HISTOLOGY

Birefringence of the Spermatozoan Tail

THE motility of the spermatozoan tail depends on the contractility of its protein fibrils, which resemble myosin threads[1]. It is not surprising, therefore, to find that the tail is birefringent. This birefringence is difficult to demonstrate by the usual means, but is easily shown by special staining, combined with the use of elliptically polarized light (EPL). The stain is chromosome red ('Michrome No. 1091'). This is haplofavioxanthic acid, a special compound of acid fuchsin, sun yellow and violamine 3*B*. It has the effect of intensifying the anisotropy of already birefringent substances, particularly with EPL[2,3].

I first saw this phenomenon in paraffin sections of rat testis, fixed in formalin and cut at 8 or 10μ. These sections were stained for 5 min in a solution of chromosome red (0·8 g), acetic acid (2 ml.) and water to 100 ml. The birefringence of the sperm tails was seen both in the seminiferous and in the epididymal tubules. In the latter it was in strong contrast to the non-birefringence of the epididymal ciliariform processes or other cilia, prepared and examined in the same way. Since all anisotropic elements appear coloured in EPL, the colour depending on their orientation in the field, the helical form of the spermatozoan tail is easily demonstrated by the colour change as the helix changes direction in its course from the neck onwards. It is more readily seen with an oil-immersion than with a dry objective, although the birefringence of groups of spermatozoa is easily perceived with a low-power objective.

The combination of myosin content and birefringence thus makes the spermatozoan tail the equivalent of a fibril of smooth muscle. The intensity of colour seen is just that of smooth muscle examined under similar conditions. An investigation into the stage of spermatozoan development at which the phenomenon is first observable is in progress.

M. A. MACCONAILL

Department of Anatomy,
University College,
Cork.

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HISTOCHEMISTRY

Uptake of Tetracycline by Human Bone *in vitro*

SINCE the demonstration by Milch *et al.*[1-3] that tetracycline is taken up in newly-formed bone after injection into the living organism, the tetracyclines have been used widely in the study of bone formation, especially by the technique of double labelling[4]. It has lately been shown by Harris *et al.*[5], comparing the uptake of tetracycline *in vivo* with the uptake of radioactive calcium, that, besides the distinct uptake in the calcification front, there is also an uptake in other parts of the bone, not related to new bone formation. In a communication to *Nature*[6], one of us showed that a diffuse uptake of tetracycline can be demonstrated in bone grafts, with the histological appearance of dead bone. We have, therefore, investigated the uptake of tetracycline in bone devitalized by different methods.

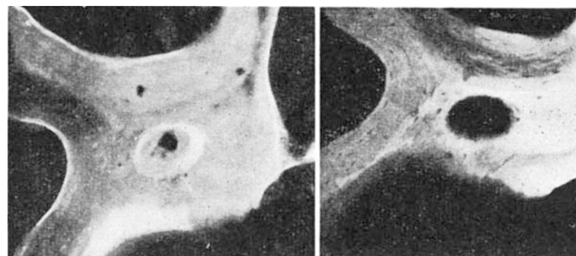

Fig. 1. Cancellous bone treated by alcohol during two weeks storage. Undecalcified specimen examined under ultra-violet light on the right and the corresponding radio-micrograph. Note the uptake on surfaces, in the demarcation zone and inside the bone in inverse relation to calcium density

Human bone, cortical or cancellous, was treated by freezing at −20° C for 2 weeks, boiling or storing in alcohol, or was kept as fresh bone. To each specimen was added a solution of 10 mg of 'Ledermycine' in 100-ml. citrated human plasma for 24 h. The bone was then rinsed in running tap water for a period of 48 h. The specimens were then fixed in formalin, dehydrated in alcohol and embedded in methyl-methacrylate. After cutting and grinding the bone specimens to a thickness of about 50μ, radio-micrographic pictures were taken, and fluorescence was observed in ultra-violet light, using a 'Reichert' filter *GG* 9/1 mm + *OG* 1/1·5 mm.

From the photographs (Figs. 1 and 2), showing the fluorescent picture and the corresponding radio-micrograph of cancellous and cortical bone, it is seen that mineralized dead bone takes up tetracycline on every surface. As in living bone, there is a greater uptake on the growing part, in the zone of demarcation, within the osteoid seam. In non-growing surfaces, like osteons which show edge-sclerosis on corresponding radio-micrographs, or in resorption cavities, there is a limited uptake. Besides this surface incorporation, there is also a diffuse uptake, which is more prominent in cancellous than in cortical bone: this is demonstrated by comparing Fig. 1 with Fig. 2. In all cancellous specimens, there is an inverse relationship between the mineralization of the bone and the degree of labelling with tetracycline. The results are not affected by the method of devitalization of the specimens.

Thus, the uptake of tetracycline by bone is evidently not dependent on the activity of living bone cells, or on the integrity of the physical properties of the intercellular components, which are greatly modified by boiling and storing in alcohol[7]. Tetracycline incorporation cannot be directly related to calcium uptake, because there were no free calcium ions in the citrated plasma used (citrate would be more likely to liberate calcium from the bone[8]). The localization of tetracycline inside the bone supports the conclusion that tetracycline uptake is not confined to growing surfaces. Its peculiar distribution might be explained by a diffusion phenomenon[5,6], but tetracycline uptake in low-density areas is less in cortical than in cancellous bone. Tetracycline staining is present whenever

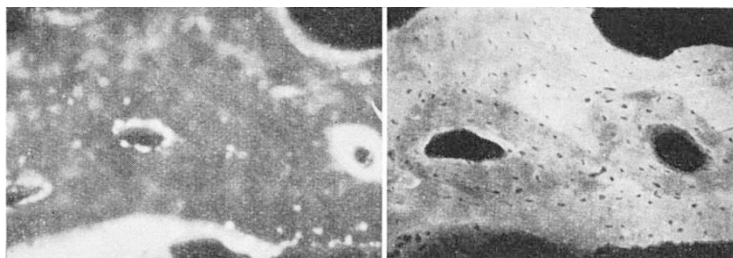

Fig. 2. Cortical bone refrigerated at 20° C and thawed after two weeks. Note the surface uptake, including the resting osteon, with the so-called edge-sclerosis and the fluorescent band corresponding to the growing osteon's calcification front. The fluorescence inside the bone is fainter and uniformly distributed. Ultra-violet light photograph and radio-micrograph as in Fig. 1

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