care, but improvement of such primary care will take time; needed vaccinations should not have to depend on these changes.

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Cytokines in adult respiratory distress syndrome

SIR—Some proinflammatory cytokines (interleukin-6 [IL-6], interleukin-8 [IL-8], tumour necrosis factor α [TNFα]) have been reported in high concentrations in blood and bronchoalveolar lavage (BAL) fluid of patients with sepsis and adult respiratory distress syndrome (ARDS);[1,2] in addition, increased plasma concentrations of interleukin-10 (IL-10) have been described during septicaemia and septic shock.[3] Since in-vitro IL-10 inhibits the production of proinflammatory cytokines, we measured IL-10 in plasma and BAL fluid supernatants from 28 ventilated patients in the intensive care unit.

14 patients had ARDS (8 with pneumonia), 5 had pneumonia without ARDS, and 9 control patients had neither ARDS nor pneumonia but were ventilated. Blood was collected onto sterile tubes treated with edetic acid (EDTA) and immediately centrifuged at 1500 *g* for 15 min at 4°C. All patients underwent BAL with a standard technique;[1] plasma and BAL supernatants were stored at −70°C. Human IL-10 (hIL-10) levels were determined by subtraction with a two-site sandwich ELISA format, as previously described:[4] one assay quantified hIL-10 and BCRF1 (viral IL-10 from Epstein Barr virus), whereas the other one was specific for BCRF1 (limit of detection 100 and 50 pg/mL for hIL-10 and BCRF1, respectively).

Our results are shown in the table. Of the 28 ventilated patients studied, hIL-10 was detected in the BAL fluid in only 1 patient with pneumonia. In plasma, only 1 patient who had neither ARDS nor pneumonia had high concentrations of hIL-10; in the 27 remaining patients, 5 had a positive reaction with BCRF1 (18·5%) which is a higher percentage than in the healthy control population (4·7%) previously described with use of the same assay.[4] This pnenomenon could be explained by Epstein Barr virus reactivation in patients in the intensive care unit.

Despite the production of high concentrations of proinflammatory cytokines (TNFα, IL-6, IL-8; data not shown), we found measurable IL-10 in blood and BAL fluid of patients with ARDS and pneumonia, or in ventilated controls, in only 2 of the 28 patients. Our results are not in accordance with those of Marchant et al who reported hyperproduction of IL-10 during septicaemia and septic shock;[3] however, although their limit of detection was lower than ours, the specificity of their assay for hIL-10 was not given. Moreover, one can hypothesise that IL-10 production could depend on the underlying pathological process and/or the type of pathogen involved. Finally, we cannot exclude the involvement of IL-10 since we did not measure the induction of IL-10 mRNA in blood or alveolar cells.

	Plasma (pg/mL)		BAL fluid (pg/mL)	
	hIL-10	BCRF1	hIL-10	BCRF1
ARDS without pneumonia (n=6)	<100 (n=6)	>50 (n=2)	<100 (n=6)	<50 (n=6)
ARDS with pneumonia (n=8)	<100 (n=8)	>50 (n=1)	<100 (n=8)	<50 (n=8)
Pneumonia (n=5)	<100 (n=5)	>50 (n=2)	<100 (n=4) 230 (n=1)	<50 (n=5)
Ventilated (n=9)	<100 (n=8) 1920 (n=1)	<50 (n=9)	<100 (n=9)	<50 (n=9)

Table: **Cytokine results**

We conclude that regulatory mechanisms, in particular IL-10 production, that control monocyte activation could be defective in patients with ARDS. This phenomenon, associated with protection of mice from lethal endotoxaemia induced by IL-10,[5] allows us to suggest that the injection of recombinant hIL-10 in such patients should be assessed as a potential therapeutic tool.

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Free radicals and antioxidants

SIR—Your series on oxygen toxicity has not emphasised the paradox that it is hypoxia that initiates the cascade of events leading to oxygen free radical injury. Zamboni and co-workers[1] postulated that the use of hyperbaric conditions to add a high dose of oxygen after ischaemia would increase reperfusion injury, but proved the opposite. They observed the microcirculation of rat gracilis muscle after 4 h of complete circulatory arrest. Without additional oxygen, they showed that reperfusion was associated with the adherence of neutrophils to the endothelium which eventually was severe enough to arrest flow. There was also severe vasoconstriction of adjacent arterioles. 1 h of hyperbaric oxygen given either immediately or after a delay of 1 h following the 4 h of ischaemia prevented both neutrophil adherence and arteriolar vasoconstriction. Zamboni et al now use hyperbaric oxygen therapy routinely to treat reperfusion injury in replanted limbs with ischaemia times up to 12 h. They have noted complete muscle survival and minimum soft tissue oedema.

