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Chairman's Summary of Session B

The antimicrobial activities and metabolic transformations of compounds which undergo reductive activation was the theme of this Session. DR I. ROWLANDS (BIBRA, Carshalton) stressed that reduction by the gut microflora plays an important role in the metabolism and toxicity of a number of drugs and other foreign compounds, particularly those containing nitro groups. The metabolism of such drugs may be modified by a variety of factors which influence the reductive capacity of the microflora including diet. In addition there are numerous differences in the gut microflora between individuals and animal species which makes extrapolation of metabolic and toxicological data from laboratory animals to man extremely difficult.

DR A. G. RENWICK (Clinical Pharmacology Group, University of Southampton) discussed sulphinpyrazone and sulindac, each of which contain a sulphoxide moiety which can undergo both oxidation and reduction to the sulphone and sulphide (thioether) analogues. The sulphide metabolites are more potent in their activities (anti platelet-aggregatory and anti-inflammatory respectively) than the parent drugs.

Comparison of the metabolism in normal subjects and ileostomy patients showed that the reduction of sulphoxide may be due to the liver and/or the gut flora. The relative importance of these two sites is dependent on the substrate and the delivery of the substrate to the hind gut flora.

DR G. T. MIWA (Merck Sharp & Dohme, Rahway) described experiments which show that *Trichomonas foetus* avidly metabolizes ronidazole by pathways different from mammalian and gut bacterial enzymes; approximately 95% of the ronidazole taken up by the protozoa had been metabolized, resulting in a substantial fraction (24% to 55%) of the total radioactivity being protein-bound. These experiments suggest opportunities for defining the mechanism underlying the specific trichomonicidal activity of 5-nitroimidazoles.

DR M. MULLER (Rockefeller University) reviewed the evidence that the antiprotozoan and antimicrobial action of nitroimidazole derivatives depends on the reductive activation of these compounds to give relatively short lived toxic intermediates. Neither the unreduced compounds nor the final products of their reduction are cytotoxic. The reduction of metronidazole, a 5-nitroimidazole, is a ferredoxin linked process, thus this compound exerts selective action on anaerobic organisms in which ferredoxin linked metabolic pathways play a major role. Aerobiosis inhibits metronidazole action in these organisms. The reduction rate of nitroimidazoles with different mid-point potentials by ferredoxin is independent of the mid-point potential whereas in the absence of ferredoxin reduction by various enzyme systems, even ferredoxin linked ones, is strongly dependent on this property. Resistance to metronidazole can occur in susceptible anaerobic species. Anaerobic resistance in trichomonad flagellates is due to a lack of enzymes responsible for reductive activation and can be developed only in the laboratory. Aerobic resistance is due to an increased inhibitor effect of oxygen on the process of drug activation and has been shown to be responsible for treatment failures in human vaginal trichomoniasis.

The first direct demonstration of O_2-quenching of the metronidazole radical anion in *Trichomonas vaginalis* under controlled and measured O_2 partial pressures was described by PROFESSOR D. LLOYD (University College, Cardiff). As well as this electron paramagnetic resonance study, he described the use of mass spectrometry for measuring the inhibitory effects of nitroimidazoles on hydrogen evolution by the parasite. This approach has enabled two biochemical lesions to be defined in a metronidazole-resistant strain of *T. vaginalis*: (a) alteration of the K_m for O_2 of the unidentified hydrogenosomal oxidase, and (b) alteration in the redox properties or concentration of the [2Fe–2S] ferredoxin of the hydrogenosome. These data also suggest that damage to hydrogenosomal electron transport components by drug reduction products is an early event leading to cell death.

DR D. I. EDWARDS (North East London Polytechnic) summarised the evidence that nitroimidazole drugs are specifically reduced in anaerobes by ferredoxin-linked redox systems and generate short-lived intermediates, which can damage DNA. Such damage is characterised by helix destabilisation and strand breakage, which is related to the % A + T composition of the DNA. Whereas 2-nitroimidazoles generally undergo a 4-electron reduction indicating reduction to the hydroxylamine, 5-nitroimidazoles yield non-integral values between 3 and 4. Interestingly, these electron values decrease in the presence of DNA suggesting possible electron transfer from DNA which could account for strand breaks and cell death.

Finally, DR P. DECLERK (Leuven and Rockefeller Universities) described *in vitro* polarographic experiments in which the influence of the four DNA bases on the reduction of 5-nitroimidazoles. This work provided evidence for interaction of reduced intermediates with guanine and adenine. On the other hand, reaction with thymine could be excluded. Electrolytic reduction of 5-nitroimidazoles in the presence of DNA clearly demonstrated the formation of adducts at the level of guanine. Evidence was also provided that the reduced species, responsible for the observed interaction, is relatively stable.

These contributions help identify some fundamental problems: we especially need (a) to gain more information on the conditions prevailing *in vivo*, with respect to pH, dissolved O_2 and thiol levels, so that we can (b) design more appropriate *in vitro* model systems, in which we can (c) identify the full range of active reduction products of nitroimidazoles, in order (d) to distinguish likely *in vivo* possibilities. Finally, (e) there is a pressing need for the development of high resolution non-invasive *in vivo* monitoring techniques for the identification of (often) short-lived radical species at low steady state levels.

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