Meetings

Carcinogenesis: Physicochemical Mechanisms

An international symposium, dealing with the mechanisms of activation of chemical carcinogens and their interaction with cellular and molecular receptors, was held at the Israel Academy of Sciences and Humanities, in Jerusalem, 21–25 October 1968. The occasion was the 50th anniversary of Hebrew University and the 10th of the foundation of the Academy.

Pullman (Paris), specializing in theoretical studies on the electronic structure of polynuclear aromatic hydrocarbons (PAH) and of purines and pyrimidines, related the physical and chemical structural properties of carcinogenic PAH to possible cellular receptors, proteins, or nucleic acids. The initial interaction would be physical attraction followed by chemical (enzymic) bond formation.

Ascoli and Liquori (Rome) showed how PAH, such as benzpyrene (carcinogenic) or pyrene (not carcinogenic), formed complexes with tetramethyluric acid by intercalation due to vertical stacking and are noted by a red shift in the spectrum. Spermine or spermidine, which tend to stabilize DNA, lowered binding of carcinogenic and inactive PAH. Model compounds related to actinomycin D, without the peptide side chains, also combined with DNA but failed to inhibit DNA transcription. Van Duuren (New York University) demonstrated the combination of acridine dyes with DNA and with other polyanions, yielding specific dye to polymer ratios and with an alteration in fluorescence or absorption spectra. Attachment by hydrogen bonding between two adjacent bases rather than intercalation within polymer strands may be involved, because denatured DNA or single strand polymers bind dyes. By nuclear magnetic resonance and vapor phase studies of interactions with purines and pyrimidines, Ts'o (Johns Hopkins) pictured hydrophobic stacking in polymers to which PAH attach. The consequent increased hydrophobic character, hence, would weaken hydrogen bonds and result in mispairing in the transcription process. Benzpyrene activated photochemically or by radiant energy reacts chiefly with nucleoprotein rather than DNA alone or histone alone. However, denatured DNA binds hydrocarbon better than native DNA.

Löwdin (Uppsala and Florida) calculated that a lower energy barrier may exist after strand opening in DNA and a 180-degree rotation. Thus, a carcinogen may open and activate a restricted part of the genome in a deep groove of double-stranded DNA covered by a protein chain. Bergmann (Jerusalem) and Meyer (Jerusalem) measured dipole moments and ionization potential to locate active hydrogens and electrons in the fine structure of substituted purines and pyrimidines. The earlier β-charge method of calculating electron density diagrams and bond lengths in PAH was refined and gave good results, especially with compounds which thus far were exceptions.

Wilk (Frankfurt) determined oxidation-reduction potentials polarographically after photoelectric activation. Upon iodine-catalyzed oxidation, involving probably one electron radical formation, benzpyrene reacted covalently with pyridine. Imidazole or 2,6-diaminopurine gave more complex interactions. However, 2,6-diacetylaminopurine acted like an unsubstituted purine. Activated PAH also combined with adenine in DNA. Laskowski (Cleveland) developed models of the interaction between PAH or heterocyclics with substituted benzo- or naphthoquinones.

Pataki and Huggins (University of Chicago) considered that three molecular parameters are crucial to the carcinogenicity of PAH—(i) electron transfer capability; (ii) geometry, optimum when like the base pairs of nucleic acids; and (iii) molecular thickness, of no more than 4 Å. Hydrocarbons depressed thymidine incorporation in liver, spleen, kidney, in limited order of carcinogenicity, 7,12-dimethylbenz-(*a*)anthracene $\gg$ 7-methyl-12-ethyl $>$ 7,12-diethyl.

According to Daudel (Paris) and Terayama (Tokyo), polylysine production, in a modified Nirenberg cell-free system containing polyadenylic acid, was inhibited by alkylating agents such as nitrogen mustard, and methyl and ethyl methanesulfonates; production was not inhibited by dimethylnitrosamine. The inhibition of transcription paralleled the extent of alkylation. Also, a stimulation of leucine incorporation into proteins by microsomes and *p*H 5 fractions of livers of rats treated with azo dyes paralleled the carcinogenic potency of the dyes in the presence of polyuridylic acid. The carcinogens lead to a deficiency of ribosome messenger units but increase appreciably the effectiveness of the *p*H 5 fraction.