The extent of reperfusion injury due to oxygen free radicals is related to the extent and duration of tissue hypoxia and seems to be mediated both by the respiratory burst generated by neutrophils[2] and by the accumulation of hypoxanthine.[3] Reperfusion injury is also associated with generalised inflammation and activation of the complement cascade. Hyperbaric oxygen has proved of value in the control of the inflammatory response in man.[4]

The observations show that hyperbaric oxygen would be a valuable adjunct to organ transplantation, since data from cardiac transplantation indicates that the same microcirculatory effects occur in the myocardium. Grines and Weaver in their Aug 20 commentary (p 490) on the importance of early thrombolysis in myocardial infarction ask "what medical treatments might augment coronary patency?" The answer, provided in a press release by the American Heart Association in 1992, is more oxygen. Hyperbaric oxygen therapy added to thrombolysis has been shown under controlled conditions to halve the time taken for pain to be relieved and for the electrocardiogram to become normal in myocardial infarction. Patients treated with hyperbaric oxygen also had lower plasma creatinine phosphokinase concentrations and an increased ejection fraction than did controls.

Oxygen given at twice atmospheric pressure reduces the cardiac workload by 20%, although increasing the plasma tension to over 1000 mm Hg greatly improves the gradient for diffusion into tissues and therefore enhances cellular oxygen availability. This improvement is not only of value to the myocardium, but also protects the brain, which often suffers from hypoperfusion in myocardial infarction. Hyperbaric oxygen therapy is of value in cardiogenic shock.[5] Hypoxia is toxic. The additional oxygen provided under hyperbaric conditions is a shield, not a sword.

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SIR—As Jenner explains (Sept 17, p 796), the potential role of free-radical reactive oxygen metabolites (ROM) in the pathogenesis of several neurodegenerative diseases is becoming more widely recognised. Although his main emphasis is on iron-dependent oxidative damage in Parkinson's disease, there is growing evidence that oxidative stress induced by an alternative cellular mechanism is more directly pertinent in the pathogenesis of Alzheimer's disease.

The initial in-vitro demonstration of ROM generation by purified brain macrophage-type microglial cells when stimulated by various chemical and immunological stimuli[1] has been complemented by studies indicating the ability of aluminosilicate particulates to stimulate the production of microglial ROM.[2] Quantitative morphometric analysis has shown increased numbers of activated microglia in the brains of Alzheimer subjects,[3] frequently juxtaposed to plaque β-amyloid fibrillar aggregates. The reported occurrence of aluminosilicate deposits within the cores of senile plaques in Alzheimer's disease brains[4] has led to the hypothesis that an analogous mechanism of β-amyloid/aluminosilicate-stimulated microglial generation of injurious ROM may be operative in vivo, and thus contribute to oxidant-mediated neurodegenerative damage.[5]

As Jenner emphasises, elucidation of the precise role of oxidative stress in neurodegeneration requires further investigation. However, the hypothesis does herald the prospect of pharmacological and micronutritional antioxidant intervention as a worthwhile therapeutic stratagem in the treatment of age-related disorders of the brain.

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SIR—Your series on free radicals and antioxidants provided an excellent overview of free radical biology and its relevance to medicine. However, one of the most commonly used terms in these articles was oxidative stress, the accuracy and usefulness of which are questionable.

Oxidative stress (or its synonym, oxidant stress) has been in use since the 1950s. Oxidative stress implies that: (1) there is a natural balance between free radicals, which are ubiquitous but toxic, and antioxidant defences; (2) damage or death result when the balance is tipped in favour of free radicals; and (3) free radicals cause non-specific or random cell damage. But free radicals, because they are so short lived, are inherently difficult to study. Thus, many events that are attributed to oxidative stress might involve specific free radicals and targets that we have not yet identified.

Unlike some of their human counterparts, biological free radicals are not always promiscuous. Indeed, many free-radical-mediated events have proved to involve specific free radicals attacking specific targets. For example, free radicals may kill *Escherichia coli* by specifically inactivating dihydroxy-acid dehydratase.[1] Similarly, nitric oxide's effects on nerve cells seem to be mediated by the alkylation of thiols on the N-methyl-D-aspartate receptor.[2]

An example of the misuse of the term oxidative stress can be seen in studies on the antimalarial drug artemisinin. Since the drug is an endoperoxide it was initially believed to work by generating oxidative stress. However, endpoints of oxidative stress, such as thiol oxidation, could only be recorded at concentrations that were more than 1000 times higher than the drug's therapeutic concentration.[3] And the oxidative stress mechanism provided no explanation for why the drug is selectively toxic to malaria parasites. Subsequently, heme and iron, which accumulate in malaria parasites, proved to catalyse the decomposition of the drug into a carbon-centred free radical, which then alkylates several specific malaria proteins.[4,5] Thus, at one point, oxidative stress served as a smokescreen for our lack of understanding of events that really involved specific free radicals and targets. This latter specific free-radical mechanism is providing guidelines for the design of second-generation derivatives in a way that an oxidative stress mechanism never could.

