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Reviews

Environmental Mutagenesis. Edited by D. H. Phillips and S. Venitt, Bios Scientific Publishers, Oxford, U.K., 1995, Pp. 403.

The book comprises 18 Chapters written by authors with the greatest expertise in their fields. It is a timely new compilation of the correlation between exposure to environmental agents and an increase in the burden of mutations in the populations of organisms which receive that exposure. The book gives background information on the relationship between mutagenicity testing, germ line mutagenesis and cancer, and describes and discusses methods in mutation research, both classical ones and new developments. These methods were designed primarily to protect the human population from germ-line mutations and cancer. Chapters 1–5 describe the current view of our understanding of environmental mutagens and carcinogens. The mechanisms of action of chemical mutagens are reviewed, together with the influence of host factors, determining genetic diversity of human populations, on these processes. Chapters 6–12 provide critical assessments of established test procedures for determining the genotoxicity of agents or chemicals. Chapters 13–18 review the present state of development of new methods for screening chemicals, for elucidating the mechanism of mutagenesis in humans and for assessing the impact of environmental mutagens on the human genome. An index of abbreviations which precedes the text is most helpful. Due to the diversity of topics dealt with by different authors the book provides an interesting mixture of views and insights into the entire area of environmental mutagenesis in a clear and comprehensive way. Each chapter provides an indivual list of references in alphabetical order of authors. The book is not only an educative introduction to environmental mutagenesis for newcomers to the field but also contains invaluable detailed information for already experienced genetic toxicologists.

Chapter 1 by S. Venitt and D. H. Phillips is entitled 'The importance of environmental mutagens in human carcinogenesis and germ line mutation'. It describes the history of environmental mutagenesis citing the first publication by Muller in 1928 as the start of the field when he demonstrated that X-rays could induce heritable gene mutations in *Drosophila melanogaster*. In the ensuing period it was shown that also chemicals were capable of inducing mutations. The first example was mustard gas, a bifunctional alkylating agent, extensively used in World War I and even during the war between Iran and Iraq. The similarity of effects to ionising radiation created the term of 'radiomimetic' chemicals for bifunctional nitrogen mustards which subsequently were discovered to be useful as anticancer drugs. The personal environment changed with the adoption of new lifestyles, including smoking, the use of over-the-counter medicines and cosmetics. From here to the realization that industrialization created a new chemical environment was only a logical step which raised the concern of scientists for environmental mutagenesis. In this context, 'mutation' was defined as deleterious germ-line mutations leading to impairment and disease in future generations and 'cancer' was correlated to somatic mutations in the present population. It is the latter concern that provoked mandatory mutagenicity screening of new chemicals in legislation of industrialized countries. Despite the absence of definite proof that ionizing radiation or chemical mutagens lead to heritable mutations in the human population, there is still no doubt that genetic disease can result from exposure to mutagens. It is ascribed to the deficiencies of current methods for detecting the induction of human germline mutations in contrast to the ample evidence from rodent experiments that heritable mutations are induced by ionizing radiation and chemical mutagens. The general philosophy is that the present generation has to protect the human genome from mutation by preventing mutagens from entering the environment. The chapter furthermore describes the sources of human exposure to mutagens, namely food, endogenous processes, radiation, tobacco smoke and man-made chemicals. It defines somatic mutations and the term genotoxicity and briefly describes the harmonization of screening for genotoxicity as hazard identification followed by risk assessment and risk management.

Chapter 2 by J. C. Barrett is entitled 'Role of mutagenesis and mitogenesis in carcinogenesis'. It describes the multistep process of carcinogenesis and discusses various models which all wind down to the succession of initiation by mutations, promotion and progression. In detail, the author then discusses mutagenesis and mitogenesis as mechnaisms of carcinogenesis. He defines non-genotoxic carcinogens as chemicals that do not directly induce DNA damage and describes the mechanisms of indirect mutagenesis. In the conclusion, he emphasises that many chemicals operate via a combination of mechanisms which may vary depending on the target cells. Some chemicals may be complete carcinogens in one tissue, promotors or initiators in another. He warns that classification of chemicals into mutually exclusive categories may be misleading and hinder our comprehension of the complexity of chemical carcinogenesis which involves both mutagenesis and mitogenesis.

