Elastosis and Cancer*

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Cryosections, Autofluorescence, Malignant Tumors, Elastic Fibers

Recently we have shown that the autofluorescence within or just outside of a malignant tumor is rather small or large resp. in comparison to healthy tissue when excited at 365 nm. Studies with unfixed, unstained cryosections of skin with melanomas have revealed bulky fiber-like structures with a high fluorescence intensity just outside of a malignant tumor. Using polarized light, the structures could be identified as elastic fibers. This was also confirmed by studies on arterial walls.

Recently we have shown that different types of biological tissue exhibit native fluorescence with a maximum at about 470 nm when excited at about 365 nm [1, 2]. The extent of the fluorescence intensity depends on the condition of the tissue. In the case of a malignant tumor, the intensity is rather small within the tumor region, while it is higher in the surrounding of the tumor than in healthy tissue. These results were confirmed by others [3–6] and by fluorescence tomographical stratigraphical measurements in which case the spatial distribution of the fluorophore has been determined by using unfixed and unstained cryosections [7–9].

These tomographical studies revealed also that there seems to be a fluorophore of low intensity which is distributed uniformly over the area outside of and in small places scattered around within a malignant tumor. Another (?) fluorophore with larger intensity is attached to bulky fiber-like structures. In the latter case, these fibers are rather thin and their fluorescence intensity weak in healthy tissue. Within malignant tumors, their intensity is very low. Right adjacent to the tumor, they seem to exhibit the highest intensity. There, the fibers seem to be broken up into smaller units forming a tight texture to prevent presumably the growth of the tumor. With increasing distance from the tumor the volume of these fibers increases first followed by a decrease to a "normal" level.

By using Elastica-van Gieson (EG) staining, preliminary investigations have shown that these fiber-like structures might be elastic fibers while collagen fibers might be involved when the fluorophore is distributed homogeneously. If so, it will confirm results according to which the degree of elastosis increases progressivly with the severity of epitheliosis and increases further with intraductal and infiltrating duct carcinoma of the breast [10]. It could also be shown that elastosis can also occur in association with carcinoma of the urethra [11], colon [12], skin [13], and malignant tumors of the salivary gland [14]. Thus, the identification of these fibers seems to be important not only for understanding the influence of normal cell-tumor cell interactions.

To address this question, the present study was undertaken to determine whether elastosis is a feature of fibrocystic disease [10]. Since collagen and elastic fibers differ in their response to polarized light, the double refraction of collagen was used to discriminate between these two types of fibers.

As reported recently, the distribution of the fluorophores seems to be the same in most of the patients with melanoma tested thus far [7]. For that reason, only results obtained from one male patient (F. P., 71 years old) with a malignant melanoma are shown. Right after surgery a sequence of at least 6 successive unfixed and unstained cryosections (10 μm each) of the excised tissue sample were prepared; each alternative one was used for fluorescence microscopy or, after hematoxylin-

