were measured. All operations were carried out in a dark room under a dim red light, at the temperature 22 ± 1 °C.

Partial bleaching. Partial bleaching of the extract was carried out by exposing the microcuvette with the extract to a source of white light against a corning interference filter of long wavelength (630 nm) for 15, 30 and 60 sec to ensure slow and progressive bleaching of the visual pigment.

Difference Spectrum. The difference spectrum was obtained after total bleaching of the extract to a white light of 100 watt source for 10 min.

Results. Figure 1 shows the optical density measurements of an extract pooled from 24 retinae shown against a wavelength range of 500–600 nm. The absorption spectrum of the unbleached extract showed a λ_{max} at 550 nm. However, a small hump around 530 nm could also be seen. The optical density of the extracts, partially bleached, at a long wavelength (630 nm), over increasing periods of 15, 30 and 60 sec showed a gradual decrement in optical density. While the shape of the curve for the extract bleached only for 15 sec more or less conforms to that of the unbleached extract, the same, when bleached for 1 min, does not show any peak. The most noteworthy feature of the results of partial bleaching is that the absorption spectrum of the extract, bleached for 30 sec, indicates a predominant hump at 530 nm, in contrast to that of the unbleached extract.

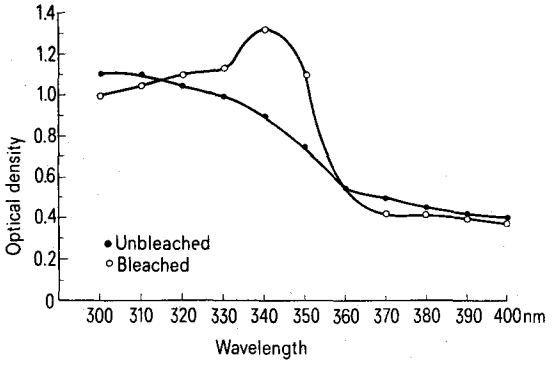

Fig. 2. Optical density of the pooled retinal extract (same as in Figure 1) at the wavelength range of 300–400 nm in unbleached (●) and totally bleached (0) conditions.

The difference spectrum, obtained between the unbleached and totally bleached extracts, shows a peak around 550 nm and a small hump around 530 nm, indicating the maximum density loss on bleaching, at the λ_{max} of the unbleached retinal extract.

Figure 2 indicates the optical density measurements of the unbleached and totally bleached extracts shown over a wavelength range of 300–400 nm. The bleached retinal extract showed a peak around 349 nm, indicating the absorption spectrum of the photoproducts.

Discussion. The results seem to indicate that *E. suratensis*, obtained from fresh water possesses 2 visual pigments, one maximally sensitive at 550 nm and the other at 530 nm, the former being predominant over the latter. The partial and differential bleaching with a long wavelength towards the red and white light has served as a reliable method for testing the presence of more than one visual pigment[2,8,13]. It is clear from the present partial bleaching experiments that the break down of the visual pigment maximally sensitive to a longer wavelength is more complete when exposed to a source of 630 nm for 30 sec. However, the approximate similarity of the shape in the curves of the absorption spectrum between the unbleached extract and the same exposed to a source of 630 nm for 15 sec seems to indicate the incomplete and inadequate bleaching of both the pigments.

The peak obtained around 340 nm in the absorption spectrum of the bleached extract confirms the photosensitive pigment, extracted, as it represents the nature of the photoproducts as a result of bleaching.

The visual pigments extracted in *E. suratensis* are comparable to those obtained from the other fishes like bleak (533, 550 nm) and carp, (523, 550 nm)[13,14]. It is of interest to note that a closely related species, *E. maculatus* apparently has a mixture of 2 pigments i.e., porphyropsin and rhodopsin[6], although their λ_{max} are far to the left (515 and 495 nm, respectively) of the same for the pigments obtained in *E. suratensis*. It should be pointed out, however, that many of the fresh water fishes possess visual pigments, whose peak sensitivity ranges from 520–550 nm[15].

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Histochemical demonstration of adrenergic nerve fibres and serotonin-containing mast cells of the knee joint synovial membrane in rats

SEISUKE TANAKA, NORIO OHNISHI and YOSHINORI TOMINAGA[1]

Department of Orthopaedic Surgery, Faculty of Medicine, Kyoto University, Kyoto 606 (Japan), 6 July 1976

Summary. Distribution of noradrenergic nerves and serotonin-containing mast cells in the synovial membrane of rat knee joints was demonstrated using the histochemical fluorescence technique of Falck-Hillarp.

Using conventional histochemical techniques, many investigators have demonstrated that the joint capsule is supplied with both myelinated and unmyelinated nerves, which are afferent and autonomic, associated with blood vessels[2], and that mast cells lie around the blood vessels and in the loose connective tissue[3,4]. Cellular localization of monoamines in various organs and tissues has been demonstrated using the histofluorescence technique of FALCK-HILLARP[5]; but demonstration in the joint capsule has not been reported.

We investigated the distribution of noradrenergic nerves and serotonin-containing mast cells in the rat, using the Falck-Hillarp method applied in such a way that the joint capsular tissue could be visualized.

The experiments were carried out using male Sprague-Dawley rats weighing 200–250 g. Each animal was decapitated under light anaesthesia and the knee joint capsule was immediately removed for routine histological and fluorescence histochemical analyses.

