Copper and disease resistance in sheep: a rare natural confirmation of interaction between a specific nutrient and infection

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'Cause and effect' relations between nutrition and susceptibility to infection on natural diets have rarely been demonstrated in any species. Such associations might be anticipated since the body responds to infection with considerable changes in protein metabolism (Baracos, 1986), basal metabolic rate (Chandra, 1980) and trace element distribution (Beisel *et al.* 1973), indicating that the disposition of nutrients plays an important role in mounting resistance to the pathogen. Nevertheless, evidence for the effects of specific nutrients on the course of infection is sparse. The object of the present paper is to review the existing literature critically and then present new evidence that a natural deficiency of copper in lambs is associated with an increased susceptibility to infection.

Non-specific effects of infection on nutrient utilization and of nutritional status on susceptibility to infection have been found in sheep as in other species. For example, Jones *et al.* (1982) showed that lambs with experimentally induced chronic pneumonia required 25% more food to reach the same body-weight as uninfected lambs; whether infection had adversely affected the utilization of several nutrients or just one was unknown. In chronic ovine nematode infections severe impairment of skeletal growth and food utilization together with marked reductions in the deposition of fat, protein and minerals in the body, have all been observed (Sykes *et al.* 1979). Evidence that nutritional status influences susceptibility to infection is contained in a paper by Eales *et al.* (1986) who found that twin-bearing ewes in poor bodily condition, indicative of undernutrition during pregnancy, are more likely to give birth to lambs which develop enteritis ('watery mouth') than those in good bodily condition. However, as with the associations between protein–energy malnutrition and susceptibility to infection in infants (e.g. Chandra, 1980), it is unclear whether deficiencies of protein, energy, micronutritients or intake of colostrum *per se* were predisposing factors.

Single nutrients and immunity: in vitro evidence

Evidence for effects of specific nutrients on disease resistance comes largely from studies in small laboratory animals fed on highly purified diets and is often indirect, being based on in vitro tests of immunocompetence. For example, experimental selenium deficiency has been associated with impaired microbicidal activity in rats (Serfass & Ganther, 1975; Arthur *et al.* 1985) and cattle (Boyne & Arthur, 1979) when phagocytes were challenged with *Candida albicans* in vitro. Cu deficiency in sheep and cattle similarly inhibited the candidacidal activity of

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peripheral blood leucocytes (Jones & Suttle, 1981); correlated reductions in the intracellular activity of an antioxidant cuproenzyme, superoxide dismutase (*EC* 1.15.1.1; SOD) gave rise to speculation that the ability to remove toxic by-products of the 'respiratory burst' was compromised.

Other in vitro studies suggest that the effects of trace elements on immune function are multifaceted. Thus, in addition to its effects on phagocyte function, dietary Cu deficiency has been associated with impaired antibody production by mouse splenocytes (Prohaska & Lukasewycz, 1981; Vyas & Chandra, 1983), altered distribution of spleen lymphocyte subpopulations (Lukasewycz *et al.* 1985), and decreased mitogen reactivity of the same cells (Lukasewycz & Prohaska, 1983; Lukasewycz *et al.* 1985) and of sheep peripheral blood lymphocytes (Jones *et al.* 1986*b*). Moreover, cell-mediated cytotoxicity (Flynn & Yen, 1981) and interleukin-1 production are suppressed (Flynn *et al.* 1984), T-lymphocyte proliferation impaired and the expression of specific T-cell surface markers altered (Flynn, 1984) in spleen cells cultured in Cu-free medium. Extensive studies on experimental zinc deficiency have demonstrated a similar variety of depressive effects on the function of lymphocytes from man (Chandra, 1980) and experimental animals (Gross *et al.* 1979; Fernandes *et al.* 1979; Chandra & Au, 1980; Dowd *et al.* 1986) as well as impaired neutrophil chemotaxis (Weston *et al.* 1977). As with Cu, Zn-depleted media alter numerous responses of cultured lymphocytes (Flynn & Yen, 1981; Flynn, 1984; Flynn *et al.* 1984).

