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The Determination of Polycyclic Aromatic Hydrocarbons

The following are summaries of four of the papers presented at a Meeting of the Analytical Division held on February 1st, 1978, in London.

Cancer from Polycyclic Aromatic Hydrocarbons (PAH)

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Certain polycyclic aromatic hydrocarbons (PAH), such as dibenz(*a*,*h*)anthracene and benzo(*a*)pyrene were, as a result of the pioneering work of Sir Ernest Kennaway and his colleagues, among the first pure chemicals to be found to be carcinogenic.[1] Although the carcinogenic natures of other classes of chemical compounds, *e.g.*, aromatic amines, azo dyes and certain inorganic materials, were recognised not long afterwards, it was not really before the mid-1950s that the full importance of biological alkylation in carcinogenesis began to be recognised, and not until well into the 1960s before the carcinogenic potency and great versatility of, for instance, certain nitrosamines was appreciated.

The notion that carcinogens act by causing mutations in somatic cells antedated the work of Kennaway and his colleagues and, when given a useful new tool in the form of pure chemical carcinogens, one of the first aims of those who favoured this theory was to show that PAH were potent mutagens. However, this was found not to be the case; at best only weak mutagenic activity was demonstrable. The carcinogenesis theorists therefore had a troublesome two decades or so. During this period biochemists, using what would now be regarded as old-fashioned techniques, set about trying to show that the carcinogenic effect of a substance may depend on its being changed in the body from an inactive pro-carcinogen to an active, or proximate, carcinogen. By analogy with simple aromatic hydrocarbons, such as benzene and naphthalene, it seemed likely that hydroxylation would occur and, eventually, numerous hydroxy and quinone derivatives or carcinogenic and non-carcinogenic PAH were isolated from the faeces or tissues of exposed animals. However, none of these derivatives was seemingly more active as a carcinogen than the parent substance in the biological test systems available at the time.

At an early stage in the research, big differences in carcinogenic activity were found between chemicals of seemingly very similar structure. The first clue to why this should be came from the work of the Pullmans[2] in France. From molecular orbital calculations on 37 unsubstituted PAH they concluded that, in those that were carcinogenic, the energy of activation at the phenanthrenoid bond, which they called the K region, must not exceed a certain limit, while the activation energy at the L, or *meso*-anthrenoid, region must exceed another value (Fig. 1). This theory of the Pullmans enabled several correct predictions of activity or non-activity to be made, but biochemists and biologists have difficulty in accepting "armchair" chemistry and would not be satisfied until they could understand activity in terms of interactions between chemicals and body constituents, preferably genetic material in the form of nucleic acids.

Great advances were made possible by the discovery of the structure of DNA[3,4] and the significance of the sequencing of the four nucleotides guanine, cytosine, adenine and thymine in determining the structure of genes. At the same time, new impetus to the concept of metabolic activation of pro-carcinogens to proximate carcinogens was given by the unexpected discovery of the Millers[5] in Wisconsin that hydroxylation could occur on the nitrogen atom of 2-acetylaminofluorene (Fig. 2), and by their suggestion that most chemical carcinogens are converted by metabolism to electrophilic intermediates that react with the nucleophilic centres present in cellular constituents.[6]

The story, as far as the PAH were concerned, was taken a stage further when it was shown by Brookes and Lawley[7] that hydrocarbons react with DNA in mouse skin and by Grover and Sims[8] that the microsomal metabolism of several different polycyclic hydrocarbons in the test-tube results in the formation of metabolites that react with DNA. Attempts to identify the