Chapter 3 by B. W. Glickman, G. Kotturi, J. de Boer and W. Kusser is entitled 'Molecular mechanisms of mutagenesis and mutational spectra'. The authors give selected definitions of mutation and discuss mutational specificity. The chapter stresses that details of the molecular nature of mutations can provide insights into the premutagenic lesions and the processes responsible for mutation. Mutations are viewed as targeted, non-random events, i.e. the results of DNA damage. Mutations reflect both the nature and the position of DNA damage which have a profound effect on the genetic consequences. The type and location of DNA lesions are dependent on the nature of the mutational treatment. Therefore, the mutational spectrum produced by each physical or chemical mutagen will be unique. The authors illustrate these statements by examples of mutational spectra induced by various agents in specific target genes. The authors then define spontaneous mutations and discuss their origin and nature. The DNA sequence directs the events of deletions, frameshift mutations and base substitutions. The authors describe how mutational specificity can be a useful tool for understanding the nature of mutation especially when accompanied by the knowledge of the potential DNA lesions. The last section of this chapter deals with the molecular aetiology of cancer and the *p53* tumor suppressor gene which has been implicated in all major human malignancies, e.g. skin, colon, liver and bladder cancer. The authors state that *p53* with more than 100 different codons is a good target for molecular epidemiology. The knowledge of the precise nature of changes in DNA at the sequence level provides insights into the nature of mutational processes in humans *in vivo*. Lastly, the authors reflect that future developments are likely to build on the knowledge of mutational specificity and new techniques such as sequencing by hybridization will relieve the technical challenge of assessing mutation in larger numbers of individuals. The ability to interpret mutational spectra will be accompanied by a broad range of demands and reflects advances in many fields besides biology.

Chapter 4 by D. G. Harden is entitled 'Inherited susceptibility to mutation'. The chapter deals with the gene-environment interactions and the mechanisms of inherited susceptibility to mutation. The latter are manifold, reaching from replication errors due to polymerase malfunction or unstable sequences, via transposable elements to variability of response to environmental agents. The latter is dependent upon access to DNA, configuration of chromatin, and DNA repair. It is also influenced by genomic surveillance factors such as monitoring and signal transduction. Again, as in the previous chapters, it is stressed that mutations do not occur at random throughout the genome. 'Hot spots' for mutations have been identified in specific genes. Spontaneous and induced germ line mutations in humans are discussed in the context of individual susceptibility. Well-defined syndromes where susceptibility to mutation is an integral part of the syndrome are described, e.g. retinoblastoma, Werner's syndrome, Xeroderma pigmentosum, Ataxia telangiectesia, Cockayne's syndrome, Fanconi's anemia and Bloom's syndrome. Genomic instability and mutator phenotypes are discussed with examples of cell lines and colon cancers. An understanding of programmed cell death, i.e. apoptosis, and the role played by failure of the apoptotic mechanism in permitting continued survival of grossly damaged cells have provided insight into the maintenance of the genetic integrity of cells. It is concluded that specific inherited mechanisms for generating genomic instability are associated with human cancers and it is anticipated that such mutator phenotypes have far-reaching consequences for other human disorders.

Chapter 5 by G. Smith, C. A. D. Smith and C.

