Meetings

Molecular Pathology and Carcinogenesis

How toxicants affect the liver and initiate cellular injury and carcinogenesis were considered at the first New Zealand international symposium on molecular pathology and carcinogenesis, held on 7 and 8 November 1966 at the University of Otago Medical School, Dunedin.

Judah (Chicago Medical School; now at University College, London) considered the fundamental factors in necrosis of the liver after treatment with hepatotoxic agents and chemical carcinogens, such as ethionine, thioacetamide, carbon tetrachloride, dialkylnitrosamines, and actinomycin D. The early morphologic and functional alterations of cells were examined. Judah reviewed attempts to define the unique biochemical or structural lesion leading to either cell death or neoplastic transformation. While the effects at the mitochondrial level, especially ion transport and energy supply, were discussed in detail, it is probable that no single event can be pinpointed. Complementing this view was the report of Gallagher (University of Sydney) on the acute toxicity of pyrrolizidine alkaloids, particularly heliotrine and lasiocarpine. He also emphasized the effects on mitochondrial metabolism. He likened the effect of these alkaloids to the neuromuscular blocking action seen after *d*-tubocurarine or some complexed cations such as iron bipyridine. These compounds inactivate NAD^+-dependent enzyme systems resulting from a loss of cofactors from mitochondria. Formerly this was thought to be caused by an increased permeability of mitochondrial membranes, but new evidence suggests that the mechanism depends on a positive displacement of NAD^+ by the alkaloids. Christie (University of Melbourne) evaluated specifically the mode of hepatotoxic action of carbon tetrachloride. This agent undergoes biochemical fission with production of ions or radicals, in turn leading to derangement of lipid metabolism, particularly in respect to peroxidative reactions. Thus, damage to lipid membranes required for transfer of essential metabolites and maintenance of intracellular ion gradients could result. In discussion, Magee (MRC Laboratories, Carshalton) referred to data by MacLean on the lack of hepatotoxicity of CCl_4 in rats on a low protein diet but in which the damaging action could be restored by injection of the enzyme-inducing drug phenobarbital. Gelboin (NIH, Bethesda) commented on his studies that microsomes from rats treated with CCl_4 mimic those stripped of messenger RNA in respect to their capacity of accepting polyuridylic acid, thus leading to increased phenylalanine incorporation. It appears that CCl_4 exerts a diversity of effects on many targets. Effects significant for hepatotoxicity still have to be sorted out.

Ultrastructural considerations were divided by Butler (Chicago Medical School; MRC Laboratories, Carshalton, England) into two types: changes in the cytoplasm and changes in the nucleus, after administration of aflatoxin, dimethylnitrosamine, carbon tetrachloride, and actinomycin D. Cytoplasmic lesions, chiefly in the rough endoplasmic reticulum, include dislocation of the ribosomes and reaction of the cisternae. With the highly carcinogenic, mold-derived toxin aflatoxin periportal changes occur before centrilobular disruption. Nuclear alterations were absent after carbon tetrachloride, but dimethylnitrosamine, aflatoxin, and actinomycin D give rise to abnormal nuclei with formation of caps, even in cells with no obvious lesions in the cytoplasm. Aflatoxin B_1 and aflatoxin G_1 caused abnormal nuclei, but only aflatoxin B_1 appears to bind to DNA. Newberne (M.I.T., Cambridge) demonstrated that a single dose of aflatoxin B_1 first reduced mitosis in parenchymal liver cells, then affected thymidine incorporation with a return to normal only one week later. Histologic damage was detected as early as 3 hours later. Newberne also related that cirrhosis and hepatoma found by a group from Auburn University 15 years ago in rats, fed diets based on peanut meal and low in choline and vitamin B_{12}, were not solely the result of these deficiencies but were now known to be largely due to the aflatoxin contained in the diets.