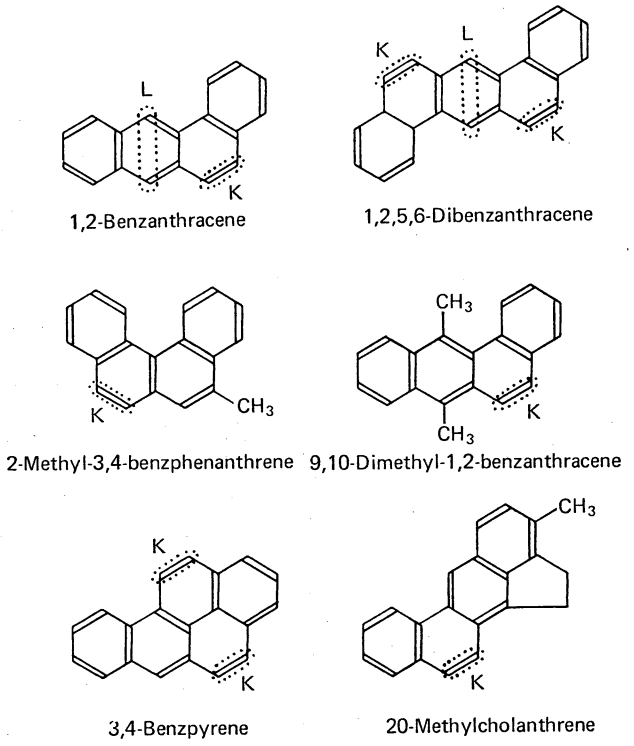

Fig. 1. Structures of some carcinogenic polycyclic aromatic hydrocarbons (earlier nomenclature) showing K and L regions.

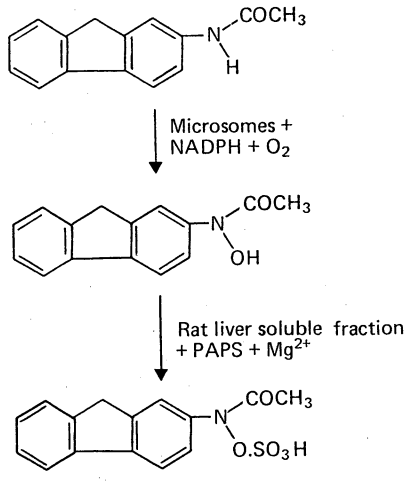

Fig. 2. The metabolic activation of 2-acetylaminofluorene in rat liver. PAPS= 3′-phosphoadenosyl-5′-phosphosulphate.

reactive metabolites have been in progress ever since. At first, simple epoxides formed at the K region came under suspicion; these compounds were known to be metabolites and they possessed properties thought to be relevant to the induction of cancer. However, during the last few years it has become apparent that another type of epoxide, known as diolepoxide, is most probably responsible for the biological effects of PAH.

A diolepoxide is formed when an atom of oxygen is added, chemically or metabolically, to the isolated double bond adjacent to the dihydrodiol grouping in a non-K region diol as shown, for benzo(*a*)pyrene, in Fig. 3. The first diolepoxide was detected as a metabolite by Booth and Sims,[9] and this type of reactive product has now been implicated in the activation of benzo(*a*)pyrene[10] and of 7-methylbenz(*a*)anthracene.[11] A similar mechanism of activation is suspected for other carcinogenic PAH. With benzo(*a*)pyrene, in particular, progress has been rapid and many of the products, such as that shown in Fig. 4, formed when the active diol-epoxide reacts with nucleic acids, have been isolated and characterised by using modern physicochemical techniques.

What is meant by saying that certain PAH are carcinogenic? The early studies of Kennaway and his colleagues involved the production of skin tumours when active compounds were applied repeatedly to the skin of mice, or of connective tissue tumours (sarcomas) when active chemicals were injected subcutaneously into laboratory animals. Later, tumours were produced by exposing animals to carcinogenic PAH by a wide variety of other routes. Facilities for exposing animals to suspect carcinogens by the inhalation route have not existed until quite recent times. Previously, the best that one could do was to instil PAH into the lungs via the trachea. Exposure to carcinogenic PAH in this way leads to the development of squamous metaplasia and benign and malignant squamous tumours.[12–14] The injection of certain potently carcinogenic PAH into newborn mice, *e.g.*, 3-methylcholanthrene, benzo(*a*)-pyrene or 7,12-dimethylbenz(*a*)anthracene (DMBA), depending on the strain, results in their developing tumours of the lung and liver, lymphoid tissues and a wide variety of other

tissues.[15,16] Huggins[17] developed a technique for producing mammary tumours in rats, which involved their being given massive doses of DMBA by gavage. Later he also reported an increased incidence of leukaemia in rats so treated.

