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Thermoluminescence of Biological Materials

THERMOLUMINESCENCE (TL) of fossil bones and of various kinds of recent biological material has been reported by Jasińska and Niewiadomski[1], who suggest that such materials could be used for dating purposes, but draw attention to difficulties which arise due to tribothermoluminescence (TTL, which is thermoluminescence derived from the mechanical energy of grinding) and to chemiluminescence (CL) associated with residual organic material.

A very satisfactory spatially uniform TL of artificially irradiated modern bone was observed in this department in 1966 and samples of fossil teeth and bones from Vérteszöllös in Hungary were supplied by the late Dr Vertes in 1967 from one of the earliest camp sites of *Homo sapiens* in Europe, perhaps 50,000 yr old.

For the past 2 yr we have been working on the elimination of the two problems independently discovered by Jasińska and Niewiadomski and, although we have not yet eliminated entirely the irrelevant sources of light, we have reduced them sufficiently to make an approximate dating of the older and therefore more heavily irradiated materials. Much of the CL is due to simple oxidation and can be avoided by the standard procedure of heating the material in nitrogen during readout. Most of that remaining can be eliminated as follows. The bone, dental enamel or other material is first crushed to a coarse powder. This is then extracted for a few hours in a series of changes of 80% aqueous ethylene diamine in a Soxhlet extractor[2] which removes more than 99% of the organic matter and reduces the CL to a level below that of the TTL. The TTL is reduced by crushing the material to a powder suitable for measurement after the organic extraction. The material is then much more friable and needs less expenditure of energy. Because it is derived from friction, TTL is a surface effect and is further reduced by rejection of the finer powder which, having a large surface to volume ratio, gives a greater ratio of TTL to TL.

Fig. 1 shows the effects of CL, TTL and recovery on the shape of the "glow curves" from fresh bone powder. Curve N

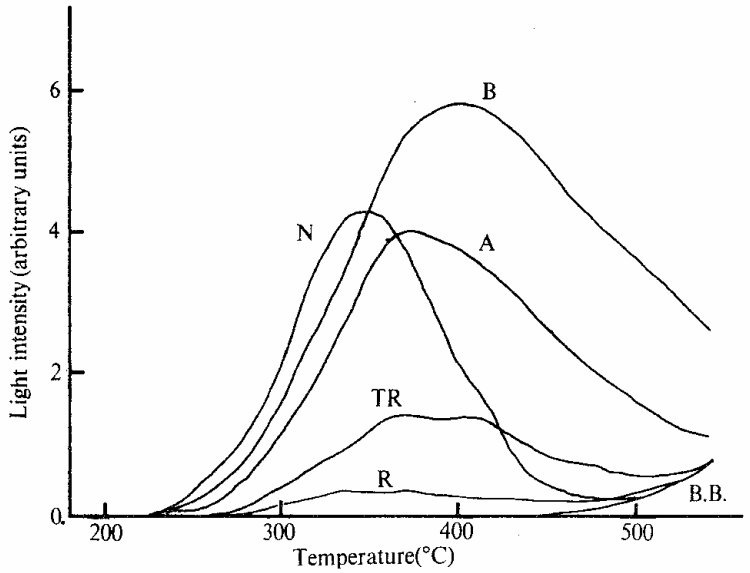

Fig. 1 Glow curves from differently treated samples of unirradiated fresh bone from the same source.

