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STUDIES OF THE VASCULAR CONNECTIVE TISSUE BY THE FLUOROCHROME METHOD IN EXPERIMENTAL CANCER OF THE LIVER

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Changes in the morphology and function of vascular connective tissue during the development of cancer still present a problem, especially with experimental tumours of internal organs. This is well shown by the fact that, while there are a number of data concerning behaviour and role of connective tissue in the genesis of skin cancer, a subject studied by us earlier, similar data on the genesis of hepatoma, for example, are very scarce.

For studying disturbances in the extravascular tissue-circulation we have applied the Haitinger-Geiser method of secondary fluorochrome staining [6], introduced into pathology by EPPINGER [3, 4], in which the sections are treated with thioflavine, euchrysine GNX and thiazine red. (Method II.).

32 white rats of both sexes, weighing about 140 g, were used in the present study. Of these, 24 were fed a diet described earlier [12] and 0.008 g of butter yellow daily, while 8 served as controls.

During the growth of hepatic tumours induced by butter yellow, carcinogenesis shows three distinct phases : the phase of toxic-degenerative damage, the precancerous phase and the malignant tumour phase. Since our purpose was to study each of these phases, the animals were sacrificed in 3 groups : the first after 2 to 6 weeks, the second after 2 to 4 months and the third group after 6 to 8 months following the beginning of butter yellow treatment. The fluorescence microscopic patterns (Zeiss) were compared with sections prepared from the same block and stained with haematoxylin and eosin.

Normal liver tissue treated by the above combined fluorochrome stain is remarkably colourful. Euchrysine stains the nuclei leaf-green, the cytoplasm yellowish-green. The intercellular part of the perilobular connective tissue stains bluish-green. The corpuscular elements of blood give a pale-green, and blood proteins a leaf-brown or a brownish-red fluorescence, both intravascularly and intracellularly. Experience has shown that, though not wholly specific, the method is suitable for the demonstration of plasma proteins.

In the toxic-degenerative phase, the connective tissue around the lobes containing dissociated liver cells exhibits extensive leaf-brown or red imbibition and the vessels in that area are dilated and congested. The dissociated parenchy-

mal cells, too, are mostly infiltrated by the brownish-red exudate (Fig. 1). The spaces of Disse are distended, and, like the ectasic lacunae in the triangles of Glisson that are believed to be lymph ducts, contain a fluid exhibiting brownish-red fluorescence.

In the precancerous liver the adenomatous areas and the epithelial cells in the cholangiocystadenomas are mostly similar to normal hepatic trabecules in colour. Their environment, however, is frequently inbibed by a protein-rich serous fluid and accumulations of macrophages often occur. In such cases the epithelial cells proper are flooded by inflammatory exudate and give consequently a leaf-brown fluorescence. A similar coloration of the contents of the cysts suggests the presence of blood proteins (Fig. 2).

The fully developed malignant tumours stain similarly. Florid, non-damaged tumour cells are of the same green hue as the liver cells, while the cells infiltrated by the protein-rich fluid are of a leaf-brown colour (Fig. 3).

The onset of butter yellow administration is followed by the appearance of degenerative changes in the liver. As the cancer develops, more and more cells are destroyed. The damaged areas usually appear in the same colour as that caused by infiltration with plasma. The areas exhibiting coagulation necrosis or total autolysis often stain sky-blue from thioflavine (Fig. 3.). The areas in which parenchymal proliferation is surrounded by fibrosed stroma (cholangiofibrosis) are poor in vessels, show no evidence of oedematous infiltration and, consequently, stain bluish-green, like normal connective tissue.

Discussion

In an earlier work [10], we studied under the fluorescence microscope the localisation of intravital sodium fluorescein in the course of the development of butter yellow cancer. During life, the distribution of dyes may be influenced by damage to tissues, changes in the circulation of fluids, as well as by an impaired function of the blood and lymph vessels. The development of cancer is accompanied by the arisal of grave vascular lesions. These changes and the presence of a protein-rich exudate ("Albuminurie ins Gewebe", 3, 4) indicate an impairment of vascular function. The disturbance of fluid circulation is already demonstrable in the early toxic-degenerative phase and persists throughout the development of cancer. Parallel with the impairment of circulation, in the perilobular connective tissue histiocytic, plasmocytic, or, at sites, leucocytic infiltration develops. Thus, the stroma exhibits changes which are definitely those of inflammation. Owing to the presence of extensive cellular infiltration, the inflammation is not purely serous in nature ; nevertheless, much more than normal amounts of plasma protein invade the intercellular space, adhere to connective tissue fibres and even to liver cells, as a sign that tissue circulation is severely impaired.