R. Wolf is entitled 'Pharmacogenetic polymorphism'. Many studies have demonstrated that phenotypic variation in the metabolism of clinically important drugs is the consequence of genetically determined differences, i.e. polymorphisms, in the activities of human drug-metabolizing enzymes. Multiple alleles at loci which encode drug-metabolizing enzymes can result in different susceptibilities of individuals within a population to the mutagenic or carcinogenic effects of drugs or environmental chemicals. As one example the cytochrome P450 enzyme family is discussed in more detail. Glutathione S-transferases and N-acetyl transferases are specifically dealt with, however, there is a whole variety of other enzymes that show polymorphism. The evidence that polymorphisms affect drug metabolism *in vivo* is unequivocal. These enzymes may be involved in activation and inactivation of mutagens or environmental toxins. A link exists between the level of expression of a particular enzyme and the incidence of cancer and of disease in general where exposure to environmental chemicals has been implicated in the aetiology. Development of allele-specific polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analyses have made the determination of individual genotypes relatively straightforward, and this has eliminated many of the problems associated with the previously phenotypic assessment of drug-metabolizing capacities. The ability to predict an individual's drug response before administration avoids the exposure of patients to drugs which have harmful side effects. Mutations in genes which encode human drug-metabolizing enzymes have been shown to have profound influence, not only on the metabolism and disposition of a wide range of commonly prescribed drugs, but also on the incidence of environmental disease such as cancer.

Chapter 6 by E. Zeiger is entitled 'Mutagenicity tests in bacteria as indicators of carcinogenic potential in mammals'. The most widespread use of bacterial mutagenicity tests is in screening chemicals for identification of mutagens and, by implication, potential carcinogens. The chapter describes details of the bacterial mutagenicity tests such as culture and mutant-selection conditions, bacterial strains of common use and the genetic endpoints observed, i.e. forward mutations, reverse mutations or frameshift mutations, at specific genes under study. A section is devoted to the discussion of metabolic activation systems and their preparation. Finally, the use of bacterial tests and data as well as the correlations between bacterial mutagenicity test results and carcinogenicity data, potency predictions and the definitions of mutagenic versus non-mutagenic carcinogens are described. It is concluded that bacterial mutagenicity tests are the most effective short-term procedures for identifying chemicals likely to be carcinogenic in rodents and, by extension, for identifying human carcinogens. In addition, these tests provide information of the mechanism of the mutagenic action of chemicals.

Chapter 7 by E. M. Parry and J. M. Parry is entitled '*In vitro* cytogenetics and aneuploidy'. Exposure to environmental agents may produce chromosomal mutations which are relevant to heritable effects and cancer. Chromosomal mutations are defined as changes in the structure or number of chromosomes of a given cell or organism. These changes are the most important causes of human reproductive failure such as sterility, low fertility, conceptus mortality or congenital abnormality. General principles of cell cycle kinetics, chromosome structure and cytogenetic analyses are described. The section on structural chromosome changes separates analyses in metaphase and interphase whereby aberration types are illustrated diagramatically and photographically. Micronucleus analysis and molecular cytogenetic techniques such as fluorescent *in situ* hybridization (FISH) and classical cytogenetic techniques are compared. The analysis of sister-chromatid exchange induction is described. The section on numerical chromosome changes, i.e. aneuploidies and polyploidies, is also divided into discussion of metaphase analysis by chromosome counting and interphase analysis by micronucleus studies. Cell division aberrations such as c-mitoses, cell-cycle delay, premature chromatid separation, chromosome lagging and spindle aberrations are mentioned as indicators of aneuploidy induction by chemical treatment of mammalian cells. The last section deals with method developments such as metabolic activation systems and FISH technology. It is concluded that the availability of new techniques, e.g. FISH, means that many more questions may be asked about chromosomal aberrations and the extent to which different chromosomes and subchromosomal

regions are involved in their formation at the level of the initial damage, after repair, at first mitosis and after several generations as stable changes.

Chapter 8 by J. A. Heddle is entitled '*In vivo* assays for mutagenicity'. This chapter is concerned with general aspects of *in vivo* tests and their protocols and contains specific recommendations for their use and improvements. '*In vivo*' in this context is defined as 'in mammals', unlike in other fields of biology. *In vivo* assays have many advantages over their *in vitro* counterparts primarily in their ability to model the human situation more closely. One of the most important considerations for genetic toxicology is the different categories of mutation which cannot always be detected with a single technique. In practice, the genetic changes are called whatever the assay is designed to detect and not necessarily what they actually are. For example, a genetic alteration involving a chromosome exchange with one breakpoint within the *hprt* locus would be identified as a translocation by cytogenetic methods and as a point mutation by thioguanine resistance selection. Mutations were assayed for the resultant phenotype with the classical techniques. Modern advances such as the use of transgenic animals, e.g. Muta™ Mouse and Big Blue™ allow the detection of mutations that are essentially neutral since the mice do not need the loci tested. These neutral mutations may accumulate upon extended exposure to a test chemical. Therefore, one of the most important recommendations given by the author is that multiple or chronic treatments be used. Further points of the discussion in this chapter are sampling times, statistics and risk extrapolation. At the end, an outlook is given into future assay developments based on techniques of molecular biology.