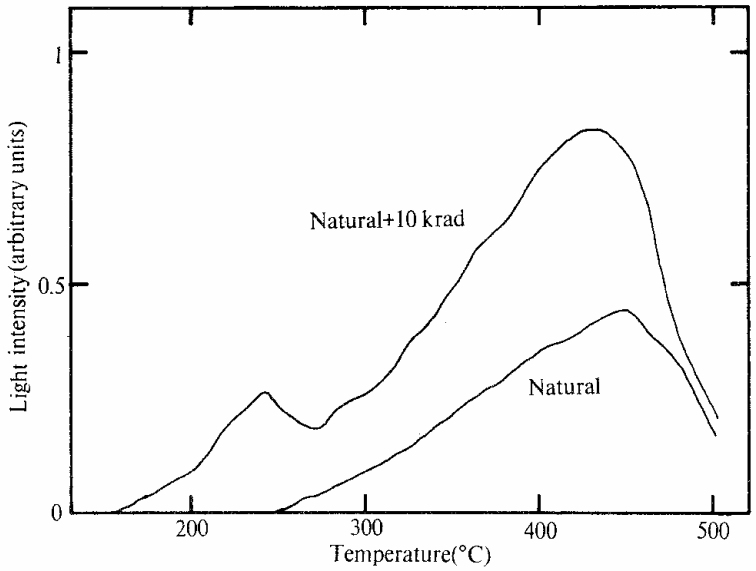

Fig. 2 Natural and artificial glow curves of an iron age bone cleaned by ethylene diamine for 17 h.

is the glow curve of untreated powder with grain diameters between 150 and 355 μm. Above 300° C the powder turns from white to black. Curve A shows the glow from bone powder of the same grain sizes but cleaned with ethylene diamine for 18 h. A decrease in CL is seen below 350° C. An apparent increase is seen above this temperature but, during the heating of untreated powder (N), the resulting blackening of the grains makes light collection very inefficient, which is why the light intensity drops above 350° C. TTL is seen by comparing curve A with B which was obtained from the same

cleaned powder but had grain diameters below 45 μm. Powder already heated for curve A was recrushed and reheated, resulting in glow curve TR which demonstrates further the importance of TTL. This powder was then left on the heating strip for 30 min. On reheating, glow curve R was obtained. This recovery of TL remains unexplained, but it is possible that the contraction of the powder after the last heating causes the observed TL by a mechanism similar to that responsible for TTL. The amount of black body radiation is shown (curve B.B.).

The combined effects of residual CL and TTL are equivalent to a radiation dose of the order of 10 krad which is still too great for good results to be obtained in the region of 10–100 thousand yr. For example, Fig. 2 shows the "glow curves" of an iron age bone after it was cleaned by ethylene diamine. The glow area between 300 and 460° C varies with dose as shown in Fig. 3. By extrapolation, the natural radiation dose absorbed by the bone since the iron age is found to be approximately 15 krad, which would indicate an age some thousands of years higher than expected. Useful measurements could be made

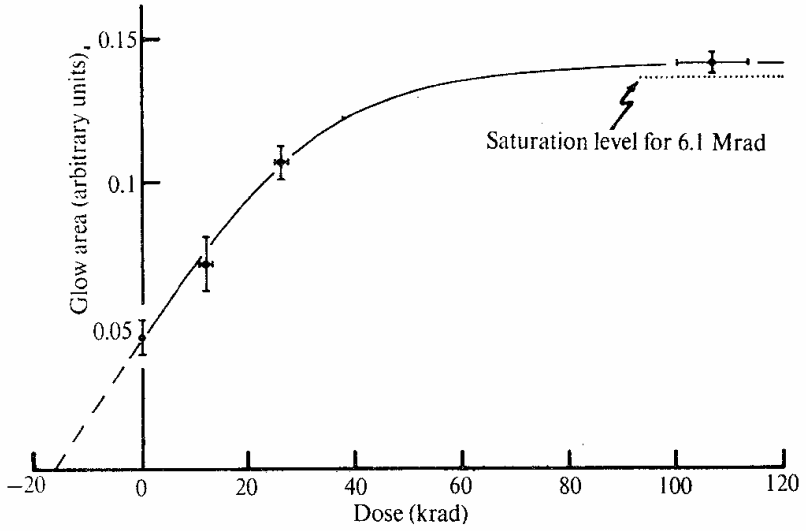

Fig. 3 Variation with γ-dose of the glow area between 300 and 460° C for the iron age bone of Fig. 2.

between 100,000 and a few million years. Above ten million years or so, saturation effects will progressively reduce the attainable accuracy.

Further research on the elimination of CL has concentrated on the use of enzymes such as collagenase and pancreatic enzymes. Limited success has been achieved, with reductions of CL by up to 50%, and more systematic work is in progress for the determination of the optimum temperature, *p*H and duration of the extraction.