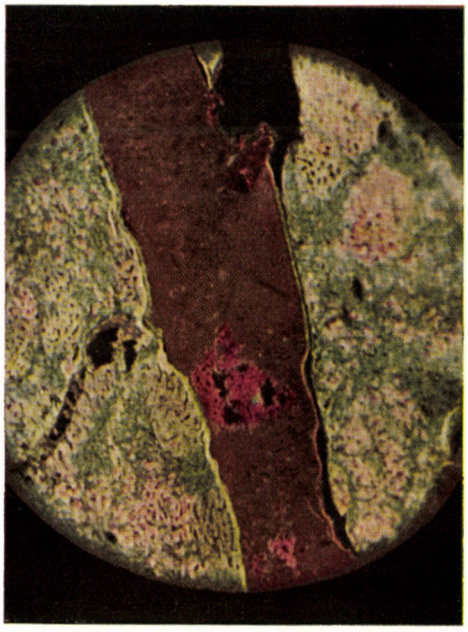

Fig. 1. Liver showing toxic-degenerative changes early during butter yellow treatment. The brownish-red areas in the green-stained liver indicate foci of infiltration by plasma protein. On thiazine red the contents of the transversal section of a vessel have taken a similar colour

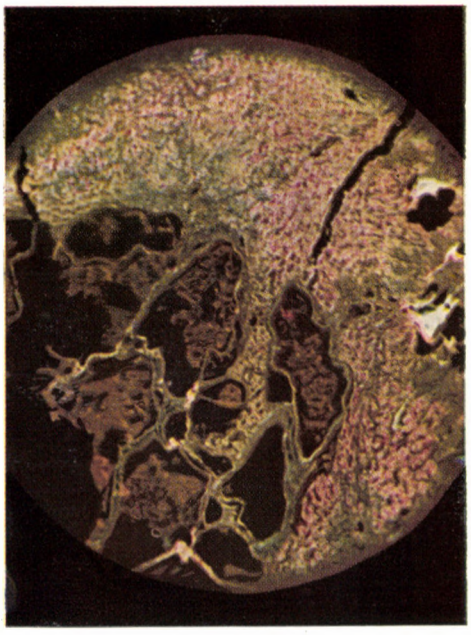

Fig. 2. Precancerous phase. The lumina of bile ducts showing cystic dilatation and the liver tissue gives a leaf brown and brownish-red fluorescencence indicative of the presence of plasma proteins

Fig. 3. Hepatocellular, partily cholangiocellular adenomatous carcinoma. Brown areas can be seen in the ground substance of the cancer, which shows a greenish fluorescence. The necrosed cells stain blue

Figs. 1—3: Fluorescence microphotographs, lens, ×8 Eye piece, ×10

The fluorochrome staining method produces no such characteristic colour effects as could decide whether the hepatic changes are precancerous or cancerous. The reddish fluorescence of epithelial cells namely depends exclusively upon the degree of infiltration by plasma proteins that had entered the tissue spaces.

VÁCZI and TARJÁN [13] have reported that the precancerous epithelial elements in the portio uteri stain differently by the Haitinger technique than those of preinvasive or invasive cancer. Our results make it probable that the tumorous proliferation of the erosion is associated with severe inflammation, which, in turn, may lead to an infiltration of the proliferating epithelium by plasma proteins ; this would explain the observation by VÁCZI and TARJÁN.

Thus, our findings indicate that the development of hepatoma is associated, early and invariably, with an impairment of circulation : focal oedema in the liver. In agreement with the results of other authors [5, 8, 11] we, too, see the cause of the congestion of fluid that has entered the intercellular space in an impairment of vascular permeability and in an insuffuciency, dynamic or, eventually, also mechanical, of the lymphatics.

The focal accumulation of vitally introduced sodium fluorescein, observed in earlier studies to occur in the course of carcinogenesis and in the developed cancer alike, may be related not only to cellular damage or cell membrane injury, but also to an impairment of circulation in the tissues, due to inflammation and degenerative changes.