A major difficulty in interpreting in vitro studies is that the results obtained can be completely dependent on the experimental conditions chosen. For example in Zn deficiency in rats, the mitogenic responses of splenic lymphocytes to concanavalin A (Con A) were impaired, but those of cells derived from the thymus were unaffected (Gross & Newberne, 1980). However, responses to a second T-lymphocyte stimulator, phytohaemagglutinin, were diminished in both preparations and differential responses of T-cell subsets were implicated. Kramer *et al.* (1986) have shown that removal of macrophages changed the pattern of mitogen responses in splenocyte populations from Cu-depleted mice, indicating that cellular interactions during in vitro tests can profoundly influence their outcome. The opportunities for such interactions are clearly multiplied considerably when the response of the whole animal to the challenge of an infection is considered.

Two observations illustrate the dangers of extrapolating from in vitro tests of immune function to disease resistance in vivo. First, strains of mice which show poor T-lymphocyte responses to mitogen stimulation in vitro are no more prone to natural infection than genetically-superior responders (I. Malavé, personal communication). Second, Esa & Reissig (1985) showed that the acquisition of immunity, as measured by survival, to mouse hepatoma virus was delayed in protein deficiency. Significantly, their parallel in vitro investigations showed that mitogen responses to Con A were enhanced, possibly due to impaired suppressor cell activity; thus in vivo and in vitro studies on the same animals gave rise to opposite assessments of the impact of a nutritional deficiency on the course of an infection.

Single nutrients and immunity: in vivo evidence

Evidence that specific nutrient deficiencies impair resistance to infection in vivo is relatively rare and again mostly limited to experiments in laboratory animals. Several decades ago Miles (1951) reported that protein-depleted mice showed poor responses in terms of neutralizing antibody production following challenge with louping-ill virus and, more recently, Bhuyan & Ramalingaswmi (1973) reported similar effects on tuberculin sensitivity. Gershwin *et al.* (1985) concluded that bacterial infections are generally aggravated when small laboratory animals are deprived of dietary protein. Turning to micronutrients, Cu deficiency in rats was associated with decreased resistance to *Salmonella typhimurium* infection, and depressed reticuloendothelial activity (Newberne *et al.* 1968). In mice, splenomegaly and thymic degeneration (Prohaska *et al.* 1983) and enhanced susceptibility to various infections including *Pasteurella haemolytica* (Jones & Suttle, 1983) and trypanosomiasis (Omole & Onawumni, 1979) are all reported consequences of Cu deficiency. Rare cases of nutritional Cu deficiency have been reported in human infants on parenteral nutrition and they were characterized by recurrent infections often leading to pulmonary sepsis (Al-Rashid & Spangler, 1971; Karpel & Peden, 1972). Zn deficiency similarly impairs disease resistance in rodents (Fernandes *et al.* 1979; Chandra & Au, 1980) and is associated with rapid and pronounced atrophy of the thymus (Chandra & Au, 1980).

The outcome of in vivo studies can also be affected by the experimental conditions chosen. Thus, the humoral responses of vitamin E- or Se-deficient chicks to sheep erythrocytes were depressed at low but not high doses of antigen and in 2-week-old, but not in 3-week-old animals (Marsh *et al.* 1981). The severity of deficiency may also influence the outcome of a nutrient × infection interaction: morbidity and mortality following challenge with bacterial pathogens are markedly increased in severely iron-deficient rats but reduced by a moderate dietary deficiency (Brock & Mainou-Fowler, 1986). Interestingly, susceptibility to infection can also be enhanced by excesses of minerals such as Fe (Murray *et al.* 1975; Kochan *et al.* 1978), Cu (Vaughn & Weinburg, 1978; Hill, 1980) and Se (Marsh *et al.* 1981). However, it should be noted that in all these examples the diets used were highly purified, and the deficiency or excess produced was often severe.