Chapter 9 by J. B. Bishop and K. L. Witt is entitled '*In vivo* germ cell mutagenesis assays'. The chapter describes germ cell assays conducted in rodents, predominantly in the mouse. These assays detect mutant individuals resulting from mutations in parental germ cells and serve two purposes. First, the frequency with which mutants occur among progeny of exposed animals provides an assessment of the impact of exposure. Secondly, mutants identified by these assays are valuable resources in the study of genetic disease, and in understanding basic genome organization and gene structure-function relationships. As a basic information underlying all germ cell assays, the chapter compares the details of mammalian gametogenesis in both sexes and gives a time-table for spermatogenesis in mice since most assays are conducted by treatment of the male parental animal. Cytogenetic assays in germ cells of treated animals are described in a separate section. Of the heritable mutation assays, the methodologies for the dominant lethal test, the heritable translocation test, the morphological specific locus test, the biochemical specific locus test and for several dominant mutation tests are presented in detail. Tests for primary DNA damage and new molecular assays such as those with transgenic mice or those using FISH analyses are briefly mentioned for completeness under reference to the respective detailed chapters.

Chapter 10 by R. Fahrig is entitled 'The mouse spot test'. The spot test is the best validated method for the detection of genotoxic alterations, namely gene mutations and recombinations, in somatic cells of mice. The test is based on the princple that embryos heterozygous for different coat colour genes are treated *in utero*. Cell clones with mutated coat colour genes are recognized as fur spots on the animals after birth. The chapter describes the genetic events that can lead to coat colour spots as well as the technical details of the test protocol and provides a table with results from spot test experiments with chemicals published since 1984. Finally, the relationship between mutagenesis and carcinogenesis and the effects of chemicals which enhance or reduce mutations and recombinations are discussed.

Chapter 11 by D. Clive is entitled 'Mammalian cell mutation assays'. The diploid nature of eukaryotic cells has long been known to permit the detection of multigenic alterations such as gene conversions, mitotic recombinations and chromosome rearrangements in addition to point mutations. Despite this knowledge, the guiding principle behind the development of *in vitro* mammalian cell mutagenicity assays for most of the past 25 years has been to detect bacterial-type mutations in a mammalian cell environment. The earliest of these assays utilized reverse mutations with nutritional markers which involved multistep biochemical pathways. These were soon replaced by forward mutations at the genetically simpler hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus, first in

Chinese hamster ovary (CHO) cells, and later in Chinese hamster lung (V79), human and mouse cells. Forward mutation to ouabaine resistance, involving the dominant Na^+/K^+-ATPase gene, was found useful for the detection of base-substitution mutations in mammalian cells. Also the heterozygous thymidine kinase locus (*tk*$^{+/-}$ mutated to *tk*$^{-/-}$) in mouse L5178Y lymphoma cells (MLA, mouse lymphoma assay) or the adenine-guanine phosphoribosyl transferase locus (*aprt*$^{+/-}$ mutated to *aprt*$^{-/-}$) in CHO cells were utilized. Later still, the heterozygous *tk* locus was redeveloped in human cells (TK-6 assay). The description of these assays is preceded by sections on the scale, organization and function of the mammalian genome and definitions of gene mutations. In the section on the scale of mutations in mammalian cells the multistep mutational model for colorectal cancer is illustrated. A point is made for the ability of the MLA to detect various scales of genetic damage from point mutation via chromosome alterations to aneuploidy. With respect to relevance for human risk, it is concluded that the MLA may be able to fill a large void which is left by other assays due to either phylogenetic distance (bacteria) or detection of a limited scale of damage (bacterial and cytogenetic assays).