The molecular interactions of carcinogenic chemicals with tissue receptors were discussed in detail by several speakers. Magee (MRC Laboratories, Carshalton) spoke on the alkylation of proteins and nucleic acids by dialkylnitrosamines, in particular the reaction of the dimethyl derivative with RNA. The chief interaction occurs at the 7-position of guanylic acid. This reaction appears possibly to be causally connected to toxic and carcinogenic events; various alternate explanations were also presented. Thus, such alkylation on DNA could result in the production of direct mutant cells, or the alkylated product could lead to coding errors. Alkylation after a single dose does not result in a stable product, possibly because of nuclear repair mechanisms. Lijinsky (Chicago Medical School) also examined alkylation of nucleic acids by aflatoxins and nitrosamines. Aflatoxin B_1 but not the G_1 derivative, bind to DNA. Both compounds combine with liver-cell proteins. Aflatoxin B_1 is the more toxic and carcinogenic material. The carcinogenic effect of aflatoxin G_1 is under study. The important observation was reported that new carcinogenic cyclic nitrosamines also give rise to 7-methyl substitution on guanylic acid in RNA. Although the precise mechanism is not yet firm, some ring splitting must be involved.

Pound (University of Queensland, Brisbane) dealt with the induction of skin cancer. Treatments leading to inflammation and hyperplasia, such as with acetic, trichloracetic or iodoacetic acids, turpentine, xylene, croton oil or cantharidin, or even scarification of the skin shortly before injection of carcinogenic urethane or application of a carcinogenic hydrocarbon, all distinctly enhanced the tumor yield. Detailed examination of the underlying mechanisms indicated that there was cellular proliferation and increased DNA synthesis measured by thymidine incorporation, which began several hours after treatment and which lasted about 3 days.

The time relation explained why old attempts to potentiate skin carcinogenesis with application of the cocarcinogen croton oil 1 week prior to hydrocarbon had failed. Pound also observed an induction of liver cancer by partial hepatectomy 1 to 6 days before administration of a carcinogenic hydrocarbon or of urethane. The general conclusion was drawn that cell proliferation and DNA synthesis favor the early events required for the action of carcinogens.

Gelboin (NIH, Bethesda), the Franz Bielschowsky Memorial lecturer, also examined the initial stages of the carcinogenic process. Two points illustrated by experimental data were developed. First, interaction of carcinogens with cell receptors results in an alteration of gene expression. Second, at least one cycle of DNA synthesis seems required for carcinogen action. 3- Methylcholanthrene, although not usually carcinogenic to liver, combines with cellular proteins, RNA, and DNA of liver. The DNA level remains invariant but the ratio of RNA to DNA increases and the synthesis of specific proteins measured by their enzyme activity is stimulated. Underlying these findings are raised microsomal protein synthesis and sensitivity to polyuridylic acid, increased nuclear RNA metabolism, and increased RNA polymerase activity. All such reactions are evidence of enhanced gene activity. Continuous feeding of liver carcinogens stimulates, then depresses RNA polymerase. Aflatoxin yields lower nuclear enzyme activity. The formulation of skin tumor is inhibited when actinomycin D, an inhibitor of DNA synthesis and of DNA-dependent RNA synthesis, is given with a carcinogenic hydrocarbon. These and related studies show that early actions of carcinogens probably involve cells in a sensitive state, as during DNA synthesis.

Reports were also made on biochemical activation of chemical carcinogens and the effect of host factors on this process. Boyland (Chester Beatty Research Institute, London) examined the pharmacologic and biochemical effects of urethane and its *N*-hydroxy metabolite. In most animal species the main compound detected is urethane whether it or *N*-hydroxy urethane are administered. On the other hand, specific biochemical and pathological effects such as chromosome anomalies, interferon production, virus inhibition, the regulation of body temperature, and methemoglobin formation are affected much more by the *N*-hydroxy derivative as compared to urethane itself. Thus, the assignment of biologic activity to either of this pair of compounds remains a challenge. Goodall (New Zealand), continuing the traditions of the late Franz Bielschowsky as director of the Cancer Research Laboratories at the University of Otago Medical School, reported on endocrine effects in liver carcinogenesis. Endocrine ablations such as hypophysectomy or thyroidectomy inhibit liver carcinogenesis with azo dyes, 2-fluorenamine and related carcinogens, and also with aflatoxin. However, the important discovery was recorded that formation of tumors was not reduced in hypophysectomized rats treated with dimethylnitrosamine. Concurrent with this the alkylation of nucleic acids at the 7-position of guanylic acid remained untouched. It was concluded that the major effect of the endocrines in liver carcinogenesis can be traced to the initial stages, with hormones controlling the effective level of proximate carcinogen. Weisburger (NIH, Bethesda) arrived at similar conclusions on the basis of an entirely different approach. He reported acceleration of liver carcinogenesis in animals with an excess of circulating pituitary hormones and treated with *N*-hydroxy-*N*-2-fluorenylacetamide. The underlying mechanism in this case rested on a finding of much higher levels of active carcinogen consequent to the alteration of the level of pituitary hormones. Modification of drug action generally, consequent to endocrine manipulation, may in part rest on alteration by biochemical means of the effective level of drugs.