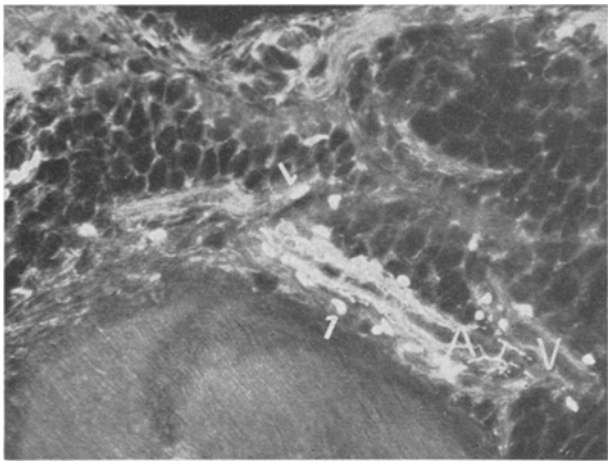

Fig. 1. Synovium of the rat knee joint. Note the network of adrenergic terminals with intensely fluorescent varicosities close to the arteriole (A). Serotonin containing mast cells (arrows) are also evident around the arteriole. × 30.

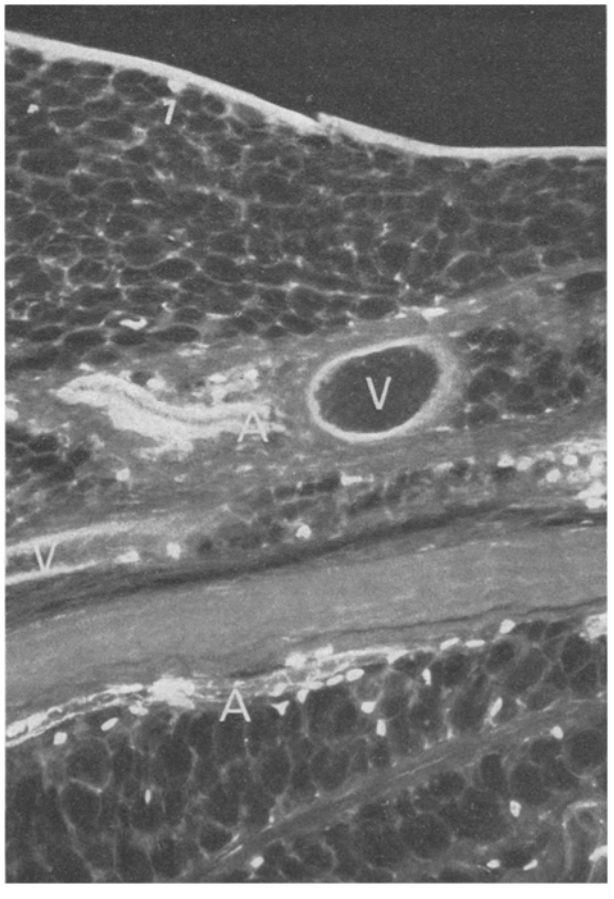

Fig. 2. Synovium of the rat knee joint. Serotonin containing mast cells are visible just beneath the lining cells (arrow) and in the subsynovial and periarticular connective tissues. The arterioles (A) receive adrenergic fibres while small veins (V) are not innervated. × 30.

Treatment with formaldehyde gas induced a strong green fluorescence in the nerve and a yellow one in mast cells in the synovium and fibrous capsule. Specificity of such fluorescence was identified following the histochemical criteria described by CORRODI and JONSSON[6]. The results indicated that the green and yellow fluorescence was due to noradrenaline and serotonin, respectively.

Adrenergic fibres are usually associated with articular blood vessels. The arteries in the knee joint capsule were surrounded with dense adrenergic ground plexus from the outside of the smooth muscle layer and veins with a loose network of thin varicose fibres. The arterioles (10–50 μm in diameter) were usually richly innervated (Figure 1), while innervation by adrenergic fibres was not evident in the venules, small veins (15–200 μm), the capillaries and lymph vessels (Figure 2). Adrenergic fibres were demonstrated mainly in the connective tissue of the deep subsynovial layer along the fibrous capsule, a few were observed in the middle subsynovial layer and retinaculum, but not in the lining layer. In the rat treated with 6-hydroxydopamine (200 mg/kg i.p.) 7 days before sacrifice, disappearance of noradrenaline fluorescence from nerves associated with the articular vessels and vasodilation was noted in the synovium.

The strong yellow fluorescence of serotonin was observed in mast cell granules which sometimes dispersed into the surrounding area. A great number of mast cells were visible around the articular vessels, particularly the arterioles and some were adjacent to capillaries and fat cells, and just beneath the lining cells. About 70% of the serotonin fluorescent granules stained metachromatically with toluidine blue but some were stained orthochromatically. Cells with orthochromatic granules were found mainly around blood vessels, while those with metachromatic granules were seen predominantly around capillaries and in the loose connective tissue.

Thus the pattern of adrenergic innervation of articular vessels is basically similar to that of blood vessels located elsewhere[7,8]. 6-hydroxydopamine causes a marked depletion of tissue noradrenaline[9] and destroys selectively adrenergic terminals[10]. In the present study, 6-hydroxydopamine-induced vasodilation suggests a vasoconstictor role of the adrenergic nerves in articular vessels.

Distribution of the mast cells is also closely associated with the articular vessels. The precise topographic relationship of adrenergic fibres, mast cells and articular vessels suggests the functional participation of noradrenaline and serotonin in the blood flow in rat synovium. Further experiments concerning the role of these amines with regard to synovial function in physiological and pathological states are underway.

[1] The authors express their gratitude to Drs M. Fujiwara and C. Tanaka for pertinent advice and to M. Ohara for review of the manuscript.

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