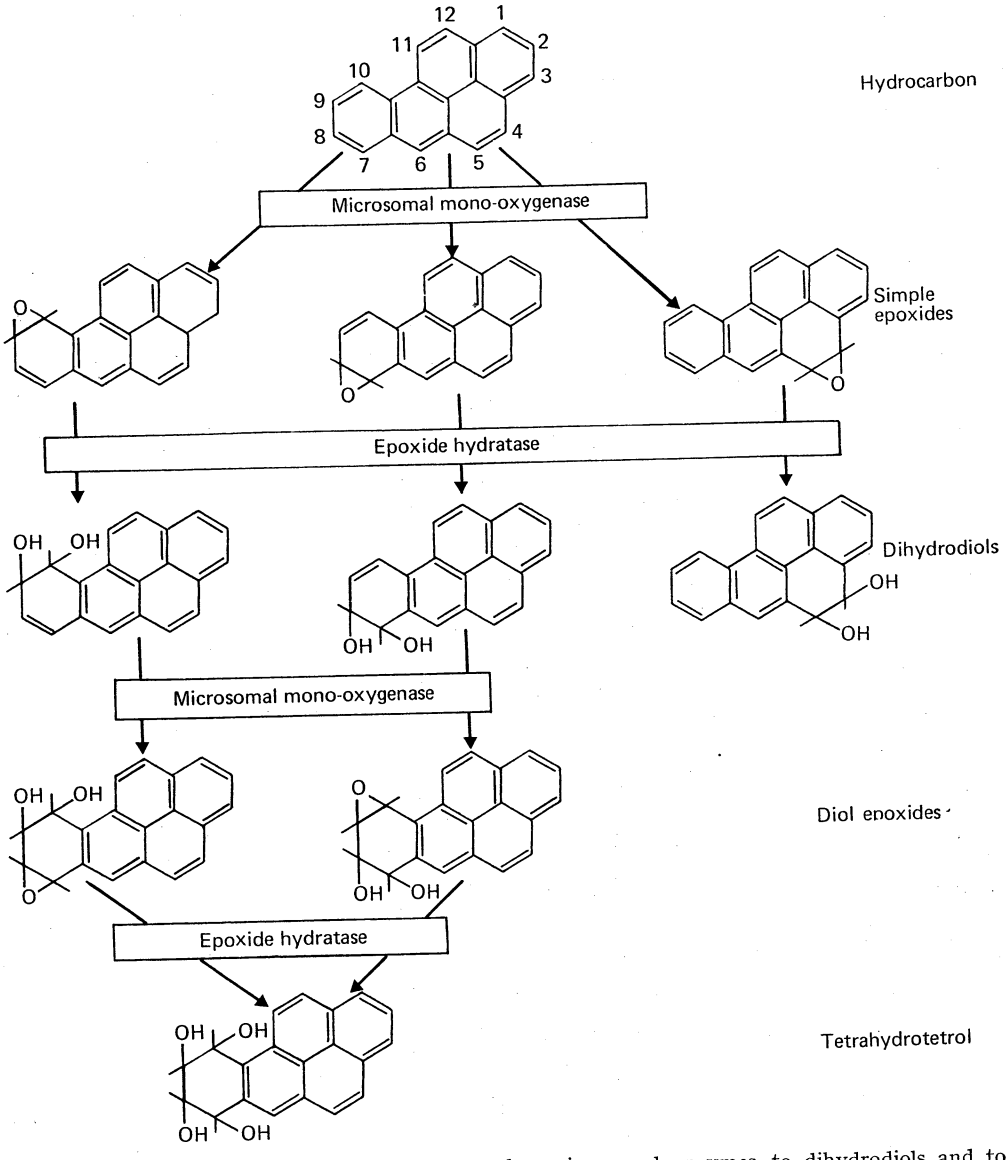

Fig. 3. The metabolism of benzo(*a*)pyrene by microsomal enzymes to dihydrodiols and to diolepoxides.

