplasmic vacuoles and exfoliation of many cells. Species may be important as shown by Holt and Keast (1972) in C57 black mice that smoke exposure daily for 34 weeks increased the number of macrophages and leukocytes recovered but did not affect the numbers of polys.

3.8.3.3.3 *Macrophages*. Increase in the numbers and in the fluorescent inclusions in the macrophages obtained from the lungs of cigarette smokers was first noticed by Vassar et al. (1960). Roque and Pickren (1968) using lung biopsies from cigarette smokers and non-smokers showed that the alveolar macrophages of the smokers had decreased activity of oxidoreductases and hydrolases by histochemical techniques and that this was proportional to the amount of fluorescent material stored in the cells. Succinate dehydrogenase, cytochrome oxidase, and NADH diaphorase were also severely suppressed. Utilizing lavage Pratt et al. (1969) showed that smokers had an increased volume of brown sediment in lavage fluid which contained pigmented macrophages and were greater in volume; Cook and Webb (1966) showed that surfactant obtained from smokers exhibited higher minimal surface tension than that obtained from non-smokers. That macrophages from smokers are stimulated has been shown by Warr and Martin (1973) who found that the migration rate was three times faster than in cells from non-smokers, whereas although the migration inhibitory factor decreased migration at least 30% in non-smokers it had no effect on the migration of macrophages from smokers. Histochemical evidence has been presented by Powell and Green (1972) that phagocytic competence of macrophages is reduced in smokers and that smoke inhibits the enzyme glyceraldehyde-3-phosphage dehydrogenase. Whether this is an effect of cyanide is not known. It is somewhat surprising in view of the lack of induction of aryl hydrocarbon hydrolases mentioned above. Cantrell et al. (1973) found that enzyme activity was low in the alveolar macrophages of non-smokers, .008 ± .004 $\mu 10^6$ cells and was higher, a mean of 0.095 ± 0.024 $\mu 10^6$ in smokers. One smoker who was studied showed low activity while not smoking and four times the activity when he resumed smoking.

3.8.3.3.4 *Ciliary beating*. This is inhibited by smoke as shown by Dalhamn (1956) and by Westergaard and Olsen (1973). This may be due to effects of formaldehyde, acrolein or cyanide at high exposure levels but to tar at lower levels. Chronic smoke exposure appears to reduce the number of cilia (Watson and Brinkmann 1964).

3.8.3.3.5 *Airway morphology*. Nitrogen dioxide, which is found in levels as high as 400 ppm in main line smoke, produces early changes in the morphology of the respiratory and terminal bronchiolar region in rat lungs with levels of 2 ppm for 1, 2 and 7 days. These included loss of cilia and increase in microvilli. Exposure to levels of 3 7 ppm for one hour produces changes in the alveolar epithelium and capillary endothelium in beagle dogs (Dowell et al. 1971).

Effects of chronic exposure to cigarette smoke on the bronchial epithelium have been shown by Auerbach et al. (1972) and Mawdesley-Thomas and Healey (1973) in lambs which smoked through tracheostomies. Tracheal and bronchial gland hyperplasia had a significant dose related response. Lamb and Reid (1969) have shown that rats have an increase in the number of goblet cells in tracheal and intrapulmonary airways over a six week period of exposure to smoke of both cigars and cigarettes. Hernandez et al. (1966) and Frasca et al. (1968) have shown changes in alveoli, particularly in the area of the respiratory bronchioles in dogs chronically exposed to cigarette smoke. These effects may be related by benz(a)pyrene and particles, as suggested by the finding of Port et al. (1973), using scanning electron microscopy of hamster tracheal and bronchial epithelium following ferric oxide and ferric oxide plus (benz(a)pyrene. The former produces loss of ciliated cells and hyperplasia of nonciliated cells, while the addition of benz(a)pyrene produced squamous metaplasia.

