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PERSPECTIVES IN THE EXPERIMENTAL APPROACH TO THE HUMAN LUNG CANCER PROBLEM

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Perspectives in the experimental approach to the human lung cancer problem

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An international conference on lung tumours in animals is a very timely event and I congratulate Professor *Severi* and his colleagues for having brought it about. I also commend the planners very heartily for having placed primary emphasis here upon experimental studies. It is my conviction that well-conceived experimental research with animals, supplemented by clinical and other observations upon humans and interpreted with caution, is the route most likely to lead us to a true perspective on the pathogenesis of human lung tumors. No experimentalist will question that the pathogenesis is a complex phenomenon involving the interaction of multiple factors, both internal and external. Comprehension of the ways in which such factors interact, and of their relative importance, should furnish us with practicable methods for reducing the toll of bronchogenic carcinoma among humans.

The methodical and systematic experimental study of lung neoplasms has, I think, been interrupted and indeed diverted by emphasis placed upon epidemiological observations in recent years. Though it is generally acknowledged that statistics alone will not solve the etiology of any disease, yet statistical reports have tempted many investigators to attempt experimental short-cuts. Though these seemed reasonable under the circumstances, they were generally less rewarding than was expected, and a return to a more orderly and methodical procedure seems indicated.

When a statistical association between cigarette smoking and lung cancer was first reported, a good deal of rather feverish activity was begun. Biologists undertook to paint the skins of mice and other animals with condensates of cigarette smoke, prepared by a variety of methods. Others introduced such condensates into the tracheobronchial tree in various media. Chemists undertook analytical studies in an attempt to identify possible carcinogenic agents, especially polynuclear hydrocarbons. Animals of several species and strains were exposed to the inhalation of cigarette smoke, generated by a variety of methods.

In smoke inhalation studies, the cancers evidently expected by many investigators failed to appear (e.g. *Campbell*, 1936; *Dontenwill* and *Mohr*, 1962; *Haag*, 1960; *Holland et al.*, 1958; *Kensler*, 1962; *Leuchtenberger et al.*, 1958, 1959, 1960; *Lorenz et al.*, 1943; *Moore* and *Bock*, 1958; *Mühlbock*, 1955; *Passey*, 1958; *Peacock*, 1958, 1960; *Scala* and *Vicari*, 1955). Many of these studies were never published *in extenso*, if at all. Some of those in which minute descriptive observations had been made were considered to justify publication despite the absence of invasive cancer (e.g. *Leuchtenberger et al.*, 1958, 1959, 1960 a, b), but the extent and bulk of this negative work is little known even today.

Of the few known carcinogens revealed by chemical analysis of smoke, that present in greatest quantity is benzpyrene, but even this occurs only in extraordinarily small concentrations (Smoking and Health, 1964, p. 56). The concentrations of any of these substances indeed, are so low that they would probably have been undetectable before the advent of chromatography, and their biological significance, if any, is questionable.

Intratracheal infusions of cigarette smoke condensates into various animals in various suspending media also failed to produce the expected tumors (e.g. *Della Porta et al.*, 1958; *Herrold*, 1962; *Passey et al.*, 1954; *Rigdon*, 1960, 1961; *Rockey et al.*, 1958). Thus, even as a method of bioassay such procedures have not provided any means of guiding the systematic fractionation of condensates for isolation of some agent hypothetically active by contact.

While long term skin painting of sensitive mouse strains with high concentrations of smoke condensates has produced papillomas, some fraction of which ultimately become malignant, the relevance of such work to the human lung cancer problem is seriously questionable (*Larson et al.*, 1961; *Stewart* and *Herrold*, 1962). Indeed the doubts and misgivings are growing. Although this procedure has become a crude tool which can guide fractionations of condensate to locate and perhaps identify some of the substances or classes of substances responsible for the activity upon mouse skins, it does, after all, employ the wrong material in the wrong form, on the wrong tissue of the wrong animal. In the area of cancer pathogenesis, strain and species differences are often great.

