RNA were examined by a procedure of labelling with 0·4 μc./ml. $^3$H-cytidine for 4 h starting 3 h after irradiation. These intervals were selected to measure the dependence of cytoplasmic labelling on nuclear and nucleolar inhibition during the stable stage following the first recovery period. In 15 cells with irradiated nucleoli, the mean of the percentage control activities of the extranucleolar areas of the nuclei was 79·9 ± 4·4 per cent, while that of the cytoplasma was 57 ± 3·5 per cent. There was no significant inhibition in the cytidine uptake, however, either in the cytoplasma of cells, the nuclei of which had been irradiated, or in the nuclei of the cytoplasm irradiated cells a few hours after the irradiation.

Effects of the irradiation on the labelling of the nucleus with thymidine were examined in the cells where either an extranucleolar nuclear area, or a cytoplasmic area, or all nucleoli in a cell, were irradiated with a total dose of 25 sec in different cells in the same fields. The results are summarized in Fig. 2. The irradiated cells without grains were excluded from the data. For all nuclear sites the irradiation caused an inhibition in the rate of DNA synthesis, which developed progressively for several hours after the irradiation. There appears to be no significant difference between the effects of the nucleolar and extranucleolar nuclear irradiations. These results are in good agreement with those obtained by Dendy and Smith[12] in their comparative study on the effects of ultra-violet and α-particle microbeams on the inhibition of DNA synthesis in mouse fibroblasts. Our results for cytoplasmic irradiation differ from theirs, however, because their rate of the reduction of DNA synthesis was smaller. This might be caused by differences in the experimental conditions or the cell species used.

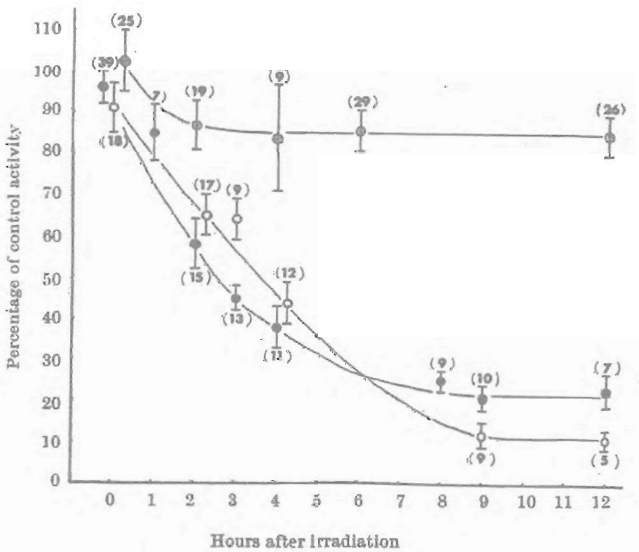


Fig. 2. Effect of irradiation on $^3$H-thymidine labelling. ○, Nucleolar irradiation; ●, extranucleolar nuclear irradiation; ⊙, cytoplasmic irradiation. Figures in parentheses show number of experimental cells.

In experiments on protein synthesis with phenylalanine incorporation, we found at most only a slight degree of inhibition for all irradiation sites, including nucleolar irradiation.

These results show that ultra-violet microbeam irradiation at one nucleolar or extranucleolar nuclear site rapidly caused an inhibition of RNA synthesis which is partly restored a short time after the irradiation. The inhibition of DNA synthesis, however, develops more slowly and is not restored within the observational period of 12 h. The experiment also shows that protein synthesis is resistant to irradiation of any cellular area at the dose used.

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## Evidence for Direct Physical Bonding between the Collagen Fibres and Apatite Crystals in Bone

The exact relationship between the mineral crystals and the collagen fibres in bone has not been determined. While the present consensus is that the collagen fibres initiate mineralization[1,2], and that the initial crystallites have a specific spatial relationship to the collagen fibres[3], evidence of the exact physical relationship between the fibres and crystals is lacking. This question pertains chiefly to the initial crystallization phase, because subsequent secondary mineralization is believed to occur on the basis of intercrystalline bonding.

In a previous communication we reported the detection of electron paramagnetic resonance signals from whole human bone as well as extracted collagen and apatite[4]. Apatite, prepared by refluxing with ethylene diamine[5] and washing in distilled water, gave a complex signal consisting of a $g$-2 resonance and several lower field resonances. The $g$-2 resonance was ascribed to an $F$ centre and the remaining resonances to various types of structured water. Because the whole bone signal consisted of a singlet at $g$-2·001 with a line width of 10 gauss, the relationship between the complex resonance of extracted apatite and that of apatite *in situ* was obscure. In an attempt to clarify this, evidence was obtained which indicated the existence of a direct physical bond between the apatite crystals of the initial mineralization phase and the collagen fibres.

Our initial hypothesis was that the complex apatite signal was largely artefactual, derived from absorption of water molecules in the washing process. Accordingly, we examined apatite after refluxing in ethylene diamine and vacuum drying but without washing in water. A weak $g$-2 singlet of 10 gauss width was observed (Fig. 1*a*). This signal was found to follow a simple Curie dependence between 114° K and 295° K. Subsequent washing of this material for 24 h in methanol or ethanol did not alter the signal. Similar washing in distilled water, however, resulted in the appearance of the previously reported complex apatite signal (Fig. 1*b*). The magnitude of the total resonance was directly proportional to the length of washing time up to 24 h; however, line width and shape remained constant. Substitution of mammalian Ringer solution for the washing medium resulted in a much distorted $g$-2 resonance with marked broadening (Fig. 1*c*).

