Agents and Actions, vol. 18, 5/6 (1986)

0065-4299/86/060560-03$2.10

Meeting Report: Free Radicals, Cell Damage and Disease

MICHAEL J. PARNHAM* and JOSEPH LUNEC†

*A. Nattermann & Cie. GmbH, Department of Immunopharmacology, P.O. Box 35 01 20, D-5000 Cologne 30, F.R.G.
†Biochemistry Department, Selly Oak Hospital, Raddlebarn Road, Selly Oak, Birmingham B29 6JD, England.

This meeting, held on 16 December, 1985 at the Royal Free Hospital, London was crammed full with invited speakers and posters, covering many aspects of Free Radicals and Disease. Here we report on presentations of relevance to inflammation.

Cancer was one of the first themes dealt with, including a presentation by Prof. T.F. Slater (Brunel University, Uxbridge) on changes in lipid peroxides and eicosanoids in human cancers. In breast tumours PGI_2 and TxB_2 production is enhanced, while the large amount of 12HPETE/12HETE formed by normal cervical tissue is reduced during cervical cancer. Perhaps this points towards a role of these 12-lipoxygenase products in the pathophysiology of the human cervix.

During chronic alcohol administration in rats and man, Dr T.J. Peters (Clinical Research Centre, Harrow) reported that liver diene conjugates are enhanced, reflecting increased lipid peroxidation. Interestingly, this increase was associated with a specific reduction in liver glutathione peroxidase (GSH-Px), but not superoxide dismutase (SOD), catalase or GSH. The author discounted the possibility of the data in rats being of direct relevance to man since the animals were ingesting the equivalent of two 1 litre bottles of whisky per day! The changes in GSH-Px and diene conjugates in man were associated with cirrhosis rather than with early liver damage or reversible fatty liver. Dr R. Fink (W. Middlesex Hospital, London) reported that 90% of all serum diene conjugates in human alcoholics consist of octadeca-9,11-dienoic acid (9,11 LA), a linoleic acid diene conjugate. Dr Fink and others have suggested that this isomer of linoleic acid is the product of free radical mediated abstraction of H* from linoleic acid followed by double bond rearrangement, which does not proceed via the classical peroxidation pathway, i.e. it most probably occurs under conditions of low pO_2 (FEBS Lett. 162, 239, 1983). The compound was not detectable in dogs, cats, guinea-pigs or rabbits. The compound was not specific to alcoholic liver damage. Furthermore, in a recent publication (Lancet ii, 774, 1985) Smith and Thompson suggest that 9,11 LA is simply a product of bacterial activity.

A role of GSH-Px in the response of leucocytes to inflammatory stimuli was indicated by a poster presented by M.J. Parnham *et al.* (Nattermann, Cologne). In both mouse peritoneal macrophages and rat pleural granulocytes, activated by inflammatory stimuli, an increase in the oxidative burst was associated with a fall in intracellular GSH-Px. These data suggest that in the initial stages of inflammation and cell damage, the natural antioxidant defenses are significantly compromised.

The role of iron in free radical generation was considered extensively in both oral and poster presentations. Dr B. Halliwell (King's College, London University) reviewed the field, emphasising the probable importance of ferritin as a source of iron for free radical generation *in vivo*, releasing its iron content in the presence of O_2^- or H_2O_2 or at $pH \leqslant 5.5$, as is likely to occur at sites of inflammation. Since synovial fluid contains very low levels of SOD, catalase and GSH-Px and an excess of ferritin, release of iron could be an important aetiological factor in

arthritic conditions. Lactoferrin, on the other hand, is probably a protective factor, mopping up catalytic iron. The iron chelator desferrioxamine has, among other effects, anti-inflammatory actions, though its use is restricted to treatment of heavy metal poisoning because of serious side-effects (Quart. J. Med., 56, 345, 1985). Dr S. Aust (Michigan University) described studies on the mechanism of iron-induced lipid peroxidation *in vitro* showing that neither Fe^{2+} nor Fe^{3+} alone is capable of inducing lipid peroxidation (of phospholipid liposomes), but in the presence of reducing equivalents (e.g. H_2O_2), a 1:1 ratio of Fe^{2+}: Fe^{3+} leads to an immediate and rapid response. He proposed that an Fe^{2+}-O_2-Fe^{3+} chelate is formed which is ultimately responsible for the lipid peroxidation. J.M. Hoepelmann and colleagues (University of Utrecht), in a poster presentation, reported that Fe^{2+} is capable of inducing human PMNL aggregation *in vitro* in a catalase-, indomethacin- or NDGA-sensitive manner, which is associated with TxB_2 generation. They proposed that Fe^{2+} (e.g. released from ferritin) may stimulate PMNLs through sequential H_2O_2 and eicosanoid release. Conversely, G. Chaudri *et al.* (Australian National University, Canberra) presented a poster in which they showed that iron chelators are able to inhibit PMA-induced lymphocyte proliferation. They suggested that this reflects a role of oxygen radicals in lymphocyte proliferation. However, since lymphocyte proliferation is dependent on the uptake of iron via transferrin receptors, chelation may affect proliferation by a mechanism which is independent of oxygen free radicals. Nevertheless, H_2O_2 has been reported in the literature to be a modulator of lymphocyte function. I.M. Allan and colleagues (University of Birmingham) provided data supporting this activity in that they found that the toxicity of UV irradiation for human lymphocytes could be inhibited by catalase, SOD and mannitol. $OKT8^+$ T suppressor/cytotoxic cells were the most susceptible to irradiation cytotoxicity.