3.8.3.4 *Effects on blood and blood vessel cells*

Effects of smoking upon platelets and blood clotting appear to be mediated by nicotine induced catecholamine release. Ardlie et al. (1966) showed that catecholamines enhance ATP or ADP induced platelet aggregation. Rowsell et al. (1966) showed thrombus formation in the extracorporeal system and clotting time in silicon tubes was increased by moderate doses of epinephrine. Schoendorf et al. (1970) found cigarette smoking, particularly of high nicotine cigarettes, reduced the silicon clotting time by 11.06 minutes and increased platelet adhesiveness. Shimamoto and Ishioka (1963) proposed an effect, other than epinephrine-like, on arterial walls causing a release of the thromboplastin-like substance leading to increased platelet aggregation. Murchison and Fyfe (1966) have shown that the rise in plasma free fatty acids following cigarette smoking is preceded by increased platelet adhesiveness and Harrison and Mitchell (1966) have suggested that the platelet adhesiveness tests *in vitro* are influenced by ADP released from damaged red cells. Kerr et al. (1965) showed that unsaturated fatty acids have a protective effect on increased platelet adhesiveness. From another angle Salky and Dugdale (1973) have found that about 10% of patients with ischemic heart disease have abnormal platelet aggregation to dilutions of plasma, as well as reduced platelet survivals. Most of these patients were smokers. Harkavy (1968) has suggested that migratory phlebitis, eosinophilia, and thromboangiitis obliterans may be related to vascular hypersensitivity to components of cigarette smoke. Although the mechanism is still obscure, the phlebitis in patients with thromboangiitis obliterans clears when they cease smoking and returns if they take it up again.

Apart from the acute effects of nicotine simulating catecholamine release with increases in heart rate and blood pressure, high concentrations of CO and cyanide appear to cause or be associated with damage to vessels in the cholesterol fed rabbit. Astrup et al. (1967) showed that such rabbits exposed to 20 ppm carbon monoxide continuously for up to 10 weeks had increased atheromatosis of the aorta compared to similarly fed controls. Kjeldsen et al. (1969) showed that increased

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concentrations of oxygen reduced the amount of cholesterol induced atheromatosis in rabbits. Webster et al. (1970) found that cholesterol fed squirrel monkeys showed significantly more coronary atherosclerosis when exposed intermittently to carbon monoxide than those who were only exposed to room air over a 7 month period.

A mechanism has been suggested by Whereat (1970) that when oxidation of nicotine amide adenine dinucleotide (NAD) is impaired, the reduced form (NADH) is available in larger quantities for fatty acid synthesis by arterial enzymes. Carbon monoxide prevents the oxidative reaction of NADH to NAD by combining with cytochrome oxidase. Cyanide is also a respiratory chain inhibitor and thus affects arterial wall fat metabolism. Nicotine sulfate depresses lipoprotein lipase activity in rats which may disturb lipid metabolism in vessels (Mitkov et al. 1971). Human studies show that pulmonary arterial collagen content are about twice as great in cigarette smokers as in non-smokers between the ages of 30 and 70 (Naeye and Dellinger 1971).

Before extrapolating from data in cigarette smokers to environmental tobacco smoke exposure, effects of low doses, 1-2 cigarettes per day must be studied.

3.8.3.5 *Effects on fetal development*

The fetus will be exposed to certain tobacco smoke products via the mother. This constitutes a special case outside the province of this review. Some evidence reviewed by Haworth (1974), suggests that there is no threshold for fetal effects of maternal smoking, but this requires verification.

Much earlier it was shown that a toxic agent, presumably nicotine, crossed the placenta to fetues to increase fetal heart rate after a human mother began to smoke a cigarette (Herriot et al. 1962). Nicotine administered to the mother rhesus monkey crossed the placenta to the fetus which responded with hypotension and brachycardia to a single injection or tachycardia to constant infusion (Suzuki et al. 1971). In mature fetuses, hypoxia and respiratory and metabolic acidosis developed when nicotine was administered to the mother. This did not occur in immature fetuses. Sawyer and co-workers (1972) have found that nicotine administered to lactating rats depressed or delayed prolactin secretion, reduced milk yield, and diminished the stimulation of prolactin by suckling.

3.8.4 CONCLUSION

Much evidence exists that smoke exposure produces adverse effects on cells, cells of the lung and the blood. The question as to which of these effect appears at the concentrations of tobacco components found in environmental tobacco smoke exposure cannot be answered by the fragmentary data which are available. Although a dependable hierarchy of effects has not yet been established, annoyance and acute changes in air distribution in the lung are shown by sensitive human subjects. Transformation of airway cells, retardation of fetal development and respiratory illnesses may be the sensitive indices to higher doses of tobacco smoke exposure. Until additional dose-response data are available we must be guided by fragmentary information from human groups at risk in

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developing criteria for environmental exposure to tobacco smoke.

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