Chapter 12 by J. C. Mirsalis is entitled 'Assays for unscheduled DNA synthesis *in vivo*'. The repair of damage to DNA involves the excision of DNA adducts and resynthesis of the excized region. This DNA synthesis occurs at times in the cell cycle other than the scheduled DNA replication during S-phase and is therefore referred to as unscheduled DNA synthesis (UDS). This endpoint has been measured in a variety of cell lines, primary rat hepatocyte cultures or in the *in vivo–in vitro* hepatocyte assay. The details of the methodologies for UDS measurement are discussed along with the analysis and interpretation of the results. The regulatory applications are mentioned and a comparison is presented for selected chemicals of responses in the bacterial test, the *in vivo* micronucleus test (MNT), the *in vivo* UDS assays and the rodent cancer bioassay which demonstrates how the MNT and the UDS assays complement each other in recognizing rodent carcinogens. It is concluded that when combined with the MNT, the UDS assay provides highly useful information on the genotoxic potential of chemicals *in vivo* in particular for hepatocarcinogens.

Chapter 13 by C. L. Crespi is entitled 'Use of genetically engeneered cells for genetic toxicology'. The focus of this chapter is the recent progress in the incorporation of xenobiotic metabolizing enzymes into target cells for genotoxicity assays via gene transfer. It is not a comprehensive review but focuses on the differences between metabolically competent cells and the traditional extracellular approach to promutagen activation, the toxicological significance of individual enzymes and the validation of genetically engineered and thereby metabolically competent cells. The superfamily of cytochrome P450 is discussed in detail. The principle of cDNA expression systems is described and an integrated approach of toxicity/genotoxicity measurment is illustrated. Theoretical and technical considerations are followed by a validation of the use of engineered cells and examples of their applications. It is concluded that metabolically competent cells, especially those expressing the human enzymes, will provide useful insights into human promutagen activation and factors which affect this activation *in vivo*.

Chapter 14 by D. A. Eastmond and D. S. Rupa is entitled 'Fluorescence *in situ* hybridization: application to environmental mutagenesis'. Cytochemical and immunological techniques detect the presence and location of specific molecules and organelles within cells. *In situ* hybridization is one of these techniques which allows the presence of specific nucleic acids to be detected and localized in morphologically preserved cells and tissues. It is based upon the specific annealing of a labelled nucleic acid probe to complementary DNA or RNA sequences followed by visualization of the labelled probe. Initially, radioisotopes were the only labels available for DNA and autoradiography was required to detect the hybridized probe. In recent years, fluorochromes have become available and the quicker and easier technique of fluorescent *in situ* hybridization (FISH) has been developed. Through the use of probes to repetitive DNA sequences or probes generated from chromosome libraries, FISH allows specific chromosomes or chromosomal regions to be recognized in interphase and metaphase cells. The hybridization procedure including probe preparation, tissue and cell preparation, hybridization and visualization are described in detail. The application of FISH in studies of environmental mutagenesis

is discussed for repetitive probes to detect aneuploidy or chromosomal aberrations in metaphase cells including micronuclei and for painting probes in metaphase cells to detect translocations. It is concluded that future developments and novel applications of existing FISH techniques promise to enhance our ability to detect environmental mutagens and understand the mechanisms underlying induced chromosomal changes.

Chapter 15 by R. Forster is entitled 'Measuring genetic events in transgenic animals'. Transgenic animals harbour transmissible new gene combinations, either exogenous DNA sequences, inactivated genes (knock outs) or replaced genes. The chapter reports the method of transgenics made by microinjection of DNA into fertilized eggs. The introduced transgenes are usually composed of regulatory DNA which determines the pattern of expression, a structural gene and a polyadenylation sequence tail. The role of transgenic assays in mutagenicity testing is discussed and transgenic assay systems are described including commercially available transgenic rodents. A tabular survey is given of the presently available data base for chemicals tested in the *lacZ* and *lac*I transgenic mutation systems. General considerations regarding transgenic models such as replication, stability, pleiotropic effects and reporter genes are presented. It is anticipated that specifically developed transgenic animal models can answer particular questions regarding toxicological mechanisms and provide data of greater relevance to human extrapolation than conventional animal experiments.

