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An Economical Microfluorescence Set-up For Detection Of Tetracyclines In Bone

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AN ECONOMICAL MICROFLUORESCENCE SET-UP FOR DETECTION OF TETRACYCLINES IN BONE

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ABSTRACT

Wratten 47 and 47b filters between microscope and light source and Wr. 8 and 9 between eye and eyepiece provide a simple, effective means of detecting tetracycline fluorescence which is economical.

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The observation of Milch, Rall and Tobie[5,6] that tetracyclines become fixed in mineralizing bone and cartilage and can be demonstrated by fluorescence microscopy has opened the door for extensive in vivo investigation of bone physiology in man. Work with tetracyclines in this laboratory was stimulated by their papers. The cost of their fluorescent equipment was an obstacle however. A fluorescent set-up used by the writer since 1953 was adapted to the present problem and has the advantages of simplicity and economy. Originality is not claimed since the technique is in sporadic use in this country although unpublished.

MATERIAL

Any tissue containing tetracycline may be examined with the following technique. Undecalcified bone sections are best made by the writer's technique.[2] Soft tissues must be cut frozen. Acid pH must be avoided. Prolonged exposure of material to alcohol or water gradually removes the tetracycline. Permanent mounts are best made in HSR (Harleco), in which no fading has occurred in 5 years.

TECHNIQUE

Wratten 47 and 47B filters are placed in series between light source and substage condenser. Wratten 8 and 9 filters are placed in series over the eyepiece. The filters are available from Eastman Kodak as 2 inch square gelatin films for $0.50 each. Kohler illumination must be used. The light source should be a high intensity tungsten filament source; a 200 watt or more 35 mm slide projector is excellent. The condenser should be opened to maximum aperture after focusing; oiling it to the slide improves illumination considerably. A monocular tube provides the brightest image.

Objectives used should have as large N.A. and small magnification as can be found and used with eyepieces of 5X-8X. Apochromats are ideal.

The individual wishing to do fluorescence microscopy but who does not understand some of the above terms is encouraged to read the references listed.[1,3,4,7] Improper adjustment of microscope optics will cause any fluorescent equipment to behave poorly.

With the above set-up tetracycline labelled bone fluoresces a strong yellow to orange color on a faint magenta background, the latter caused by some leakage in the filters. The bone itself fluoresces a faint blue. Since the energizing band of the above set-up is at 430 mu in the visible blue no special glass is necessary in the optics. Fluorescence is detectable when the drug is present in less than microgram amounts.

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Strong fluorescence of bone labelled in vivo by drug dosages of 5 mg/kg of organism appears. Fluorescence of tetracyclines in other tissues and of other fluors may be observed with the above equipment.

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