Doubts are reinforced by the observation that cigar, pipe and cigarette smoke condensates all show a comparable albeit feeble activity on the skins of experimental inbred mouse strains (*Homburger et al.*, 1963), whereas the statistical incidence of lung cancer among pipe or cigar smokers is scarcely greater, even when they report that they inhale, than among non-smokers (Smoking and Health, 1964, p. 112).

Smoke condensates are not smoke, either in chemical composition or in physical properties. There can be no assurance that removal from smoke condensates of any agent to which mouse skin tumors might be attributed would influence in the least any effects of whole smoke inhalation that might be significant in human experience.

The first intense wave of experimental studies stimulated by the statistical reports has thus awakened us to several realities. We now realize, I think, that much more sophisticated approaches will be necessary. The early smoke inhalation studies were qualitative in that a satisfactorily accurate method of measuring exposure of lung surfaces was lacking. Moreover these studies were originally launched with almost no basic or background information about squamous or bronchogenic carcinoma in most animal species. Such cancers had rarely been described or reported. Virtually nothing was known about the conditions under which they can occur. There was even a question whether some species were biologically capable of experiencing this type of lesion. The realization began to develop that it might be well to back up, and begin at the beginning.

Gradually some of the gaps in basic knowledge are being filled by systematic experiments. Some years ago, *Lisco* and *Finkel* (1949) showed that substantial doses of inhaled radioactive cerium can produce squamous lung cancers in mice. Subsequently a considerable series of studies have demonstrated that many other radioactive substances, in substantial doses, can do so in rats and mice (*Cember* and *Watson*, 1958a, b; *Gates* and *Warren*, 1960; *Lisco*, 1959; *Temple et al.*, 1958; *Scott* and *Thomas*, 1957). These investigations have thus answered an important basic biological question, that mice can develop squamous lung cancer. In this field, we still need animal studies to determine dose-response relationships and thresholds, when exposures are chronic or repetitive at very low levels. Species differences will also be of interest.

Another basic study of significance was that of Dr. *Marvin Kuschner* and his collaborators (1956), in which they exposed mice to inhalation of methylcholanthrene in several air-borne forms, including a pure fume. Lung cancers did not develop in these animals.

The logic of this experiment is obvious. If the tiny traces of polynuclear hydrocarbons present in tobacco smoke are to be suspected of playing a role in lung cancer genesis, for example, it is reasonable to observe the effects of a greatly increased dose of a similar hydrocarbon, introduced in a similar form into the lungs of experimental animals. The strategem is old, simple and still useful. Once we learn from exaggerated doses what the effects of an agent can be and where to look for their manifestations, we can hope to

solve the problems of dose response, thresholds and tolerances by systematic follow-up experiments.

Kuschner's initial lack of result did not demonstrate any basic immunity of rodent lung tissues to the action of this potent carcinogenic hydrocarbon. Rather, the experiments showed still further the complexity of the situation. His animals were intact, free from known infections and well-fed. Presumably their mechanisms for lung cleansing and for detoxification were working efficiently. Efficient lung cleansing could be expected to minimize contact time between lung cells and the hydrocarbon.

Some years ago *Andervont* (1937) demonstrated that lung cells of rodents can respond to certain carcinogenic hydrocarbons by developing squamous cancers. In 1937 he transfixed the lungs of C-57 black mice with threads impregnated with 1,2,5,6-dibenzanthracene. Squamous carcinomas of the lungs developed in these mice. This was, to my knowledge, the first experimental production of such tumors in rodents. Since the threads passed through skin before entering the lung, there was a theoretical possibility that skin cells might have been introduced into the lung. Conceivably these could have developed into skin cancers within the lung area.