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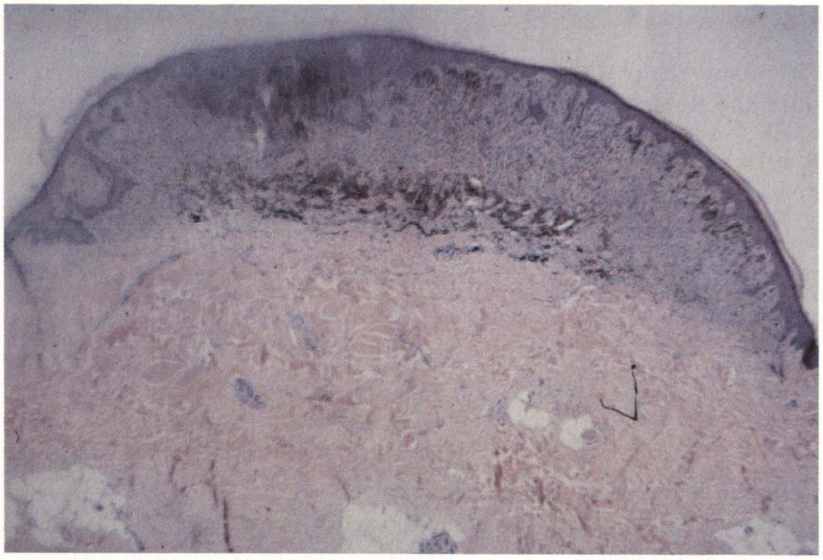
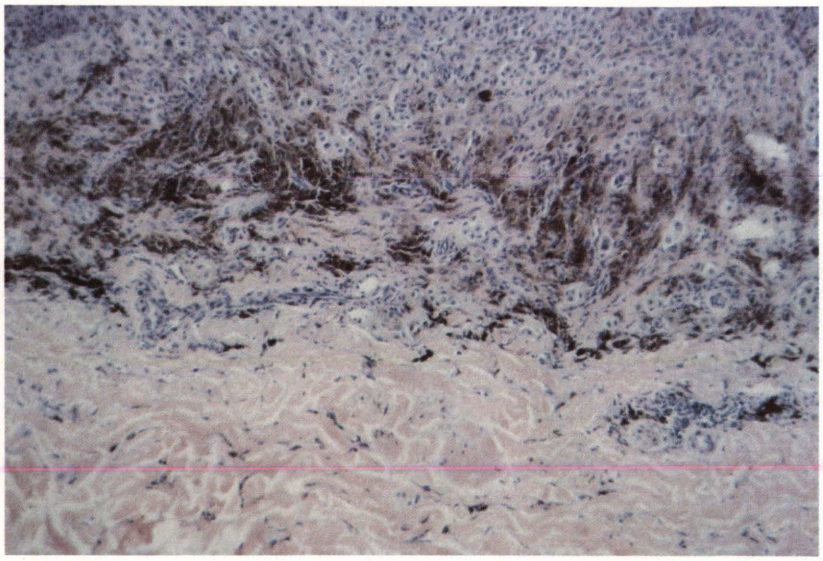


Fig. 1. Image of an HE stained, unfixed cryosection (10 µm thick) of a melanoma. Magnification: × 21.5.



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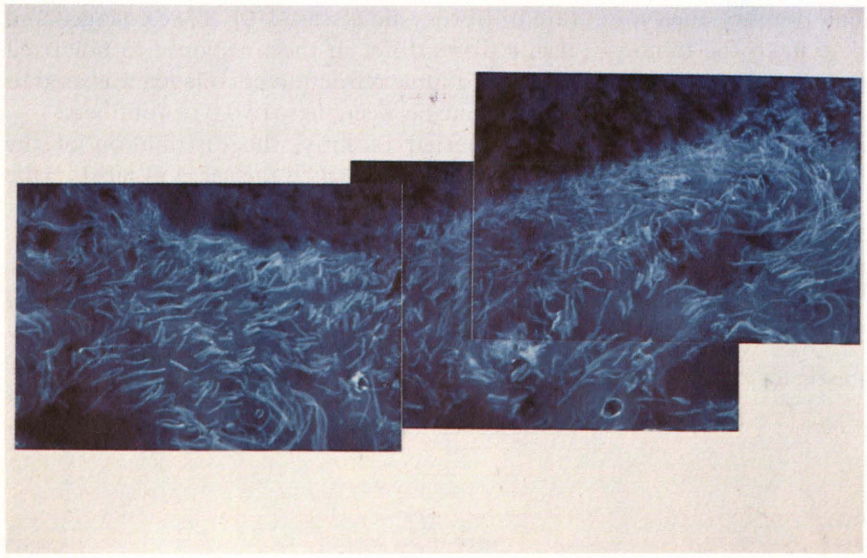