Chapter 16 by R. R. Tice is entitled 'The single cell gel/Comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cells'. Originally, DNA damage was quantitated in individual cells by lysing cells embedded in agarose gel on slides under mild alkaline conditions to allow the partial unwinding of DNA. After neutralization, the cells were stained with acridine orange and the extent of DNA damage was quantitated by measuring the ratio of green fluorescence for double stranded DNA and red flourescence for single stranded DNA. To improve the technique for detecting DNA damage, a microgel electrophoresis method was developed commonly called the Comet assay. Cells are embedded in an agarose gel on a microscopic slide, lysed by detergents and high salt, and the liberated DNA is electrophoresed under neutral condition. Cells with increased DNA double strand breaks display increased migration of DNA towards the anode, visualized by ethidium bromide staining. Using electrophoresis under alkaline conditions detects also single strand breaks and alkali-labile sites. This latter version of the assay offers greatly increased sensitivity because it detects a larger range of DNA damage. The details of the methodology are described and discussed and a list of chemicals tested with the assay is presented. It is warned that the relationship between the DNA migration and the process which leads to cell death needs to be understood since the effect of toxicity is the most important artefact associated with the assay. It is concluded that the Comet assay is of almost unlimited application in the areas of radiation biology, genetic toxicology, environmental biomonitoring and human studies.

Chapter 17 by R. J. Albertini and J. P. O'Neill is entitled 'Human monitoring for somatic mutations in humans'. The chapter considers the methods currently available for investigating *in vivo* somatic mutations in humans such as the erythrocyte assays and the T-lymphocyte assays with different reporter genes. Quantitative results of studies in human populations and molecular analyses of the underlying mutational events are summarized. Evidence is presented that mutations in reporter genes may serve as valid surrogates for at least some pathogenic mutational mechanisms. At present, biomonitoring for somatic mutations defines the genetic effects in populations with a given exposure and may serve as an impetus to reduce or abolish the exposure. The challenge of validation of these mutation assays is seen in the step from populations down to individuals. Three approaches are envisaged. Mutations in reporter genes and cancer genes can be measured in the same individual, i.e. in cancer patients receiving chemotherapy. In human cancer chemoprevention trials mutagenicity studies can be added to demonstrate a reduction in reporter gene mutations along with a decrease of the cancer incidence. For individuals with radiation or chemical exposure through therapy or by accidents longitudinal studies can be designed to compare the results of the biomarkers with the clinical outcome of disease. Positive correlations between mutations in the reporter genes and in

cancer genes can be meaningfully used for human risk assessment and in designing strategies for prevention.

Chapter 18 by D. H. Phillips and P. B. Farmer is entitled 'Protein and DNA adducts as biomarkers of exposure to environmental mutagens'. In many cases, DNA damage occurs through the formation of electrophilic species that can react covalently with nucleophilic sites on the purine or pyrimidine bases. Monitoring adducts in DNA isolated from human or animal tissue will determine the levels of exposure to environmental mutagens. The same electrophilic sites also react with nucleophilic sites in proteins and the hemoglobin and albumin readily accessible in human red blood cells makes these alternatives to DNA for monitoring carcinogen exposure. This chapter describes the methods available for detecting and characterizing protein and DNA adducts. The relationship between adduct formation and carcinogenic activity is described, together with examples of the formation of these lesions in humans and other species as a result of environmental exposure to genotoxic agents. Experimental studies are discussed under the aspects of dose–response, persistence of adducts and accumulation of adducts under acute and chronic exposure conditions. Human studies are discussed with examples of tobacco smoking, occupational exposures to ethylene oxide, acrylamide, or polycyclic hydrocarbons, medical exposure to chemotherapeutic drugs or environmental exposures. While DNA and protein adducts are useful markers of human exposure the demonstration that a chemical forms adducts indicates its genotoxicity. The biological significance of adducts formed endogenously as opposed to those formed by exogenous exposure remains to be determined.