Kuschner (1956) repeated this thread transfixion maneuver with mice using both dibenzanthracene and methylcholanthrene, and with similar results. Dibenzanthracene proved to be the more effective of the two. He also performed similar experiments on rats. With both species, the possibility of introducing skin cells into the lung was minimized by opening the skin and transfixing the lung directly. Hence it seems almost certain that native lung cells interacted with the carcinogen rather than any extraneous ones. *Kuschner* also implanted carcinogenic hydrocarbons into the lungs of rats in the form of pellets enclosed within stainless steel wire cages. Squamous cell carcinomas arose in many of these cases also.

A number of other investigators (*Della Porta et al.*, 1958; *Rigdon*, 1960; *Herrold*, 1962), as well as *Kuschner*, have instilled infusions of carcinogenic hydrocarbons in various kinds of media, by the intratracheal route, into several species of animals. In certain cases, depending in part upon the nature of the medium, cancers were obtained, some of which were squamous.

All these experiments are obviously very artificial in character and do not remotely resemble any conditions encountered by mankind in ordinary life. They appear, however, to have given a definitely affirmative answer to one preliminary question with important bearing on the suitability of certain animals for experimental study of lung cancers of the types prevalent among humans. We can, I think, feel confident that the lung tissues of several species are biologically

able, under the action of certain contact carcinogens, and under suitable conditions, to produce tumors similar histologically to the types prevalent in man.

Kuschner's experiments suggest to me that an inhaled airborne carcinogenic substance may not easily be able to produce neoplasm unless some combination of precursor conditions is favorable. Perhaps it may not even then do so unless the physical state and chemical condition of the inhaled agent is such as to favor retention, absorption, tissue contact or delayed elimination or alteration. The experimentalist seems to have no alternative to the systematic test of all such factors until the picture of their interaction becomes clear (*Hockett*, 1962).

It must be noted that while several of these various experimental strategems contributed toward prolonging contact time between lung and hydrocarbon, all of them also introduced the element of trauma. The traumatic effects of thread transfixion and pellet implantation in steel cages with hooks, are obvious. Even empty imbedded cages produced metaplasia. Intratracheal infusion solutions also produce severe and extensive inflammation. Trauma is succeeded by regeneration. Hence the presence of young, regenerating tissue may have been a common element among the successful experiments in squamous lung cancer generation by chemical carcinogens.

Beginnings are being made toward a systematic study of trauma and regeneration in this context. *Stanton* and *Blackwell* (1961) have produced lung infarctions in rats by intravenous injection of hexachlorotetrafluorobutane. When this was accompanied by injection of methylcholanthrene, invasive epidermoid carcinomas were produced. These were considered, from observations of transitional lesions, to have arisen from regenerating epithelium. The hydrocarbon alone, in tricaprylin, produced only keratinized cysts that did not become invasive. Somewhat similar experiments involving lung damage with a hot wire have also been reported (*Takeda et al.*, 1959).

While the intravenous administration of hexachlorotetrafluorobutane is in itself unphysiological in the sense that probably no human has ever had this experience, yet infarctions of the human lung, from one cause or another, are common (*Baló et al.*, 1956; *Raeburn*, 1951; *Raeburn* and *Spencer*, 1953; *Spencer* and *Raeburn*, 1954; *Yokoo* and *Suckow*, 1961). There is, as a matter of fact, a rather impressive literature reporting human lung tumors that developed in infarcted areas and around old tubercular lesions (cf. *Schwartz*, 1960).

The *Stanton* method promises to become a useful experimental tool since it provides a method for traumatizing the lung that can be controlled and reasonably well standardized. Experiments are now under way to observe the effects of inhaled agents by animals with such infarcted lungs. Many combinations and permutations of con-

ditions are possible which may produce the lung carcinomas that *Kuschner's* healthy, intact animals failed to experience when they inhaled methylcholanthrene. We may hope by such studies to get some clarification of the role of lung trauma and regeneration in the total picture.