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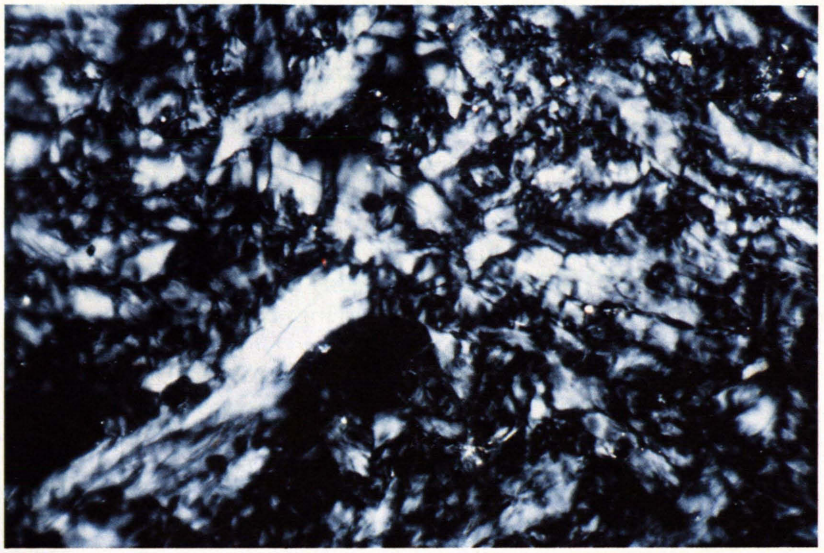

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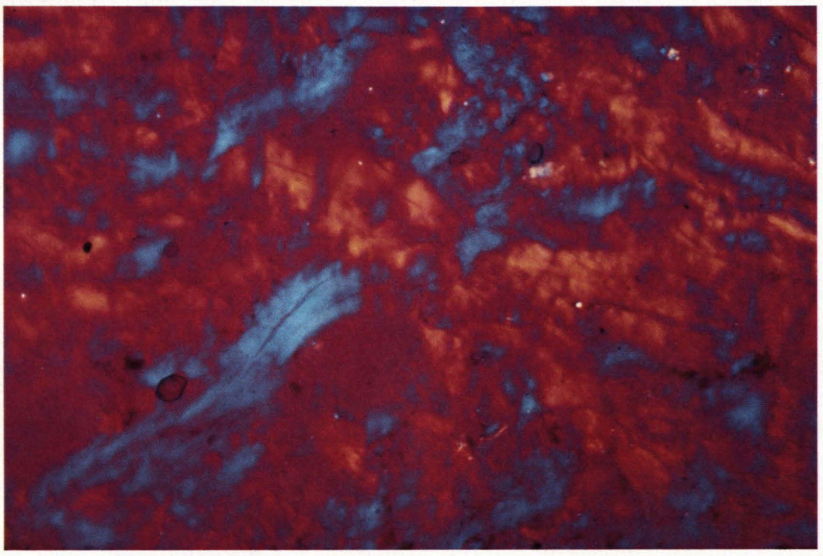

2d

Fig. 2. Fluorescence image of an unstained and unfixed 10 μm thick cryosection of an enlarged section of Fig. 1 (Fig. 2b, magnification × 50) compared with an HE stained section (Fig. 2a). Fig. 2c: Illumination of the cryosection used in 2b with polarized light. Fig. 2d: Same as in Fig. 2c, however, a λ plate was inserted. Magnification of Fig. 2a, c, d: × 86; Fig. 2b: × 43.

eosin (HE) staining, for histological examination. Details of the technique used have been published elsewhere [7–9]. The unstained and unfixed cryosections were illuminated with a 50 W Hg high-pressure lamp with an added BP A filter (transmission between 340 and 380 nm). The HE sections were illuminated with a bright-field transmitted light by using a 12 V, 100 W halogen lamp. The fluorescence and the histological investigations as well as the photodocumentation were done with a Zeiss Axiophot or a Leitz Aristoplan microscope.