The term oxidative stress also has a curiosity-numbing effect. When an event is attributed to oxidative stress, it

implies that it is fully understood. By contrast, when one recognises that free-radical-mediated processes involve specific identifiable structures and reactions, one cannot be satisfied with merely demonstrating the involvement of free radicals. New questions are raised, such as what are the free radicals, how are they generated, what are the targets, and what is the mechanism of attack? Recent experience has shown that we can identify specific important free radicals and their targets. Therefore, the paradigm of oxidative stress could now be impeding progress.

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SIR—We read Bulkley's review (Oct 1, p 934) of ischaemia-reperfusion injury with interest, but with some surprise. He attributes the description of post-hypoxia-reoxygenation injury to an article by Granger and co-workers.[1] However, the history of this hypothesis is longer and better documented than Bulkley suggests.

The oxygen paradox itself was described at least 40 years ago and has been acknowledged in clinical medicine for decades. Starting in 1975, we showed that the concentration of hypoxanthine in body fluids increases during hypoxia, making it a sensitive indicator of this condition. Since hypoxanthine is a potential generator of oxygen radicals we went on to speculate that the oxygen paradox was due to a burst of oxygen free radicals generated by the hypoxanthine-xanthine oxidase system during reoxygenation, and we presented this hypothesis at several meetings. In our first published report[2] we stated that "free radicals, which may destroy cell membranes seem to be of importance during and after hypoxia. It is well known that xanthine oxidase combined with oxygen, produces free radicals. The new observation that the damaging effect is higher when hypoxanthine is present as well, focuses our interest on what happens when hypoxia is relieved and large amounts of hypoxanthine are present in the tissues. Will the damaging effect be smaller if the hypoxia is relieved gradually without surplus of oxygen being present?". Granger's paper[1] was published the following year. It describes how McCord's group in Alabama adopted and modified our hypothesis without, however, acknowledging where it came from. At the time the Alabama group had a close working relationship with Scandinavian researchers with whom we had discussed the hypothesis in the 1970s.

Bulkley seems unclear about the possible role of allopurinol in the reoxygenation of the myocardium, kidney, and brain. Since the 1960s it has been known that the distribution of xanthine oxidase varies considerably according to species and organs. Neither Granger and co-workers[1] nor McCord[3] refer to this relevant work, which could be one reason for confusion about the role of hypoxanthine-xanthine oxidase in hypoxia-reoxygenation injury. Although allopurinol has a certain antioxidant effect per se its main function is as a xanthine oxidase inhibitor, and as such one cannot expect it to have a protective effect against injury caused by oxygen radicals generated by hypoxanthine-xanthine oxidase if no xanthine oxidase is present. Therefore, how could it have a protective role in reoxygenation of the myocardium, kidney, or brain under circumstances referred to by Bulkley since these organs in man probably contain negligible xanthine oxidase activity? A possible explanation is that xanthine oxidase is released from liver and intestine during hypoxia or shock and circulates throughout the body. This means that during the post-hypoxic-reoxygenation period, oxygen free radicals generated by hypoxanthine-xanthine oxidase might attack several organs simultaneously. This hypothesis of xanthine oxidase release, which we introduced in 1982,[4] has been confirmed by others (eg, ref 5).

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Risk of recurrent abortion after appearance of a chorionic sac or heart rate on vaginal ultrasound

SIR—Simpson and colleagues (Oct 1, p 964) report the risk of recurrent pregnancy loss (first and second trimester spontaneous abortion) in patients with normal fertility whose pregnancies were diagnosed during the fifth week after the last menstrual period. Their results were inconclusive because they included only 4 patients with 3 or more previous losses. Accurate tables of chorionic sac diameter (CSD)[1] and crown-rump length (CRL)[2] have been produced for the first ten postmenstrual weeks, which encompass the embryonic period of human development.[3] By use of vaginal ultrasound, estimates of the risk of abortion can be made on the basis of whether the size of the CSD or CRL is above or below the 50th centile for healthy individuals.[1,2]

We have reported the association between CSD and pregnancy loss in 820 patients attending an infertility clinic because of infertility or previous pregnancy loss.[1] 322 of these patients had previously had a first or second trimester pregnancy loss and 60 had had 3 or more losses. The overall first and second trimester pregnancy loss rate was 17·2% (95% CI 14·6 to 19·8%) after detection of a chorionic sac during the fifth week. We have now reanalysed these data with respect to previous pregnancy outcome (table). The risk of recurrent loss was increased after 3 but not after 1 or 2 previous losses. When the initial CSD was below the 50th centile, pregnancy loss for all patients was 28·1% (23·5 to 32·6) and for patients with 3 or more previous losses 46·4% (27·9 to 64·9), which were higher than equivalent values for