The whole-body response to microbial invasion often includes specific or non-specific inflammatory reactions, or both, and there is evidence that these can also be influenced by nutritional status. Jones (1984) reported that both acute, histamine-induced oedema and delayed hypersensitivity reactions to oxozalone and sheep erythrocytes were enhanced in Cu-depleted mice. Cu deficiency similarly enhanced acute inflammatory reactions in rats (Denko, 1979; Velo *et al.* 1982; Kishore *et al.* 1984) although the effects on chronic inflammation are more contentious (Velo *et al.* 1982; Kishore *et al.* 1984). Effects of Cu deficiency on the inflammatory response may be mediated by the two Cu-dependent enzymes, ferroxidase (*EC* 1.16.3.1) and SOD, since both exhibit anti-inflammatory activity when administered exogenously (Denko, 1979; Huber *et al.* 1977), as do a variety

of Cu complexes (Sorensen *et al.* 1983). It is interesting to note that some injectable Cu preparations used for the treatment of deficiency in grazing animals can cause local tissue reactions (Suttle, 1981), acute toxicity and anaphylactic shock: this may reflect the impaired ability of some Cu-deficient animals to cope with a cytotoxic agent, be it free Cu^{2+} ions, chelating agent or the suspending matrix.

Natural nutrient deficiences and infection

Examples of enhanced susceptibility to infection on natural diets are largely confined to young infants or animals with rare genetic defects in trace-element metabolism. Infants with Menkes' disease, an inherited defect in Cu absorption, have a history of recurrent infections particularly of the respiratory tract (Danks *et al.* 1972) which has been associated with a variety of impaired immune responses (Pedroni *et al.* 1975; Sullivan & Ochs, 1978). Similarly, children with an inherited defect of Zn absorption (acrodermatitis enterohepathica) often suffer from recurrent diarrhoea and infections (Hambidge *et al.* 1978). The inherited defect in Zn deficiency in man has its equivalent in the A46 trait in Friesian cattle: affected animals show thymic atrophy, depressed cell-mediated immunity and diarrhoea (which all respond to Zn therapy) on natural diets that provide adequate Zn for normal animals (Brummerstedt *et al.* 1974, 1977). However, recent studies showing reductions in mortality in Indonesian children given supplementary

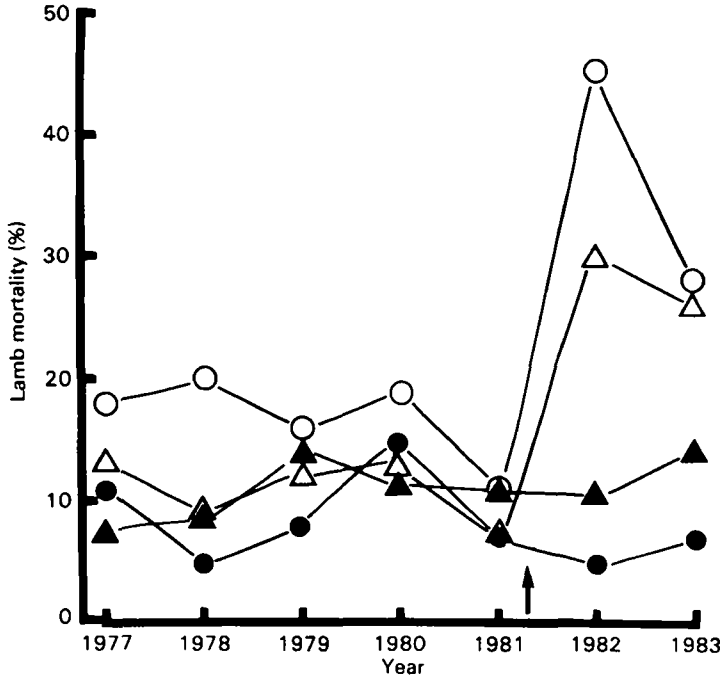

Fig. 1. The effect of pasture improvement (↑) on annual mortality (%) up to 24 weeks in lambs of four different genotypes: Scottish Blackface (○), Welsh Mountain (●), low line (△) and high line (▲). Lines were produced by selecting for low or high plasma copper within a Scottish Blackface × Welsh Mountain inbred cross (from Woolliams *et al.* 1986).

vitamin A, are believed to involve improved resistance to diarrhoea and respiratory disease (Sommer, 1986) without a component of inherited susceptibility to vitamin A deficiency. Also of relevance is the observation that Cu supplementation markedly reduces the incidence of infections in infants recovering from marasmus (Castillo-Duran *et al.* 1983).