Several problems remain other than the further reduction of these irrelevant glows. These include determination of the relative contributions to the total natural irradiation by (*a*) the internal radioactivity of the samples themselves (usually small),

(*b*) the surrounding material in which they have lain, and (*c*) cosmic rays. This may be laborious but is not difficult. Also needed is an estimation of the extent to which chemical change or accretion of thermoluminescent material (for example, calcium carbonate) during storage may affect the results. This estimation may vary from very easy to quite impossible and will involve careful selection of material. The internal enamel of the complex molar teeth of herbivores and the shells of some molluscs seem to be the most promising of the materials which we have tried so far. The removal of organic matter was found to be unnecessary for dental enamel from a 2–3 million year old rhinoceros tooth and samples of this nature seem to present little difficulty to TL dating workers[3].

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[1] Jasińska, M., and Niewiadomski, T., *Nature*, **227**, 1159 (1970).
[2] Williams, J. B., and Irvine, J. W., jun., *Science*, **119**, 771 (1954).
[3] McDougall, D. J. (ed.), *Thermoluminescence of Geological Materials* (Academic Press, New York, 1968).

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NEWS AND VIEWS

New Baskets for Old

UNICELLULAR organisms reflect the extremes to which cellular morphogenesis can be stretched, as any protozoologist will testify. However esoteric their appearance they also retain the ability for clonal growth—to generate repeatedly two "finished" cells from one. They, and indeed most cells, contain organelles made up of microtubules—arrays of globular protein subunits which form hollow tubes of diameters around 250 Å. Protozoa should therefore spell out the range of possible structures which can be formed from microtubules and yet be reduplicated in a spatially precise manner at each cell division. The control of such a system presents intriguing problems. On page 387 of this issue of *Nature* J. B. Tucker of the University of St Andrews, Scotland, raises questions about the spatial discrimination that must underlie the formation of microtubular components in the ciliated protozoan *Nassula*.

The largest organelle in *Nassula* is the feeding apparatus the pharyngeal basket), a palisade of rods made up from longitudinal microtubules and attached to the cell surface. At division the old basket detaches from the surface and two new ones appear. Each new rod begins its assembly close to a basal body, another and more ubiquitous microtubular structure. The rods first appear in short rows; they align and detach from the basal bodies and then roll up to constitute the circular palisade. The completed baskets make microtubular connexions to other surface basal bodies. The two new baskets do not always include all the new rods, and these "errant" rods, after moving away, break down as do the old rods, while the integrated new rods elongate by addition of microtubular protein to their tips. How can rods in one area of the cell add subunits while apparently identical rods elsewhere in the same cytoplasm are being dismantled?

Like flagella elongation in *Chlamydomonas* the synthesis and assembly of rod microtubules are separable events. Cycloheximide inhibits synthesis and colchicine blocks assembly. Errant rods do not move away from the new baskets in the presence of vinblastine, nor are they resorbed, although the old rods continue to break down. Tucker erects two alternative hypotheses to explain this effect. Further research relevant to this study will be the resolution of the number of different types of microtubule subunit involved, and whether the microtubular assemblies appearing at different times and in different places are composed of identical subunits, and if so whether these share a common subunit pool, and are recycled when the structures are broken down.

The questions that Tucker raises—how the subunits are localized at the points of assembly and whether random diffusion of subunits synthesized throughout the cell accounts sufficiently for the observations—are two among several problems. They include the nature of the information system specifying the location of "nucleation" centres for organelles, the number of such centres and the regulation of the dimensions of the realized structures, and the self-assembly properties of the subunits. Tucker has previously observed in living cells that rod length fluctuates with feeding and starvation and that when the number of developing tubules is reduced they reach greater than normal length; both these facts are consistent with the available subunits determining the length. Dingle can upset the control of flagella number in *Naegleria* by heat shock at the moment of amoeboid-flagellate transformation (*J. Cell Sci.*, **7**, 463; 1970). The attraction of this system is that high developmental synchrony can be obtained in populations of these amoebae. Tilney (*Devel. Biol.*, **2** (Suppl.), 63; 1968) and Roth (*J. Ultrastr. Res.*, **30**, 7; 1970) have suggested that the pattern of microtubules in the axopod of the heliozoan *Echinosphaerium* is a function of cross-linkages of fixed length and orientation between tubules rather than of an "orientation centre". Heat-shock disruption of rod tubules in *Nassula* and the subsequent abnormal arrangements of tubules argue in favour of the initiating sites as the determinants of the final pattern.