On the occasion of the symposium, six former associates of Sir Roy Cameron, the late professor of experimental pathology, University of London, were assembled in Dunedin. The influence both of Cameron and of Bielschowsky, whose memory was honored by this assembly, pervaded the proceedings as the data presented and concepts derived therefrom stemmed from the inspiration of these two leaders in studies on the toxic and carcinogenic effect of drugs acting on the liver. The proceedings of the symposium will appear in the *New Zealand Medical Journal.*

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Calendar of Events

National Meetings

January

15–18. **Environmental Sciences** Research Symp. (Solid Waste Disposal, Air Pollution, Agricultural Pollutants, Water Quality, Corrosion), New Orleans, La. (E. Klein, Director, Physical Chemistry, P.O. Box 26500, New Orleans 70126)

16–18. **Reliability** Symp., Boston, Mass. (V. R. Monshaw, Astro-Electronics Div., RCA, Box 800, Princeton, N.J. 09540)

17–18. American Soc. of **Cybernetics**, Chicago, Ill. (G. T. Jacobi, IIT Research Institute, 10 W. 35 St., Chicago)

17–19. **Nuclear Medicine**, postgraduate symp., St. Louis, Mo. (E. J. Potchen, Washington Univ. School of Medicine, St. Louis, Mo. 63110)

17–19. **Process Industries**, instrumentation symp., College Station, Tex. (R. G. Anthony, Texas A&M Univ., College Station)

18–20. **Pediatrics**: Diagnosis and Treatment of Disorders of Perception, Speech and Learning, Gainesville, Fla. (Division of Postgraduate Education, P.O. Box 746, J. Hillis Miller Health Center, Gainesville 32601)

19–20. American **Rheumatism** Assoc., mtg., Baltimore, Md. (M. M. Walsh, ARA Headquarters, 1212 Ave. of the Americas, New York 10036)

19–20. American Soc. for **Surgery of the Hand**, annual mtg., Chicago, Ill. (R. M. Curtis, The Society, 2947 St. Paul St., Baltimore, Md. 21218)

19–20. **Blood**, 16th annual symp., Detroit, Mich. (W. H. Seegers, Chairman, Dept. of Physiology and Pharmacology, Wayne State Univ. College of Medicine, Detroit 48207)

20–25. American Academy of **Orthopaedic Surgeons**, annual mtg., Chicago, Ill. (J. K. Hart, AAOS, 29 E. Madison, Chicago 60602)

22–23. **Industrial Research**, 3rd annual, Chicago, Ill. (V. H. Disney, IIT Research Inst., 10 W. 35 St., Chicago 60616)

22–24. **Aerospace** Sciences mtg., New York, N.Y. (Meetings Manager, American Inst. of Aeronautics and Astronautics, 1290 Ave. of the Americas, New York 10019)

22–24. **Coal and Coke**, Philadelphia, Pa. (American Soc. for Testing and Materials, 1916 Race St., Philadelphia 19103)

22–24. **Radioisotopes and Radiation Effects**, New Orleans, La. (American Soc. for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103)

22–26. **Basic Electronics**, Hopatcong, N.J. (Saul Gordon Associates Center for Professional Advancement, P.O. Box 66, Hopatcong 07843)

22–26. **Marine Sciences Instrumentation,** 4th natl. symp., Cocoa Beach, Fla. (M. Reed, Instrument Soc. of America, 530 William Penn Pl., Pittsburgh, Pa. 15219)

22–26. **Powder X-Ray Diffractometry**, Austin, Tex. (D. E. Griffith, Program Director, Taylor Hall 153, College of Engineering, University of Texas, Austin 78712)