To determine the presence of asymmetrical regions or of oriented molecular structures the object was examined between crossed polarizers. A rotating stage (360°) was used for determining the optical behaviour of the fibers being present in the samples by setting the polarizer and analyzer so that the directions of the oscillating electric fields of the halogen light waves transmitted by them are at right angles. A first order red plate (λ plate) was inserted in diagonal position between polarizer and analyser for introducing a known phase lag.

To demonstrate the response of elastic fibers to polarized light, an unfixed and unstained cryosection (10 μm) of a femoral artery was prepared. This sample was taken from an autopsy (86 year old female) 37 h after her death.

In Fig. 1*, the HE image gives a survey of a malignant melanoma. The area indicated in Fig. 1 has been enlarged and shows a more detailed structure in Fig. 2a. The melanocytes as well as the tumor cells are located in the upper portion of the figure. They have been determined histologically.

For comparison, the fluorescence response obtained from the same region using an unfixed and unstained cryosection is shown in Fig. 2b. This figure agrees very well with our previous findings according to which the tumor region exhibits a rather low fluorescence intensity. Right adjacent to the tumor, the fluorescence intensity is the highest one followed by a decrease towards the healthy tissue. According to this image, there seems to be fluorescent fibers of different volume and length superimposed to a homogeneous fluorescence of less intensity. It is interesting to note that the fibers located adjacent to the tumor are thinner and shorter. They exhibit, however, a higher density. They seem to form a tight texture probably as a defence against the tumor.

There is a strong indication that the homogeneous area represents a fluorophore which is attached to collagen fibers, while the fiber-like structures are elastic fibers with, presumably, the same fluorophore attached to them. To prove this assumption, unstained and unfixed cryosections were illuminated with polarized light. As expected, only the homogeneous fluorescence region shown in Fig. 2b responds (Fig. 2c** taken from an area adjacent to the tumor). This effect is due to the strong double refraction of collagen while elastic fibers exhibit an extremely weak effect, at the most. This effect can be seen even better, when a λ plate is inserted. Then, collagen fibers exhibit a positive double refraction (+ 45°: blue; −45°: yellow) (Fig. 2d). When the cryosection is turned about 90°, the blue region turns yellow and *vice versa* (not shown).

From the results obtained thus far, it might be concluded that the fibers seen in Fig. 2b do represent elastic fibers. To provide an additional prove, the same experiments conducted with the melanoma sample were repeated with an unfixed, unstained cryosection of a femoral artery.

All layers of the arterial wall can be seen by the fluorescence image when illuminated with 365 nm (Fig. 3a)***. The dark area at the bottom is the lumen followed (from bottom to top) by the intima, the double layer of the lam. elastica interna, the t. media, the lam. elastica externa, and finally the adventitia. As is known, the adventitia is composed mainly of collagen which is indicated also by the fluorescence image: there are zones with a rather homogeneous fluorescence light. A few elastic fibers are present in the media also.

It is interesting to note that not only all layers of the arterial wall can be detected by the fluorescence response but that elastic fibers exhibit the highest fluorescence intensity.

These findings are confirmed by the results obtained with polarized light (Fig. 3b). The elastic fibers can be seen hardly (see especially the double layer of the elastica interna). The collagen fibers, as expected, are rather bright. This effect can be seen better in Fig. 4a, b (see Plate on page 229)

* Figs 1, 2a, 2b see Plate on page 224.

** Figs 2c, 2d see Plate on page 225.

*** Figs 3a and 3b see Plate on page 228.

which is an enlargment of Fig. 3a, b. It is interesting to note that the elastic fibers of the elastica externa seem to be arranged perpendicularly to the longitudional direction.

When the λ plate is inserted, the collagen fibers will respond like in the case of a melanoma: depending on the collagen fiber direction, the color will be blue or yellow and *vice versa* after turning the object for about 90° (not shown).