Infarctions, of course, are only one form of trauma. Many inhaled chemical agents that are only too abundant in the air of cities, such as sulphur dioxide, produce chronic or recurrent inflammation of the lungs. The possible role of chronic or recurrent inflammation in modifying the susceptibility to carcinogenic agents received by inhalation or other routes must certainly be elucidated by systematic, step-wise studies. Such systematic study is hardly beyond its infancy at the present time.

But the most important source of lung irritation, inflammation and regeneration, probably lies in the infections — both viral and bacterial. A history of chronic or repetitive lung infection is quite common in lung cancer cases.The reports of the late Dr. *M.C. Winternitz* (1920) on his pathological examinations of the lungs of influenza victims in the 1918-1919 epidemic aroused interest in and concern over the possible relation of virus infections to carcinoma of the lung, which has persisted (e.g. *Leuchtenberger et al.*, 1965; *Steiner* and *Loosli*, 1950; *Straub*, 1937; *Wagner*, 1956).

The numerous current studies in this field, including lung cancer production in animals by human viruses, are too well known to require any detailed account here, even if time permitted. In the present context, however, the report of *Wisely et al.* (1961; *Kotin*, 1963) stands out as particularly relevant. These investigators produced lung damage in C-57 black mice by infection with a succession of three mouse-adapted influenza virus strains. When this was followed by inhalation of a carcinogenic aerosol of ozonized gasoline vapors, invasive and metastatic lung carcinomas were obtained at a substantial level of incidence. This result has obvious significance. Nevertheless, it does not justify incautious conclusions. Its main value may lie in its use as a technique, albeit a laborious, slow, and expensive one, for studying the contributory effects of still other variable factors to the end result.

Such another variable, for example, is the functional condition of the liver. The well-known role of this organ in detoxification has been shown to apply to certain, at least, of the carcinogenic hydrocarbons (*Berenblum* and *Schoental*, 1942, 1946; *Berenblum et al.*, 1943; *Falk et al.*, 1962; *Kotin et al.*, 1962). The prevalence of cirrhotic or pre-cirrhotic livers, at autopsy, among victims of oral-cavity cancer, suggested that disturbance of the detoxification efficiency of this organ might increase susceptibility to the action of carcinogenic

agents (*Trieger et al.*, 1958). It seems to have been established experimentally by *Protzel et al.* (1964) that benzpyrene treatment of the oral cavity in mice will produce cancers much earlier if their livers have previously been damaged with carbon tetrachloride, alcohol or a combination of these. Liver damage by dietary deficiency is now under study in this connection. When standardized methods have been perfected, the possible role of liver damage in lung cancer genesis should undoubtedly be studied as an added factor by techniques based upon the *Stanton* procedure, the *Wiseley-Kotin* method and others.

There has been much discussion of ciliastasis as a possible factor in lung cancer genesis. The postulate is that any influence which can slow down the clearance of inhaled materials from the lung, might tend to prolong the period of tissue contact. If such inhaled material contains carcinogens, a prolonged contact might be presumed to increase the opportunity for cell transformation, but considerable rigorous investigation remains to be done before ciliastasis can really be evaluated as a factor. Mucus flow is not the only mechanism for removal of extraneous matter from the lung. *La Belle*, basing his conclusions on rates and pathways of clearance of radioactive tagged dusts from the lung, was of the opinion that the activity of roving phagocytes accounted for as much as eighty-five percent of its removal, as compared to fifteen percent attributable to the flow of mucus. He observed experimentally that inhalation of carbon particles could markedly stimulate phagocytosis, and recommended this as a treatment in cases of accidental inhalation of radioactive materials. He also observed that the inhalation of cigarette smoke, though this may have ciliastatic effects, had no influence on the overall clearance rates for inhaled radioactive dusts by rabbits (*La Belle et al.*, in press). Hence he reasoned that if mucus flow was impeded, phagocytosis must have been stimulated commensurately. *La Belle's* untimely death has interrupted these studies for the time being, but it is to be hoped that they will be resumed.