Cu deficiency and infection in lambs

It is against this background of limited information on specific natural nutrient deficiencies influencing disease resistance that we present a summary of an experiment which shows a clear connection between Cu deficiency and disease resistance in a large flock of 400 lambs, albeit with an important genetic component (Woolliams *et al.* 1986). Early investigations had shown that two breeds of sheep reared under identical conditions on hill pasture differed in their susceptibility to Cu deficiency: the Scottish Blackface ewe often had lower plasma Cu concentrations than the Welsh Mountain ewe and, in years of high swayback incidence (swayback is an enzootic ataxia which is prevented by Cu supplementation), Welsh Mountain lambs were rarely affected whereas many Scottish Blackface lambs succumbed (Wiener & Field, 1969).

When the hill pasture was improved by liming and reseeding, the Cu status of ewes and lambs declined and there was a sudden upsurge in lamb mortality amongst Scottish Blackface but not Welsh Mountain lambs (Fig. 1). That this breed × environment interaction involved a Cu × infection relation was shown by the changes induced by genetic manipulation and Cu supplementation. For four generations ram lambs had been selected for high or low plasma Cu concentrations from within an inbred Scottish Blackface × Welsh Mountain cross to produce high

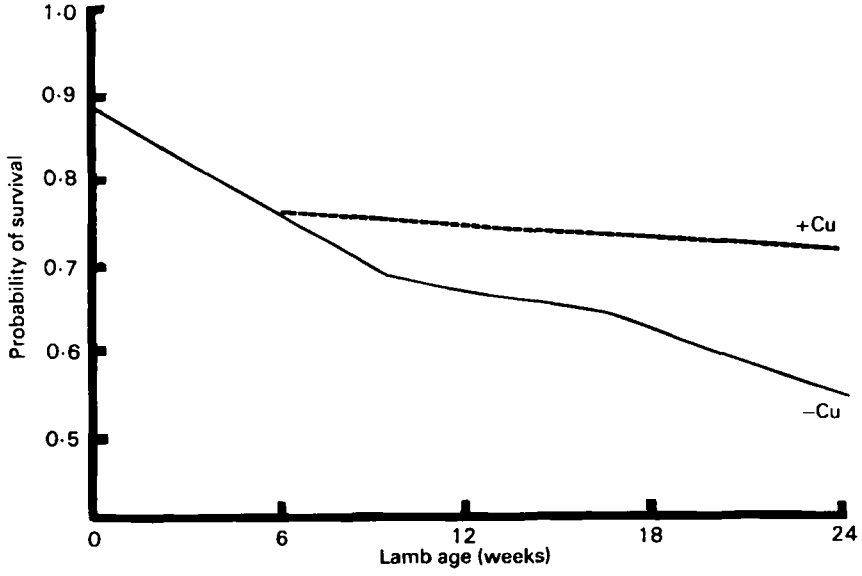

Fig. 2. Treatment of lambs, selected for low plasma copper, with an oral Cu supplement at 6 weeks of age (---) improved their probability of surviving risks other than swayback when they grazed improved pastures in 1983 (after Woolliams *et al.* 1986).