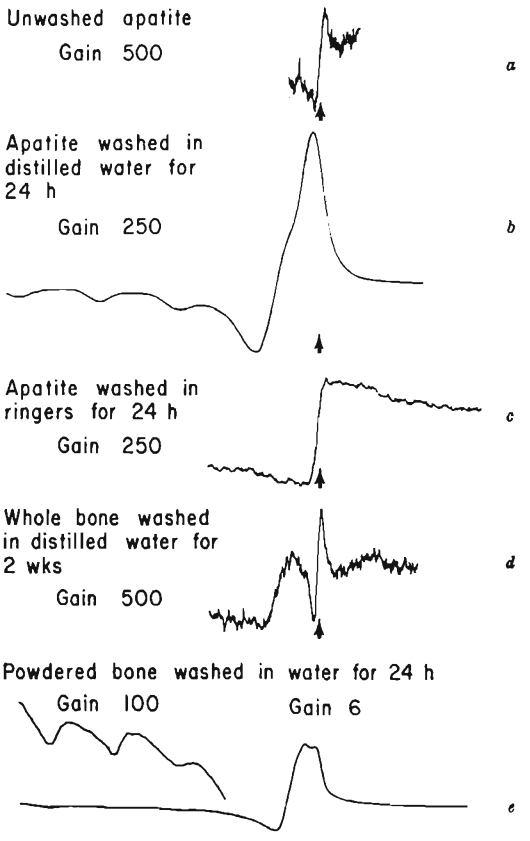


Fig. 1. Electron paramagnetic resonances of extracted apatite and bone. Instrument gain is as noted and the arrow denotes $g$-2·000. The broad resonance, just to the low field side of $g$-2 in the water washed whole bone spectrum, is probably the result of water absorption by the collagen fibres, not by apatite. In the water washed, powdered whole bone spectrum (lowermost in illustration), the three, equally spaced low field resonances are shown at a gain of 100 while the whole trace is at gain 6. The higher gain tracing is sweep synchronized with the whole trace.

These results indicated that apatite, prepared by removal of the matrix collagen fibre, was capable of taking up a variety of inorganic ions from solution. The method of preparation used dissolves all the organic matrix leaving the mineral crystals undisturbed. In this fashion free surfaces are created at the original sites of attachment between the crystals of the initial mineralization phase and the organic matrix. Because collagen constitutes more than 95 per cent of the organic fraction of bone, it is reasonable to expect that the majority of the corresponding binding sites are located on the collagen fibres. The intercrystalline bonds of the secondary phase of mineralization are assumed to be unbroken. It therefore appeared most likely that the ions removed from solution by apatite prepared in this fashion were bonding to sites of previous attachment to the collagen fibres. If this attachment was a direct physical bond, then whole bone subjected to prolonged washing in distilled water should not demonstrate the complex signal. Powdered whole bone (in which a significant number of free surfaces are produced by disruption of the intercrystal bonds of the secondary calcification phases), however, should demonstrate the complex signal. This was found to be the case; whole bone washed for 2 weeks in distilled water displayed an unchanged $g$-2 resonance (Fig. 1*d*) while powdered whole bone similarly washed for 24 h revealed a complex signal very similar to that of apatite (Fig. 1*e*). The broad low field resonance in Fig. 1*d* is probably a result of absorption of water directly on to collagen fibres[4].

We conclude, therefore, that the experiments described can be interpreted as supporting the thesis that a direct physical bond exists between the initial apatite crystallites and the collagen fibres. The constancy of the $g$-2 singlet from apatite under all experimental conditions would seem to indicate its presence in apatite *in situ* as well. In this case, it would contribute to the $g$-2 resonance displayed by whole bone.

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## Thermo-voltaic Radiation Dosimetry

The most familiar process of solid state radiation dosimetry makes use of trapping processes in phosphorescent materials by monitoring thermoluminescent glow curves as a function of dosage received[1]. In previous papers[2–4] it has been shown that this process can be advantageously modified—at least in principle—in a number of ways. Its sensitivity, for example, can be increased by the application of high electric fields which accelerate thermally released electrons and thereby lead to impact ionization. Alternatively, it is possible to use "current-glow" or photo-stimulated currents[3,4] for dosimetric purposes. Because of the absence of light measurements, this leads to a considerable simplification of technique.

It has now been found that thermo-electric properties can be used for dosimetric purposes in a similar way. The principle is simple, though in practice a number of complications are observed. One would expect the incoming radiation to charge electron traps in proportion to the dosage received. Subsequent heating of the specimen in the presence of a thermal gradient should then yield a temporary thermopower which is lower than that associated with unirradiated specimens because of the release of free carriers from the traps. Once these carriers have recombined (whether thermally or radiatively), the original value should be restored.

In the exploratory work reported here, specimens of zinc sulphide in the form of a single crystal clamped between metal electrodes have been irradiated with ultra-violet light. One electrode is rapidly heated to produce a temperature gradient across the sample. In due course the temperature of the second electrode likewise increases, as a result of heat conduction through the crystal. Under final steady state conditions, the temperature difference is again small. The average temperature gradient thus passes through a maximum with time, and so does the corresponding thermo-voltage. Fig. 1 shows a set of results for three different dosages of ultra-violet. Irradiation by X-rays gives quite similar results. After each run, the specimen must be returned to its equilibrium condition. This is done by maintaining it at 400° C for 1–2 h. This procedure ensures repeatable results.

The process is essentially thermo-electric in character, but the conditions are complicated by at least two (probably related) factors: space charge regions arise