A series of papers dealt with the biochemical activation of carcinogens and their interaction with specific targets in the cell. Most chemical carcinogens, except alkylating agents, require an activation mechanism. Polynuclear aromatic hydrocarbons were often considered as direct-acting, but Brookes (London) visualizes an oxidized intermediate ionic structure which can react a number of ways to give a dihydrodiol, an epoxide, or a phenol. More important, the intermediate provides an electrophilic center active toward appropriate intracellular targets. This concept now opens the way to search for enzyme systems yielding such intermediates in the tissues susceptible to PAH. 7-Bromomethyl-12-methylbenz-(*a*)anthracene which leads to a model carbonium ion is as carcinogenic as 7,12-dimethylbenz(*a*)anthracene, and also reacts rapidly with DNA chiefly at guanylic acids residues. Gelboin (Bethesda) showed that PAH bound very little to DNA in vitro, but substantial amounts were fixed in the presence of a liver microsome fraction capable of oxidizing benzpyrene [Sims and Grover, *Biochem. J.* **110**, 159 (1968)].

Warwick (London) reported on the kinetics of labeling of DNA, RNA, and proteins of rat liver with tritiated 4-dimethylaminoazobenzene. Bound metabolites remained longest, up to 3 months, on DNA. Prior treatment with phenobarbital, increasing microsomal detoxification, led to lower binding. Labeling was lower in hamsters than in rats, and DNA-bound material was lost more rapidly. The 2-methyl azo dye, not considered carcinogenic, labeled DNA more than the parent compound, thus reflecting reaction with target. Tumors did not develop because of lack of cell duplication. Stimulating cell division by partial hepatectomy gave tumors with 2-methyl azo dye. Hence, carcinogen must react with receptors, and cell multiplication is also required for tumor induction.

Magee (London), who with Barnes discovered the carcinogenic nitrosamines, reviewed the conceptual ad-

vances stemming from them. Label from dimethylnitrosamine attached to the 7-position of guanylic acid in DNA and RNA, and minor amounts also to the 1- and 3-positions in adenylic acid and in cytidylic acid. The significance of these interactions in the carcinogenic process remains to be fully documented, but that carbonium ions produced in vivo may lead to neoplasia has led to other tests. Thus, after a single intravenous injection, methyl methanesulfonate gave brain tumors, and the ethyl ester gave kidney tumors.

Boyland (London) discussed activation processes with chemical carcinogens; he emphasized the intermediates derived from the simple molecule ethyl carbamate. A single electron carbethoxy radical, which reacts with cytidylic acid in DNA, may be involved. Also, epoxides from PAH cannot be disregarded as active intermediates despite unfavorable test data in animals. He concluded that DNA is the primary molecular target with a variety of carcinogens.

A key presentation by Miller (Wisconsin) provided evidence that chemical carcinogens ultimately are converted to electrophilic reactants. Carcinogenic alkylnitrosamines yield carbonium ions; urethane, a one-electron or carbonium ion intermediate; aromatic amines and azo dyes, *N*-oxy compounds (which as esters yield reactive amidonium ions); and pyrrolizidine alkaloids, a carbonium ion (also with an ester-leaving group). Some metals, such as chromium, cadmium, cobalt, lead, and nickel, are electrophilic.

Troll (New York University) showed that melting temperature, buoyant density, and template activity of DNA with respect to RNA polymerase was lower after treatment in vitro with the carcinogens β-propiolactone and *N*-acetoxy-*N*-2-fluorenylacetamide. *N*-Acetoxy-*N*-1-naphthylacetamide, from noncarcinogenic 1-naphthylamine, did not alter the properties of DNA. Kriek (Amsterdam) discovered an interesting difference in the binding of *N*-hydroxy-*N*-2-fluorenylacetamide with RNA and DNA of rat liver in vivo. Whereas the residue combined at C-8 of guanylic acid in RNA was an acetylamino derivative, it was the amino derivative on DNA.

Roberts (London) examined DNA in HeLa cells damaged by alkylating agents such as nitrogen mustard, methyl methanesulfonate, or nitrosomethylguanidine after labeling with bromodeoxyuridine (heavy chains) or thymidine (light chains). Elimination of label gave a clue on repair mechanisms which may also play a role in the carcinogenic process.

Sorof (Philadelphia) spoke on the binding of carcinogens to a specific subfraction of the cell sap of tissues, the h_2 proteins. In several tissues carcinogens labeled proteins with similar charge and molecular size, thus suggesting that the functional consequences of this binding may be alike. Liver h_2 proteins had physical properties like those of arginase, and inhibited certain cells in culture. Heidelberger (Wisconsin) noted that mouse skin had h_2 proteins as found in liver. However, mouse skin arginase had different electrophoretic mobility.