The solution of these problems promises to be of interest to all cell biologists and it may in addition shed light on the phenomenon of "cortical inheritance" in ciliates—alternative patterns of the same microtubular structures existing stably over hundreds of cell divisions in the absence of gene differences. Finally, it would be a glaring omission not to point out the close affinity in interest and attack between this field and that of phage morphogenesis (Levine, *Ann. Rev. Gen.*, **3**, 323; 1969).

Filling the Dating Gap

THE dating of the earlier part of the Pleistocene period still presents considerable problems but further exploitation of the various "radioactive clocks" should eventually overcome these difficulties. It is particularly encouraging that thermoluminescence dating may soon be extended to date bones and teeth from archaelogical deposits (Christodoulides and Fremlin, *Nature*, **232**, 257; 1971). The use of this method is well established for the dating of baked clay obects, and its potential for solving questions of authenticity has been illustrated in the past week by Martin Aitken and his colleagues at the University of Oxford who have demonstrated clearly that some "Etruscan" wall paintings on terracotta in European and American museums are at the most 12 years old (Aitken *et al.*, *Archaeometry*, **13**, 89; 971; Fleming *et al.*, *ibid.*, 143).

The promise of the thermoluminescence dating method of Christodoulides and Fremlin is that it will span the gap which still separates the time range of radiocarbon dating and the clocks of longer half-life. At the early end of the human time scale, potassium-argon dating has been useful in establishing a chronology for artefacts or hominid remains. The youngest dates obtainable from the uranium-lead and rubidium-strontium methods are about 10 million years, so that these isotopes, which are useful for longer geological time spans are not helpful here. The half-life of ^{40}K is 1.03×10^9 yr, however and mass spectrometry allows the determination of one of its decay

products, the gas ^{40}Ar, in low concentrations. It is more difficult to date more recent ages than older ones because of the small quantities of argon involved, and the lower limit of the method is at present half a million years or a little less.

At the other end of the scale is radiocarbon dating. Radiocarbon dates now provide the essential basis for prehistoric chronology from Upper Palaeolithic times on and, until recently, radiocarbon seemed the most reliable of radioactive clocks. For a radioactivity-based age determination, it is usually necessary, of course, both to measure the quantity of the decay product present and the quantity left of the parent isotope. Radiocarbon dating was able to dispense with the former, because the original concentration of the parent isotope, ^{14}C, was assumed to be the same as the modern atmospheric concentration. The discovery that the atmospheric concentration of ^{14}C has fluctuated in recent millennia by up to 8 per cent of the present level has come therefore as something of a shock. The radiocarbon determination of dendrochronologically dated samples is now allowing the investigation of this effect back to 6000 BC, but its magnitude before this time remains uncertain.

The half-life of radiocarbon, approximately 5.7×10^3 yr, sets a more fundamental limitation on the age of samples which can be dated. The short half-life means that after a few tens of millennia there is very little radioactivity left in the specimen, and the counting of disintegrations in the sample is always complicated by a background activity caused by cosmic radiation which cannot be eliminated. Even the isotopic enrichment of gas samples only increases the effective time range of the method to a maximum of 70,000 yr.

Between the potassium-argon and radiocarbon dating methods lies a gap from 50,000 to 500,000 yr where other methods are required. Fission track dating is a possibility, especially when applied to volcanic glass. But like the potassium-argon method it gives the date of formation of the rocks under examination so that for more recent times, where the archaeological remains are not likely to be interstratified with eruptive deposits, this is a serious limitation. It is over this time range that thermoluminescence dating would be most useful.