A more decisive experimental test of the possible role of ciliastasis would be a long term or life-time study with animals. This would require a basic system that could be counted upon to produce a relatively high and predictable incidence of squamous lung cancers in the population, so that the superimposition of agents that either stimulate of depress ciliary activity could be carried out, and any effects assessed in terms of actual increase or decrease of tumor yield. If the system used for this purpose employed only influences that occur in human experience, as in the case of the *Wiseley-Kotin* study, so much the better. Even then, some interspecies comparisons

would be required to give assurance that observed effects were of general significance.

Similar comments apply to the possible role of promoting agents in the pathogenesis of lung carcinoma, The whole concept of promoter action has arisen from skin carcinogenesis studies. Efforts should be made to design methods whereby the action of agents suspected of being promoters could be studied directly in the lungs of intact animals following exposure to initiating agents.

Time does not permit discussion of other possible factors in any detail. Certainly stimulation or repression of the reticulo-endothelial system would be expected to play some part in carcinogenesis. The composition of mucus, its possible anti-carcinogenic properties, its known or suspected role in the resistance to virus infections and changes in abundance and properties, are all candidates for systematic study, once suitable test systems can be devised. Age, sex, nutritional state and hormonal excesses or deficiencies remain to be evaluated in the context we have been discussing.

Some chemical and physical factors influencing the retention and absorption efficiencies of inhaled polynuclear hydrocarbons have been described by *Shabad et al.* (1964), *Saffiotti et al.* (1964) and others, but such studies are still in a rather preliminary stage.

It will be clear that in this brief paper, nothing resembling a complete review is possible. It has been my purpose merely to illustrate some of the requirements of an orderly, systematic, step-wise program to elucidate the pathogenesis of lung carcinoma. The Council for Tobacco Research is dedicated to such a program.

I have said relatively little specifically about tobacco smoke in animal studies of lung carcinoma. It should be implicit in my remarks that until we have learned more about the conditions for action of carcinogens in the lung, the role of trauma, regeneration, non-specific irritation, virus infections, ciliastasis, hormones, mucus, nutritional factors, liver damage, and the like, we really possess no methods for adequately assessing the possible action or function of this particular complex material. Until we have an adequate background picture of the factors at work in lung cancer pathogenesis, it is difficult to determine whether tobacco smoke actually has dangerous properties or to determine what these might be (cf. *Passey*, 1962).

Though my hair is already gray, I am very hopeful of seeing the day when the incidence curve of human lung cancer turns downward and its toll diminishes (*Gilliam et al.*, 1961). Outlines of experimental approaches that may lead to that result are taking shape, and pathways, though long and complicated, are beginning to appear. I suspect that this down turn will eventuate in a world where tobacco is still widely used.

SUMMARY

Wide publicity to statistical associations between cigarette smoking and human lung cancer incidence tempted many investigators in years past to undertake experimental shórt-cuts in search of the major factors contributing to pathogenesis of this disease. These short-cuts have not in general been very rewarding. A return to more methodical, systematic and step-wise research with animal models, supplemented by clinical observations upon humans, and interpreted with caution, is indicated.

By such methods, significant progress is now being made in determining the combinations of conditions under which squamous lung cancers can be produced in animals at a substantial yield level. Systems need to be developed which employ only agents encountered by humans in normal life. Such experimental systems should permit assessing the relative significance of the many factors which may influence the yield level. Among such factors, now being studied or candidates for study, are lung trauma, including infarction, infection and inflammation, the detoxification efficiency of the liver, ciliary function and phagocytosis, composition, properties, abundance and flow rates of mucus, hormone excesses or deficiencies, and nutritional status, sex and age, as well as physical and chemical properties of agents which may influence their retention and absorption when they reach the lung by inhalation or other routes.

Only methodical studies of this nature can provide the background against which any potential effects of cigarette smoke can, for the first time, be assessed realistically. Comprehension of the ways in which the various, possibly influential factors interact, and of their relative importance, should furnish us with practicable methods for reducing the toll of bronchogenic carcinoma among humans.

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