The results obtained suggest that the fibers exhibiting a high fluorescence intensity and which can be seen especially near malignant tissue are elastic fibers. Their overproduction might be caused by a stimulation of fibroblasts by melanoma cells. This would confirm results obtained by others [10]. In a more recent publication it could be shown that the discriminatory fibroblastic influence is mediated by soluble inhibitory and stimulatory growth factor(s) [15] depending obviously on the stage of tumor progression.

The distribution of elastic fibers observed might be explained also by compressing the elastic fiber network of the corium, an effect exerted by the growing tumor resulting in a higher density of the fibers. Thus, all facts known so far suggest that fibroblast cell interactions with human melanoma cells affect tumor cell growth as a function of tumor progression and mediated by elastic fibers.

The fluorescence response of the two types of fibers is obviously not caused by the fibers. Both types of fibers exhibit a fluorescence light at about 400 nm when illuminated at 365 nm. In the tissue investigations described above, the fluorescence band is located at about 470 nm. Type and strength of the attachment of the fluorophore(s) to the fibers is still unknown.

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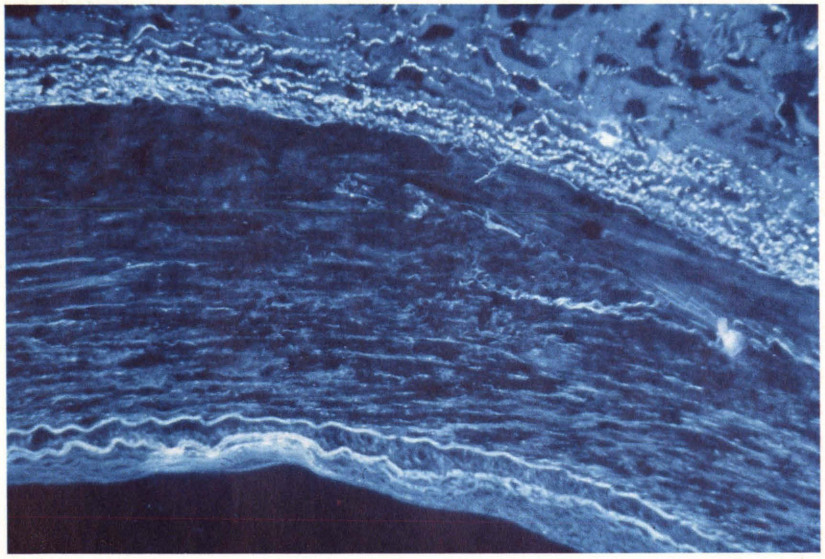