Goldblum (Jerusalem) dealt with viral carcinogenesis, in particular, the incorporation of the genome from SV-40 agent into the DNA of host cells. Infected cells exhibited (i) increased DNA synthesis; (ii) induction of a viral replicase; (iii) induction of an

intranuclear "T" antigen; and (iv) induction of surface transplantation antigens. The purified T antigen had a molecular weight of more than 200,000 and contained RNA. Removal of RNA lowered the molecular weight, thus yielding a pure basic protein containing no sulfur amino acids. The T antigen might be the viral DNA polymerase.

Heidelberger (Wisconsin) reported on a quantitative in vitro system of hydrocarbon carcinogenesis with cells from adult C3H mouse prostate. Transformed cells formed piled-up colonies which gave transformation frequencies related to the carcinogenic potency of eight hydrocarbons, and produced tumors in unconditioned mice.

Clayson (Leeds) found that the mitotic rate in normal bladder measured by direct count or thymidine incorporation was low, and exhibited two peaks—one at 5 hours and one at 18 to 24 hours. Cells were usually diploid, but in bladder tumors they were often heteroploid. After treatment with a new bladder carcinogen, 4-ethylsulfonylnaphthalene-1-sulfonamide, an increased rate of mitotic waves in the epithelial fraction showed peaks at 36 hours. The increased DNA synthesis was preceded by ribosomal RNA synthesis.

Systemic carcinogens were discussed by Weisburger (Bethesda)—in particular the circulatory pathway of carcinogenic *N*-2-fluorenylacetamide and its active metabolite, *N*-hydroxy-*N*-2-fluorenylacetamide. After absorption the compounds were metabolized chiefly in the liver. The many products were transported by blood as loosely and as firmly bound metabolites. Urinary excretion occurred after renal filtration. Passage from liver to bile led to additional metabolism in the gut by bacterial action, particularly splitting of conjugates such as glucuronides and sulfate esters. Resorption of free metabolites from the gut explains enterohepatic circulation. Unresorbed materials constitute fecal metabolites.

Furst (San Francisco) dealt with induction of cancer in rodents by metal and metal ions, in particular the induction of sarcomas at the site of injection of certain nickel and titanium derivatives. Titanium powder also gave rise to lymphomas.

Berenblum (Rehovoth) saw the induction of tumors as a complex process involving many variables such as chemical structure of agent, biochemical activation and detoxification processes, dosage, mode of administration, and host factors such as species or sex.

Thus, quantitative carcinogenic potency is a relative term valid in a controlled experimental setting.

At this symposium important conceptual and practical advances were reported. Theoretical and experimental approaches have led to a consensus that many different types of chemical carcinogens may be considered as electrophilic reagents, produced by synthesis or by biochemical activation. New experimental developments should help pinpoint the target specifically related to carcinogenicity. It need be a cellular or molecular entity with a preference for such electrophilic centers. Additional events in the carcinogenic process leading to tumors deal with host-specific modifying factors, concerned with the multiplication of cells altered by action of an electrophilic reagent with its receptor.

The meeting was held under the sponsorship of the Hebrew University of Jerusalem, the Foundation Edmond de Rothschild of Paris, and the Israel Academy of Sciences and Humanities.

The complete proceedings will appear as a monograph published by the Israel Academy. This first symposium dealing with carcinogenesis will be followed by others in which significant reports on broad multidisciplinary aspects of research develop into advances of an entire field.

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National Meetings

September

2–4. **Comparative Virology**, intern. conf., Montreal, Canada. (K. Maramorosch, Boyce Thompson Inst. for Plant Research, Yonkers, N.Y. 10701)

2–6. **Molecular Structure and Spectroscopy**, 24th annual symp., Columbus, Ohio. (K. N. Rao, Physics Dept., Ohio State Univ., Columbus 43210)

2–6. **Tuberculosis**, intern. conf., New York, N.Y. (J. E. Perkins, Natl. Tuberculosis Assoc., 1790 Broadway, New York 10019)

3–5. **Weather Forecasting and Analysis**, 3rd, Virginia Beach, Va. (E. C. Kindle, Navy Weather Research Facility, Bldg. R 48, Naval Air Station, Norfolk, Va.)