In general, the experimentalist remains suspicious about the interpretation of carcinogenicity studies unless there is clear evidence of a dose-response relationship. With many carcinogenic PAH there is abundant evidence of this nature. The evidence that some PAH are potent carcinogens for virtually all of the species of laboratory animal in which they have been adequately tested is overwhelming, and although there is no direct proof of it, there can be no doubt that PAH are also carcinogens for man. No-one has had the temerity to expose themselves or anyone else to pure PAH known to be carcinogenic in animals. However, there are

many examples of increased cancer risk in humans exposed to mixtures of chemicals including carcinogenic PAH.

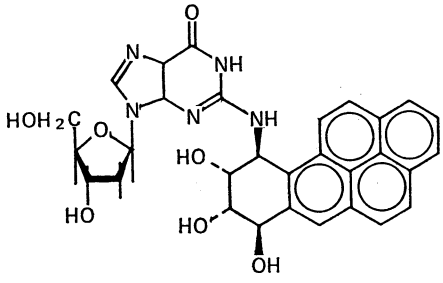

Fig. 4. Structure of one of the guanine products formed when benzo(*a*)pyrene-7,8-diol-9,10-oxide reacts with DNA.

The oldest example is the increased risk of scrotal cancer in chimney sweeps exposed to coal tar and soot. During the latter half of the nineteenth century and first half of the present century, there was a major epidemic of skin cancer among mule-spinners in the cotton and wool industries. Inadequately refined mineral oils, which contained various PAH with four to six or more condensed benzene rings, were used for lubricating the spindles and this oil contaminated the skin and clothing of the operatives. Skin cancers of the hands and arms, but more especially of the scrotum in men and vulva in women, occurred in high incidence among persons thus exposed.[18] The identification of carcinogenic PAH in mineral oils was reported by the Medical Research Council's Carcinogenic Action of Mineral Oils Committee in 1968.[19] This committee concluded, ". . . there is a strong presumption that the active constituents are more or less closely related to the known polycyclic aromatic carcinogens." In any case, long before this report appeared it became compulsory for millowners to use a solvent-refined mineral oil for spindle lubrication, and the cotton industry had gone into decline anyway. However, these developments did not prevent cancer hazards from mineral oils cropping up in other industries. Kinnear *et al.*[20] reported an incidence of precancerous and cancerous skin lesions in jute workers in Dundee. Jute has to be softened by a batching process, which involves the exposure of workers to poorly refined mineral oils. In 1967 Roe *et al.*[21] found a sample of jute-batching oil, currently in use at that time, to be highly carcinogenic when applied to the skin of mice. The most recent event in this saga has been the minor epidemic of scrotal cancer among tool-setters and automatic lathe operators exposed to inadequately refined cutting oils, used as coolants, which led to the famous court case known as Stokes *v* Guest, Keen and Nettlefold in 1968.[22]

Another occupation in which observed raised incidences of cancer are thought to be attributable to PAH is the coal-gas industry. Men who used to work in retort houses developed three kinds of cancer in excess, *viz.*, cancers of the bladder, lung and skin.[23] The excess of bladder cancer is thought to be a result of exposure to β-naphthylamine and possibly other carcinogenic aromatic amines, but the responsibility for the skin cancers and lung cancers has been laid at the door of the PAH.

Because it has a characteristic ultraviolet absorption spectrum, and because many kinds of researchers like to measure whatever they can measure irrespective of whether what they are doing is meaningful, benzo(*a*)pyrene (BP) has received far more attention than any other PAH in relation to human cancer. BP has been blamed for the excess of respiratory tract cancer in urban compared with rural dwellers[24] and in cigarette smokers, and for the high incidence of stomach cancer in consumers of home-smoked fish and other foods.[25] Almost certainly BP is nothing more than a minor contributor to the cancer risk from industrial air pollution or cigarette smoke and it may not be implicated at all as a carcinogen for the gastro-intestinal tract. Wynder and Hoffman[26] listed many carcinogens, not all of them PAH, that have been found in tobacco smoke condensate. Similar lists could be drawn up for vehicular exhaust fumes and for polluted air.

