pigment aggregation. Aggregation of pigments in the pigment cells of the retina and the choroid would allow light to diffuse out and sensitize more photoreceptors, or permit light that passed the retina to be reflected back and sensitize the photoreceptors again.

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Sensitivity of human lymphocytes to bleomycin increases with age

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Summary. The sensitivity of human peripheral blood lymphocytes to bleomycin and mitomycin-C was assessed by measuring the inhibition of phytohemagglutinin stimulated proliferation. The sensitivity to bleomycin, and not to mitomycin-C, increased with the age.

Aplastic anaemia, a marrow disorder characterized by chronic marrow failure, shows some features which have led to the suggestion that it represents premature or induced ageing of haemopoiesis[2]. We recently observed[3] that lymphocytes from some patients with the disorder were abnormally sensitive to bleomycin and, since this agent acts by causing strand breaks in DNA[4-6], we suggested that abnormality of DNA structure or repair was involved in the disease. During the course of these studies we observed that lymphocytes from normal individuals showed an increasing sensitivity to bleomycin with age and in this paper we report these observations and discuss their possible significance for the aetiology of ageing.

Studies were performed in 89 individuals. Cord blood was obtained at the time of delivery from 16 full-term normal babies and venous blood was obtained from 32 males and 41 females between the ages of 2 and 86 years. All of the subjects studied were healthy and, except for some females on oral contraceptives, none were taking any medication. Lymphocytes were stimulated with phytohaemagglutinin (PHA) and the proliferative response was measured after 72 h by pulsing with ^{3}H-thymidine. Lymphocytes from all 89 subjects were exposed to bleomycin and lymphocytes from 63 subjects to mitomycin. Repeat studies with bleomycin were performed on a total of 44 occasions in 15 individuals.

The drug concentrations producing 50% inhibition of ^{3}H-thymidine uptake were logarithmically transformed and the relationship with age was analysed by linear regression analysis. There was a significant correlation between the sensitivity of lymphocytes to bleomycin and the age of the individual ($r = -0.54$; $p = < 0.0001$) (figure 1). The variance about the regression line was 0.33 whereas the variance of repeated estimations in 15 individuals was 0.18; these results suggest that, of the observed variation in bleomycin sensitivity unrelated to age, approximately 55% was due to within-individual experimental variation and 45% was due to true between-individual variation. There was no correlation between the sensitivity of lymphocytes to mitomycin-C and the age of the individual (figure 2) ($r = -0.17$; $p =$ not significant).

The observed increase in the sensitivity of lymphocytes to bleomycin with age could be due to a membrane phenomenon since an age-related increase in passive diffusion or active transport of the drug across the cell membrane would result in an age-related increase in sensitivity to it. Such an explanation would be of great interest for the understanding of ageing but it seems unlikely since there is little other evidence that a primary membrane alteration is involved in ageing (reviewed in Masoro[7]). Most hypotheses on the nature of ageing have stressed the importance of alteration of critical cellular macromolecules, and particularly of DNA. There is evidence to suggest that ageing is associated with an increase in DNA strand breaks[8-10] and a decrease in DNA replication[11-13]. Several observations can conceivably be interpreted as indicating a decrease in DNA-protein cross-linking with age[14-16], and Hart and Setlow reported that the extent of DNA repair in a variety of animal species was related to longevity of those species[18]. Since DNA is the primary target for bleomycin, the increased sensitivity to bleomycin observed in lymphocytes from aged individuals suggests an age-related alteration in DNA in these cells. This could be a structural alteration of DNA which by some mechanism results in production of a