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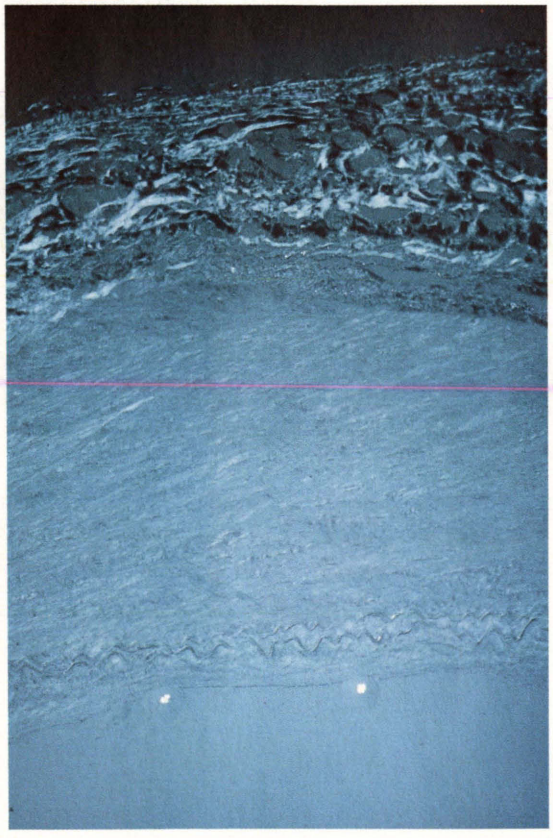
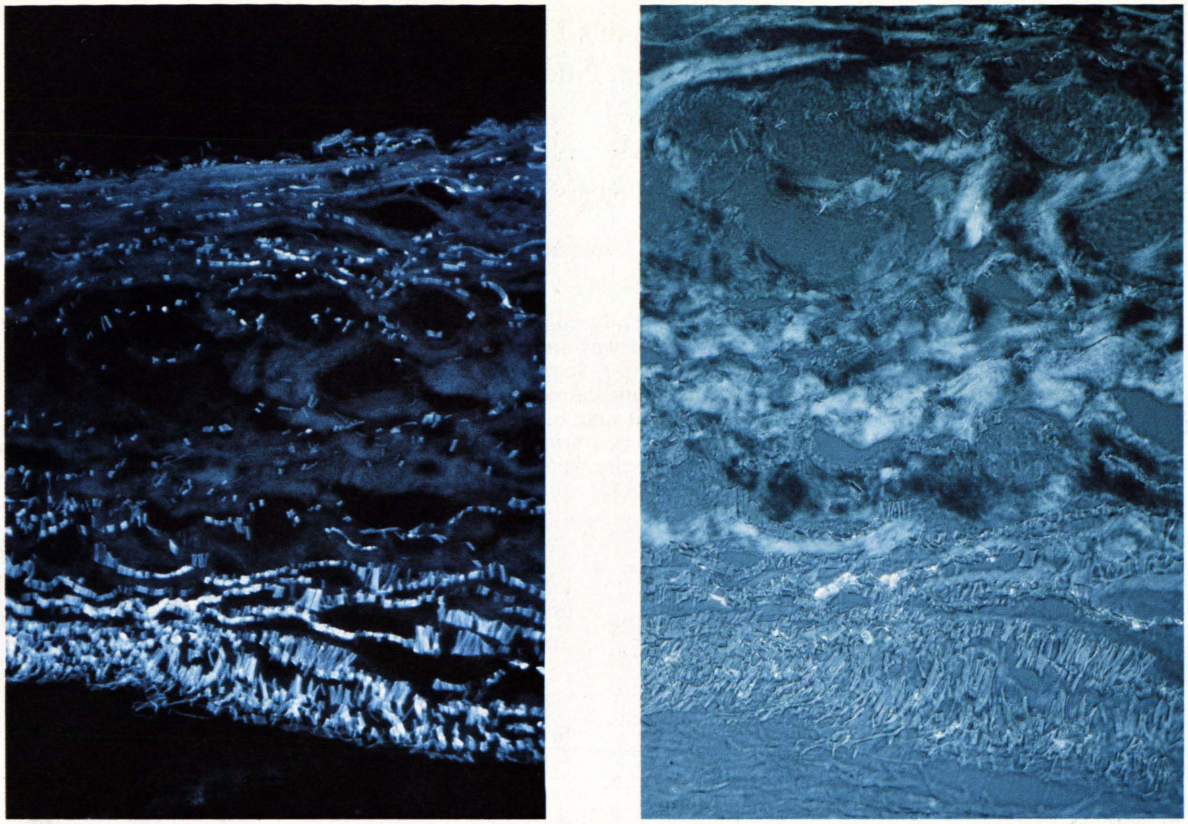


b

Fig. 3. Fluorescence image of an unstained and unfixed 10 µm thick cryosection of a femoral artery illuminated with 365 nm (Fig. 3a) compared with the same section, however, illuminated with polarized light of a halogen lamp (Fig. 3b). Magnification: × 86.



a b

Fig. 4. Enlarged section of the elastica externa and adventitia shown in Fig. 3a, b. Fig. 4a: Fluorescence image, Fig. 4b: Illumination with polarized light. Magnification: × 72.