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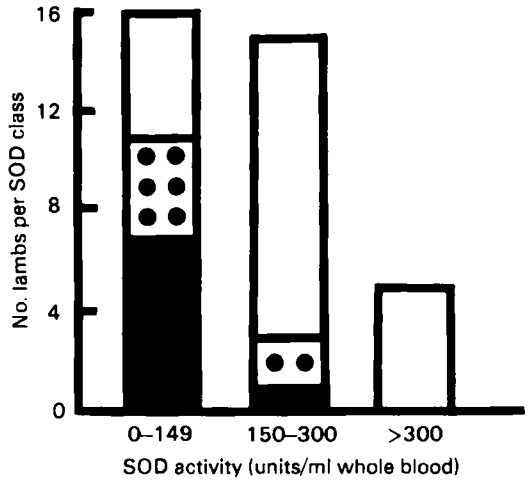

Fig. 3. Deaths due to swayback (●●) and other causes (■) in unsupplemented Scottish Blackface lambs occurred predominantly in those of lowest copper status as measured by the erythrocyte cuproenzyme, superoxide dismutase (*EC* 1.15.1.1; SOD) at 6 weeks of age. Total height of column gives the number of lambs at risk per SOD class.

and low lines differing only in their plasma Cu status. The low line, like the Scottish Blackface, showed an upsurge in mortality following pasture improvement not seen in the high line (Fig. 1). Swayback was the cause of 40% of lamb deaths in 1982 but only 2% in 1983. Post-mortem findings indicated that most other deaths in both years were associated with bacterial infections, the organisms most commonly isolated being *P. haemolytica* and *Escherichia coli*. Survival of the lambs could be enhanced by administering Cu at 6 weeks of age: the effect was clearcut even in the year when swayback was not a problem (Fig. 2), and when it was important both swayback and other causes of death (mostly infections) were reduced. Final evidence for the association between Cu deficiency and susceptibility to infection was provided by the inverse correlations within a genotype between Cu status and mortality, the most Cu-deficient individuals being those most likely to succumb to infections or swayback (Fig. 3).

Thus the increased losses of Scottish Blackface and low-line lambs following pasture improvement were largely attributable to an induced Cu deficiency enhancing susceptibility to swayback and infection, both of which should be regarded as specific consequences of Cu deficiency. Confirmation for these conclusions can be found in other, less detailed, reports (Whitelaw *et al.* 1979; Yeoman, 1983). The mechanism for reduced disease resistance is unclear but using conventional immune function tests it was found that the responses of peripheral blood lymphocytes to stimulation with mitogens, particularly pokeweed, were markedly depressed in low-line animals and that this immunosuppression was abolished by Cu supplementation (Jones *et al.* 1986*b*). Further studies of acquired immunity in the lambs are in progress. However, the possibility that early lamb

mortality in particular (Fig. 2) may have reflected an impairment in the transfer of passive immunity from ewe to lamb via the colostrum cannot be ignored.

The association between Cu deficiency and disease resistance was not confirmed in the incidence of pneumonic lesions (mean 76·0%) in lungs of eighty-eight lambs examined at the abattoir: indeed there was some indication that Cu-supplemented lambs were more prone than unsupplemented lambs (D. G. Jones, N. F. Suttle and J. A. Woolliams, unpublished results). The correction of a concurrent Se deficiency, which had been sufficiently severe to retard lamb growth (Suttle *et al.* 1984), also had no effect on the incidence of chronic pneumonia (Jones *et al.* 1986*a*). When the level of parasitic infections in the lambs was assessed in terms of faecal parasite egg counts, there were indications that they were decreased in the most Cu-deficient groups (Jones *et al.* 1986*a*) and subsequent work has shown that a challenge dose of nematode larvae are depleted of Cu more rapidly than their ovine hosts when a Cu-deficient diet is given (M. Clarke and N. F. Suttle, unpublished results). These findings indicate that the impact of a single nutrient deficiency on the incidence and outcome of an infection will depend on the nature of the infection.

Conclusions

Proven associations between specific nutrient deficiencies and increased susceptibility to infection have rarely been found under natural dietary conditions. The relation between Cu deficiency and increased mortality in lambs on improved hill pastures described here, to which infections substantially contributed and from which Cu supplementation afforded protection, therefore represents an important advance. Improved survival in Indonesian children given vitamin A supplements provides a parallel example of a specific nutrient influencing susceptibility to infection in man. Further examples of nutrition × infection interactions under natural dietary conditions would help to justify the continuing extensive studies of experimental depletion and immune defence mechanisms in vitro.

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