Traces of BP and, wherever they have been looked for, other PAH and related heterocyclic compounds have been found in all sorts of foodstuffs. They find their way on to food crops

as a result of atmospheric fall-out and into the crust of bread cooked in direct-fired ovens, in which the bread is cooked in the gases generated by the combustion of oil.[27] When such ovens are working well the amounts of PAH produced are minute, but under conditions of inefficient firing combustion becomes incomplete with the result that PAH are formed by pyrolysis. During the era of intense activity in the detection of BP it was found in, among other things, charcoal-broiled steaks and smoked salmon,[28,29] roasted coffee,[30] soil,[31] water[32] and fresh olives.[33] Dr. A. J. Lindsey of the Sir John Cass College showed that PAH, including BP, can be formed readily by pyrolysis from a variety of organic starting materials, such as methane, ethane, propane, ethylene, etc.[34] He also showed that several different components of tobacco (*e.g.*, cellulose, lignin, pectins, glucose and malic acid) may give rise to BP and other PAH on thermal decomposition.[35]

Experimentalists have long been aware that carcinogenesis may be a complex process, usually requiring multiple steps. The logarithmic increase in the age-standardised risk of many forms of cancer is consistant with there being from three to five steps in the process. It has also been apparent that the carcinogenic effects of PAH can be greatly enhanced by concomitant or subsequent exposure to other substances which, in the broad sense of the term, are irritant. Concomitant enhancement is referred to as "co-carcinogenesis" and the most striking example of this is the up to 1 000-fold enhancement of the tumour-producing effects of BP by the aliphatic hydrocarbon dodecane, reported by Bingham and Falk.[36] Where enhancement of the effects of carcinogens such as BP is brought about by subsequent exposure to an irritant, the second stage is referred to as "tumour promotion." Co-carcinogenesis, tumour promotion and interactions of other kinds are thought to be very much involved in carcinogenesis by mineral oils, air pollutions and tobacco smoke condensate. Thus, we found that the phenolic fraction of tobacco smoke condensate promoted skin tumour development following a sub-carcinogenic dose of DMBA.[37] Also, we[38] and others[39] have shown conclusively that BP cannot, by itself, be responsible for more than a small part of the carcinogenicity of tobacco smoke condensate.

A few years ago a report by Kellerman *et al.*[40] stirred up much interest. These workers found higher levels of arylhydrocarbon hydroxylase (AHH) inducibility in cultured lymphocytes from patients (most of whom had been smokers) with lung cancer than in those from smokers or non-smokers without lung cancer. They suggested that a capacity for AHH induction constitutes susceptibility to lung cancer. The hypothesis, is, in fact, a very loose one because there are several enzymes that fall within the definition "AHH" and no-one is quite sure whether such activity favours the production of carcinogens or their destruction. Most other groups who have tried to repeat the work have failed. An exception to this are recent reports[41,42] of high AHH inducibility in the circulating lymphocytes of patients with cancer of the larynx or oral cavity.

In this brief survey I have tried to put the consideration of PAH as carcinogens into perspective biologically and medically. Personally, I have no doubt that PAH contribute to the burden of human cancer. What is not clear to me or to anyone is how concerned we need to be about the presence of traces of PAH in many aspects of our environment. I suspect that with PAH and other carcinogens triumphs in the field of analytical technique are tending to divert our energies towards removing traces that are of negligible importance biologically, whereas we would be better occupied trying to find entirely new kinds of causes of cancer.

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Analytical Aspects of Polycyclic Aromatic Hydrocarbons in Food

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Potential sources of polycyclic aromatic hydrocarbon (PAH) contamination of food are atmospheric pollution, soil and water, food processing, endogenous synthesis and the use of petroleum-based food additives (mineral hydrocarbons).

General Analytical Methodology

There are several problems that are unique to this class of compounds and, on the basis of permutations and combinations of ring structure and number, type and position of substituent groups, this class, even taking into account probability factors, can number thousands of compounds. The problems are further exacerbated by the complex structures of these compounds, the difficulty of isolating them from a food matrix, their low levels of occurrence and their occurrence as complex mixtures.

These factors, together with legislative needs, have resulted in two broad approaches to PAH analysis in food: the development of collective or total methods, which do not involve separation or identification of individual components; and the development of chromatographic systems for the separation and identification of selected or total components of the PAH extract.