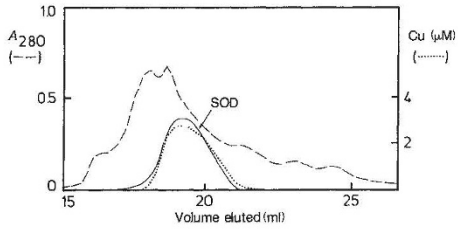
Mummified enzymes

SIR—Although it is known that many biopolymers in mummified tissues remain structurally intact, no functional evidence of an isolated protein has yet been found. The availability of a well defined air-dried mummy of a male aged 16 (±2) years, from 1200 BC[1] prompted us to search for the presence of functional Cu_2Zn_2 superoxide dismutase or its remnants. A sample



Fast protein liquid chromatography of mummified brain extract on Superose-12. The column was calibrated with BSA, horse heart cytochrome *c* and glucagon, and was operated at 0.5 ml min^{-1} at 20 °C; PBS was used as equilibration buffer. Protein elution was traced at 280 mm (A_{280}). The fractions were analysed for copper on a Perkin-Elmer Zeeman 3030 atomic absorption spectrometer. Superoxide dismutase activity was monitored using the nitro blue tetrazolium assay[2]. The reaction volume (0.5 ml) contained 0.62 mM nitro blue tetrazolium, 20 mM Tris-HCl buffer, pH 7.8, 1 mM EDTA, 0.2 % (w/v) gelatine, 50 μM xanthine, 0.18 μM xanthine oxidase and the sample. Readings were taken at 540 nm in a 10 mm light-path cell at 23 °C. SOD, relative superoxide dismutase activity.

of brain tissue taken at autopsy and stored completely dry in the presence of solid NaOH, was minced and suspended in phosphate-balanced saline buffer overnight. Using the xanthine, xanthine-oxidase-dependent nitro blue tetrazolium assay[2], we obtained unequivocal proof of a specific Cu_2Zn_2 superoxide dismutase activity in the extract; 66 per cent of the copper present in the soluble fraction could be assigned to this enzymatic activity. The presence of 1 mM EDTA did not affect this activity, but 0.1 mM cyanide completely abolished the observed inhibitory reaction. Unfortunately, the concentration of the active component was too low to be detected by nitro blue tetrazolium staining on polyacrylamide gels after electrophoresis, but fast protein liquid chromatography of the extract on Superose-12 revealed a Cu-containing polypeptide of relative molecular mass 5,000 (5kD) to which we ascribed all the activity (see figure). It is assumed that during the ageing process, considerable portions of the original 31.3kD homodimer were cleaved leaving the active core.

To the best of our knowledge, this is the first case of an enzymatically active component surviving 3,000 years of mummification. Secondary microbial infections after excavation and autopsy were excluded as a source of the superoxide dismutase activity, as no fungal or bacterial population was seen even when tissue extracts were incubated for more than 16 days. Other methods of mummification, specifically those using natron, resins, oils and especially bitumen, cause deterioration of many biopolymers. Muscle tissue samples taken from several mummies pretreated in this manner showed considerably less activity, or none at all, probably because reactions of the resin or bitumen with intact tissue proteins, converted them into catalytically inactive polymers.

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Screening for cystic fibrosis

SIR—P.N. Goodfellow, commenting on the probable identification of both the cystic fibrosis (CF) locus and the most common of the recessive alleles leading to the clinical condition, advocated an immediate start to carrier screening[1]. Before prenatal screening can be implemented, I suggest that several issues have to be considered.

The defined mutant allele seems to account for about two-thirds of CF chromosomes, and hence is found in the homozygous state in about four-ninths of affected individuals[2]; these proportions may well vary in different populations. In the UK and North American populations, the frequency of affected individuals is around 1 in 2,000 so that about one affected pregnancy in every 4,500 of the screened population could be detected. The most likely strategy would be to screen one partner in each pregnancy; if he or she is a carrier, the second partner would be screened; and if both partners are carriers, prenatal diagnosis would be offered, with a view to termination of affected pregnancies.

In round terms, for first pregnancies the risk of CF would currently progress from the initial 1 in 2,000 (of which 1 in 4,500 are detectable), through 1 in 90 (1 in 140 detectable) for those screened and found heterozygous, to 1 in 4 where both partners are heterozygous (1 in 1,100 screened couples), with a residual risk of 1 in 250 where only one partner is finally found to be heterozygous for the known mutation (1 in 35 of screened couples). (The risks of not detecting an affected child are higher if whole families, rather than just the first child, are considered.) These figures will obviously be improved as further mutations are identified.

To enable this progression, informed consent to testing would be required from both partners; if screening were carried out on the first partner but consent was refused by the second, a positive test would raise the risk to a level that might produce anxiety but would not necessarily justify prenatal sampling procedures. False-negative tests (unexpected affected children), as well as unnecessary prenatal sampling, would result from non-paternity of putative fathers; the frequency of non-paternity is variable, but can be 10 per cent or higher. The timing of the tests is difficult if screening is initiated at the first hospital ante-natal clinic (around 9–14 weeks gestation in Britain), because little time is left for obtaining the second and third samples if a reasonably early termination of pregnancy is to be offered. Earlier sampling, which implies the involvement of general practitioners, would be preferable.

Prospective parents would need education and counselling to give informed consent. Education should also include the relevant medical and para-medical specialities so that accurate and timely information could be provided. Community health workers would be needed to ensure appropriate timing, the co-ordination of tests with availability of facilities and continuity of counselling. High-risk couples should be referred to clinical genetics services experienced in ensuring that appropriate counselling and social support are available; and the outcome of the programme should be regularly reviewed.

Programmes of community health education and action have been instituted for ethnic groups with high incidences of haemoglobinopathies. Coping with genetic disease in this way would be a new and major undertaking for most of the European and North American population, and the uptake is hard to predict; but screening could not be justified without this kind of integrated approach.

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