Dr J. Lunec (University of Birmingham) reported on studies showing that H_2O_2 generated by stimulated human PMNL alters human IgG to a fluorescent product which is antigenic and reacts with IgM or IgA rheumatoid factors (RF). The altered IgG also generates further H_2O_2 from PMNL providing a positive feedback loop for the formation of antigenic IgG. The formation of this altered IgG is inhibited by catalase which also totally inhibited the binding of this antigenic IgG to RF. Dr Lunec has also detected increased levels of fluorescent IgG and RF binding in rheumatoid synovial fluid (SF) compared to normal SF, and treatment with d-penicillamine leads to a reduction in these levels in close association with relief of disease symptoms. In one patient in whom treatment exacerbated symptoms, the levels of fluorescent IgG in SF were also increased, further indicating the close association of these two parameters. These data offer a possible explanation for the appearance of RF in rheumatoid arthritis and suggest that inhibition of H_2O_2 formation may be effective in the treatment of this disease. A further role of H_2O_2 in rheumatoid arthritis was indicated by the finding of Dr I. Emerit (Institut Biomedical des Cordeliers, Paris) that lymphocytes from rheumatoid arthritis patients have increased chromosomal aberrations. Such aberrations could be produced in normal lymphocytes incubated with monocytes from RA patients and inhibited by SOD or catalase.

In a poster presentation, E.J. Dowling and colleagues (Surrey University, Guildford) demonstrated the presence of free radical activity at the site of foot pad oedema (Koch model) using a novel *in vivo*-chemiluminescence procedure. Inhibitor studies indicated the generation of hydroxyl radicals, hydrogen peroxide and singlet oxygen at the site of inflammation. Although the method needs further validation it promises to be a sensitive procedure for following oxidative reactions occurring in animal models of inflammation.

Another area of growing interest with regard to the role of oxygen free radicals is their involvement in reperfusion damage to transplanted organs. Dr B. Fuller (Royal Free Hospital, London) introduced this topic towards the end of the meeting and underlined the potential use of antioxidants in preventing the damaging effects of reperfusion of blood through transplanted organs. He also outlined a procedure based on Schiff-base measurements of lipid peroxidation which clearly demonstrated the involvement of free radicals in transplanted livers and kidneys in animal models.

In conclusion, it is clear that there is a growing awareness of the importance of reactive

oxygen species, particularly peroxides, for the aetiology of inflammation and a variety of other pathological conditions. Whether the peroxides arise as a result of increased catalytic iron levels in extracellular fluids or as a result of the leucocyte oxidative burst, therapy with scavengers of these reactive compounds is becoming increasingly attractive. The following song was composed by Prof. H. Baum (King's College, London University) to sum up the subject matter of the meeting:

Received 12 April 1986

Free radicals and disease
(Tune – Abdul Abulbul Amir)

Now, once things were simple, we thought it was best,
Excited states were to be feared
Triplets were babies, a singlet a vest
A radical wore a red beard

Reduction of oxygen gave energy
And carbon tet. dry-cleaned off mud
To stop getting colds you took vitamin C
And iron was good for your blood

But transition metals now terrify men
Reductants they view with alarm
For partial reduction of dioxygen
Can end up by doing you harm

From Fenton reaction through to Haber-Weiss
And on to hydroperoxides
Those chain propagations are not very nice
And sometimes are fatal besides

The species involved though seem not to be clear
Hydroxyl? Perferryl? Perhaps.
But superoxide they say you shouldn't fear
Well, 'SOD that!' is my answer chaps

From Kwashiokor to excesses of gin
For ageing to cancer, the same
The moment the unpleasant symptoms begin
Oxy radicals take the blame

Disorders of blood, emphysema, R.A.,
and paraquat toxicity
Involve the same species, at least so they say
So should we take vitamin E?

Is desferrioxamine all that they claim?
And what is selenium's role?
If chelation therapy gets a bad name
At least it keeps us off the dole!