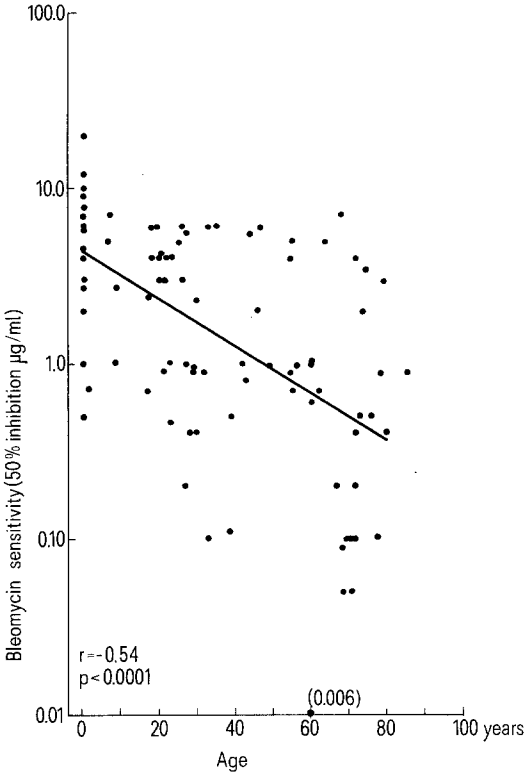

Fig. 1. Relationship between age and sensitivity to bleomycin. The lymphocytes were separated by Ficoll-Hypaque centrifugation and were washed 3 times in McCoy's modified medium 5A (Gibco, New York, USA). The lymphocytes were suspended at 1×10^6 cells/ml in McCoy's medium containing 15% heat inactivated foetal calf serum and were aliquotted into tubes which, with the exception of one control, contained phytohaemagglutinin (PHA) (Burroughs Wellcome, reagent grade) at a final concentration of 5 µl/ml. Varying amounts of bleomycin or mitomycin-C being tested were added to each tube, and 0.2 ml aliquots from each tube were then pipetted into 4 wells of a microplate. The final concentrations of bleomycin ranged from 10^{-4} µg/ml to 40 µg/ml, and those of mitomycin-C ranged from 10^{-4} µg/ml to 5×10^{-2} µg/ml. The microplates were cultured for 3 days at 37 °C in a humidified atmosphere of 5% CO_2 in air and were then pulse labelled for 4 h with ^{3}H-thymidine 1 µCi/ml (Radiochemical Centre, Amersham, England). The cells were harvested onto a glass fibre filter disc by a cell harvester ('Titretek', Skatron, A.S., Norway) and counted in a liquid scintillation counter. The mean and SE of the radioactivity of quadruplicate cultures for each drug concentration were calculated and expressed as a percentage of the control value which did not contain any drug. The results were plotted on probability paper and the concentration of the drug producing 50% inhibition of ^{3}H-thymidine uptake was estimated visually and was used as the measure of drug sensitivity.

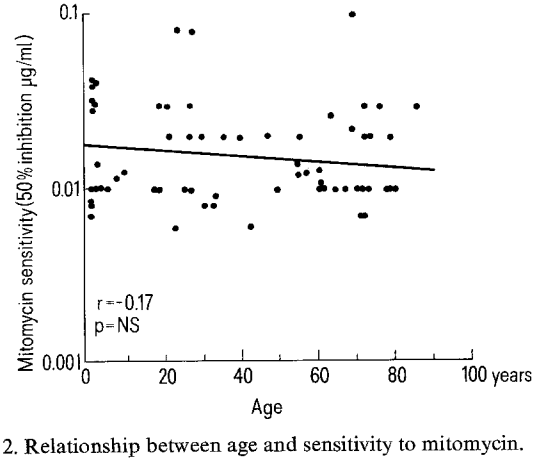

Fig. 2. Relationship between age and sensitivity to mitomycin.

greater degree of DNA damage by bleomycin. One possibility is that a structural change of DNA and/or chromatin leads to readier access of bleomycin to its site of action. The studies of Kuo and Hsu[19], indicating that bleomycin cleaves the linker DNA between nucleosomes, suggest that this site could be the linker DNA. Alternatively, impairment of a pathway repairing DNA damage produced by bleomycin would also lead to increased sensitivity to the drug. The failure to observe a relationship between age and sensitivity to mitomycin suggests that if impairment of DNA repair occurs with ageing, the impairment involves only some, rather than all, repair pathways.

Many experiments on ageing have studied cells 'senescing' as the result of prolonged maintenance and repeated division in tissue culture. The analogy between this in vitro system and in vivo cellular ageing, let alone whole body ageing, is unclear and many of the findings on senescing cells might be artefact of the experimental system. Again, other studies on ageing have employed cells obtained from individuals suffering from syndromes believed to represent premature ageing and the relevance of these syndromes to physiological ageing might be questioned[20]. By contrast, the technique used in our study involves cells which have aged physiologically in vivo and thus is not open to the above objections. Although measurement of proliferation is a nonspecific endpoint and tests a variety of intracellular processes, the technique is simple, can be used as a screening procedure, and is of value precisely because it tests a variety of functions. Bleomycin is a valuable probe since its mode of action is at least partly defined and since its effects on DNA can be studied at the molecular level. We, therefore, suggest that study of sensitivity of proliferating cells to bleomycin with subsequent molecular analysis may prove to be a valuable approach to further investigation of ageing and possibly of other disorders which may be related to abnormality of DNA.

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