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Wilson's Disease, vol. 30 in the series Major Problems in Neurology. By Tjaard HOOGENRAAD. London, Philadelphia, Toronto, Sydney, Tokyo: W. B. Saunders. Co Ltd. Pp. 209. £45.00.

A similar volume was produced in the series Major Problems in Internal Medicine, By Scheinberg and Sternlieb in 1984, at that time the price was £34.50 – such is inflation. In reviewing that volume I commented that the price 'does seem high for such a slim volume' and in this respect the situation does not appear to have changed. There are two main differences between these two volumes, first and most important there has been a significant and most important advance made in our understanding of Wilson's disease since Scheinberg and Sternlieb's volume was published in that the gene has been identified simultaneously by three groups working independently, two in the U.S. and one in Canada and this was published in *Nature Genetics* in December 1993. This new work has been included in the present volume in a chapter written jointly with Dr Houwen bringing the subject right up to date, unlike many multiauthor textbooks which are dated before they are published. The book consists of seven chapters, History, Genetics, Copper metabolism, Pathology, Clinical manifestations, Diagnosis and Treatment. The other main difference with the earlier volume is that Drs Scheinberg and Steinlieb based their volume on their experience of 500 patients whereas Dr Hoogenraad's experience is confined to only 50 so that he, inevitably, is more reliant on the literature. While the list of references is very extensive, and will therefore be most useful, it does seem to ignore some of the important advances made in the 1950s and 1960s. Furthermore, some of the quotations are not quite accurate; if I may take an example, on page 18 he quotes Walshe as estimating an incidence for Wilson's disease in the U.K. as 30 per million. However, the actual incidence calculated was 20 cases per million in East Anglia. It was Bachmann (1979) who estimated the higher figure for East Germany, but this work is not quoted in the chapter on disease prevelance.

In one chapter diagnostic problems are well covered but too much stress is placed on the use of radiocopper incorporation into caeruloplasmin. As most patients are severely deficient in this protein, despite an extraordinary statement on p. 112 that a normal concentration of caeruloplasmin may be found in 15 to 25% of patients, the isotope can not be incorporated. In the U.K. radiocopper is now too expensive to be used as a simple diagnostic test, but things may be different in the Netherlands. There is another curious statement in this chapter that Kayser Fleischer rings may be absent in the majority of

patients at the time of first consultation. While this is true of those presenting with hepatic disease, it is virtually unknown for patients with neurological disease not to have the rings. Whilst on this subject it can be said that the colour pictures of the rings (Plates 1 and 2) are quite excellent, one of the black and white pictures (Figure 1, 6) is so out of focus as to have better been omitted. Further in this chapter Dr Hoogenraad is right to use caeruloplasmin determination as a first screening test and he is also correct to stress its lack of sensitivity but what he means by a 'homozygous carrier' I do not know.

However, it is when I come to the chapter on treatment that I have the greatest difficulty. The author points out that there are two main strategies to be followed, either the use of chelating agents (penicillamine, trientine, dimercaprol) or zinc sulphate to which might be added tetra thiomolybdate. He goes on to point out that the former approach is favoured by Walshe in the U.K. and Scheinberg and Sternlieb in the U.S.A. Between them these workers must have seen close on 1000 patients by the present time. This, however, Dr Hoogenraad does not allow to disconcert him despite the small number of patients he has seen for he goes on: 'As we shall explain below, following the advice of experts is not always the best way to solve the therapeutic dilemma.' In clinical medicine, unfortunately there is no satisfactory substitute for experience and this reviewer finds it difficult to see how to evaluate the difference between workers with so much experience against Dr Hoogenraad's fifty patients. Nevertheless he strongly defends his view that zinc is the only permissible treatment because of potential chelation toxicity against zinc's alleged safety and efficiency in his own experience, though not of this reviewer.

The book is well produced and will, undoubtedly, find its place in the medical library, whether the individual physician will wish to purchase his own copy is another question.

J. M. WALSHE