Autofluorescence of bone tissues

A. I. D. PRENTICE

From Notley Hospital, Braintree, Essex

SYNOPSIS The phenomenon of autofluorescence of bone is due primarily to the collagen itself rather than to incidental substances adsorbed on to it. Fluorescent microscopy is a convenient method of dating the different microanatomical components of bone, and in this respect the same conclusions can be reached from the study of microradiographs or autofluorescent photographs, which is proof of the intimate relation between the development of matrix and the progression of mineralization.

It has been known for many years that collagen, in common with many other biological tissues, shows autofluorescence when irradiated by ultraviolet light.

The colour when viewed with a green-yellow ultraviolet stopping eyepiece filter, is a steely blue with a greenish component.

During the course of examination of bone sections for tetracycline marks (Prentice, 1965) it was seen that there were variations in the autofluorescence of the bone itself, and it is the extension of these observations which is the subject of this paper.

METHODS

The microscope arrangement consists of a 200 amp mercury vapour light source and an exciter filter with a main peak at 360 mμ. A red component does not interfere if a low-power dark ground condenser is used. The eyepiece filter transmits from 450 mμ upwards.

Photographs were taken with Agfa colour film, exposures varying between four and eight minutes.

The specimens were prepared by the method previously described by Jowsey (1955) from femur shaft bone obtained at routine necropsy. Sometimes the bone was cut unfixed and sometimes after short fixation in alcohol or formalin, but prolonged fixation was avoided because of the possibility of altering the autofluorescence. When dealing with cortical bone embedding in methacrylate is not necessary.

RESULTS

The bone sections show that not all the osteons autofluoresce to the same extent. From examinations of the relative positions of the poorly fluorescent osteons and from the study of bone previously marked by tetracycline it can be seen that the oldest bone components fluoresce most and the newest least (Fig. 1). Removal of the mineral from the section does not affect the autofluorescence (Fig. 2).

It was thought possible that the differences in fluorescence might be due to some substance that was adsorbed on to the bone, but immersing the sections in weak acids, alkalis, or organic solvents had no effect. Nor did the application of heat to the point of destruction. It seems likely, therefore, that the differences are due to factors inherent in the bone collagen itself.

Recent observations of Armstrong and Horsley (1966), who have isolated material from alkaline hydrolysates of bone with the same fluorescent characteristics as collagen, support this conclusion.

Observations which have been made from examining microradiographs, such as those of Amprino (1952) and Sissons, Jowsey, and Stewart (1960) and Jowsey (1960), indicate that initial mineralization of a new osteon reaches about 75% quite rapidly, but further mineralization is a slow process taking months or years. Bone from children contains a high proportion of osteons which are incompletely mineralized. In the aged there is an accumulation of bone with high mineral content in which are scattered new osteons. The contrast between the different bone components is therefore very great.

All these changes can equally well be observed in autofluorescent photographs. Microradiographs compared with autofluorescent photographs show a close correlation (Figs. 3a and 3b). Bone from young individuals shows generally poor autofluorescence. Indeed, where for any reason the turnover of bone is rapid the number of new osteons is increased and the general autofluorescence is reduced. Such a picture is seen in hyperparathyroid disease.
FIG. 1. There is a variation in the amount of fluorescence from different osteons. The 'background bone', which is the oldest, fluoresces most. A tetracycline ring (T) surrounds one osteon which indicates that this particular osteon started to form 810 days before death.

FIG. 2. Removal of the mineral from a bone section does not affect the fluorescence (decalcified section).

FIG. 3a

FIG. 3b

FIGS. 3a and 3b. An autofluorescent photograph and a microradiograph of the same piece of bone indicate that the degree of autofluorescence and the degree of mineralization are closely related. The tetracycline ring (T) in Fig. 3a indicates that the osteon was forming 150 days before death.
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In bone from the aged there is an accumulation of highly fluorescent bone, particularly poorly fluorescent osteones scattered about. Osteoid can be distinguished by autofluorescence from recently calcified bone even after the decalcification of the tissue, which must indicate an irreversible alteration of bone matrix on mineralization.

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