Thermoluminescence dating depends on the principle that many minerals give off light when heated—the light emitted being proportional to the radiation dose experienced since the formation of the material. Archaeologically it has so far been principally applied to pottery and baked clay and the dating involves determining the radiogenic minerals in the clay as well as the thermoluminescence itself. The necessity of determining very small quantities of decay product (as in the potassium-argon method) or of the parent isotope (as in the radiocarbon method) is thus avoided. An early problem was how to eliminate light during the readout on heating, which derived from the energy used in pulverizing the sample. Fortunately, heating in an atmosphere of nitrogen considerably reduces this problem. Further improvement of this method came from the separation of different grain sizes in the pottery and an accuracy of less than 10 per cent is claimed for fine grain dating (Zimmerman, *Archaeometry*, **13**, 29; 1971).

Until recently the chief application of thermoluminescence dating has been in testing the authenticity of museum objects. The magnitude of the variations in atmospheric concentration of ^{14}C, suggested by tree-ring work, indicated a further useful line of approach, and the first TL results of Zimmerman and Huxtable on early neolithic pottery from the Balkans (*Antiquity*, **44**, 304; 1970) lend some support to the long chronology indicated by tree-ring work. Pottery was unfortunately not manufactured before about 7000 BC, but the same authors have dated baked clay figurines from the Gravettian site of Dolni Vestonice to 33,000 BP (*Archaeometry*, **13**, 57; 1971). The thermoluminescence age is 15 per cent higher than the radiocarbon age, with a probable error of 10 per cent. The method thus offers a useful check on radiocarbon dates.

The materials most readily available from Palaeolithic deposits are flint and bone, and it is these which make the work reported by Christodoulides and Fremlin, and the work which is currently in progress on thermoluminescence dating of flint, so promising. Once again the chief problem is luminescence arising independently of the radiogenic thermoluminescence which is to be measured. Christodoulides and Fremlin have reduced this effect to the extent that useful measurements can now be made on samples older than 100,000 yr.

If further work succeeds in reducing this background thermoluminescence by another order of magnitude, the method may yet yield useful dates in, and beyond, the 8,000 to 50,000 yr range at present covered by radiocarbon dating, but not yet checked by dendrochronology.

Mechanism of Polarity in Dispute

THE mechanism of genetic polarity has of late become the centre of one of those controversies which occasionally enliven the literature and the coffee room. One camp holds that nonsense codons, by stopping the movement of ribosomes translating nascent messenger RNA molecules, prevent further transcription because transcription is coupled to translation. Members of the other camp argue, however, that nascent messenger RNA chains continue to be elongated after a nonsense codon at some early position has prevented ribosomes translating the messenger. They claim that because the newly made stretches of messenger are devoid of ribosomes they are rendered susceptible to attack by nucleases and are degraded.

In next Wednesday's *Nature New Biology* there are two articles which present conflicting data to succour members of both camps. Imamoto and Kano, who favour the first hypothesis, have studied the synthesis of *tryp* operon mRNA in a strain of *Escherichia coli* with a temperature sensitive mutation in the structural gene for one of the 30S ribosome proteins, P10, which must be present for ribosomes to initiate translation of natural messengers. They present evidence which suggests that the overall rate of transcription of the *tryp* operon in this strain parallels the rate of protein synthesis. At the nonpermissive [illegible] synthesis is impaired and correlated with this synthesis of *tryp* messenger RNA is arrested.

By contrast, a few weeks ago Morse and his colleagues reported in *Nature New Biology* (**231**, 214; 1971) experiments which suggest that messengers devoid of ribosomes are degraded by a previously undetected endonuclease. Morse and Guertin now report new experiments on the effect of starving *E. coli* of amino-acids on the polarity of polar nonsense mutations in the *tryp* operon. Polarity is relieved when stringent cells carrying such mutations are starved; in other words, the *tryp* mRNA distal to the polar nonsense mutation, which is rapidly degraded in the presence of an adequate supply of amino-acids is stabilized when amino-acids are in short supply. By contrast, when relaxed strains are similarly starved there is no relief of polarity; indeed polarity is enhanced.

Morse and Guertin suggest that because starvation of stringent cells results in the cessation of ribosomal RNA synthesis as well as protein synthesis nucleotides must accumulate, and this might well result, by some feedback control mechanism, in the inactivation of nucleases. Messengers although exposed might therefore survive. By contrast, when relaxed cells are starved ribosomal RNA synthesis continues even though protein synthe-