The role and importance of chronic oedema and infiltration of cells by fluid in the development and growth of cancer is a further problem. Relying on evidence described by BAITSELL [1], DOLJANSKI—ROULET [2] and others, some authors [8, 9, 3] have arrived at the conclusion that fibrotic changes in the connective tissue are due to an accumulation in the intercellular space of aphysiologic protein-rich fluid, rather than to a direct involvement of cells. We, too, have frequently seen an increase in connective tissue or fibrosis in the course of carcinogenesis. The exudate, and the infiltration by protein-rich fluid of the cells impair cellular function and may lead to necrosis. All these considerations appear to indicate that the increase in connective tissue associated with exudation and impairment of nutrition may affect the relation of epithelium to connective tissue and may thus be an important factor in the morphology and biology of tumour. This is especially plausible in the light of the so-called trophic concept of carcinogenesis put forward by LARIONOV [7].

Summary

The behaviour of vascular connective tissue and of the internal circulation in tissues has been examined in the various phases of experimental carcinogenesis using the secondary fluorochrome staining technique of Haitinger.

As a result of a pathological alteration in vascular permeability, an oedematous infiltration of tissues is a common occurence throughout the development of cancer. In the stroma of

developing tumours, in the perilobular connective tissue, excessive amounts of plasma proteins are present. The plasma proteins, which stain reddish-brown or leaf-brown, infiltrate the parenchymal cells, too, and thus the cells, both hepatic and tumour cells, adjacent to the areas of inflammation also stain brown. Otherwise, both normal and tumorous liver cells present a yellowish-green fluorescence. The internal circulation in tissues is particularly impaired in areas adjacent to degenerative foci. These phenomena probably influence the relationship of epithelium and connective tissue during carcinogenesis. The impairment of circulation affects also the distribution of fluorochrome stains, introduced into the organism because of their affinity to tumour tissue.

Even this staining method fails to produce such a colour effect as would facilitate a differentiation between malignantly proliferating cells and normal liver cells.

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ИССЛЕДОВАНИЕ СОЕДИНИТЕЛЬНОЙ ТКАНИ КРОВЕНОСНЫХ СОСУДОВ ФТОРОХРОМОВЫМ ОКРАШИВАНИЕМ В ПРОЦЕССЕ РАЗВИТИЯ ЭКСПЕРИМЕНТАЛЬНОГО РАКА ШЕЙКИ МАТКИ

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Авторы исследовали в различных стадиях экспериментального печеночного канцерогенеза поведение соединительнотканного вещества кровеносных сосудов и внутритканевого сокообращения с помощью методики вторичного фторохромного окрашивания по методу Хайтингер.

Вследствие патологической проницаемости кровеносных сосудов начиная с ранней стадии канцерогенеза вплоть до развития рака отечное пропитывание тканей представляет часто наблюдаемое явление. В строме возникающих опухолей в перилобулярной соединительной ткани можно выявить большое количество белков крови. Окрашивающиеся в краснобурый и бурый цвет плазматические клетки пропитываются также и в клетках паренхима, причем печеночные или опухолевые клетки, расположенные вокруг этих воспалительных областей также окрашиваются в бурый цвет. Впрочем, как неповрежденные, так и опухолевые клетки флуоресцируют в желтовато-зеленом цвете. Нарушение тканевого сокообращения особенно интенсивное вблизи дегенеративных очагов. Все эти явления играют роль по всей вероятности при образовании взаимоотношений эпителиальной и соединительной тканями в процессе канцерогенеза. Изменение сокообращения играет роль также и в распределении фторохромных прижизненных красящих веществ, введенных в организм ввиду их сродства к опухолям.

Злокачественные, разрастающиеся клетки и при данном способе не обладают таким специальным эффектом окрашивания, на основании которого возможно было бы их различать от нормальных клеток печени.

UNTERSUCHUNG DES BINDEGEWEBES DER BLUTGEFÄSSE MIT FLUOROCHROMFÄRBUNG WÄHREND DER ENTWICKLUNG VON EXPERIMENTELLEM LEBERKREBS

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Das Verhalten der Bindegewebesubstanz der Blutgefässe, und der Gewebesäfteströmung wurden in verschiedenen Stadien der experimentellen Leberkarzinogenese nach sekundärer Fluorochromfärbung laut Haitinger untersucht.

Infolge der pathologischen Permeabilität der Gefässe vom frühen Stadium der Karzinogenese bis zur Entwicklung des Krebses ist die ödematöse Durchtränkung der Gewebe eine häufig beobachtete Erscheinung. Im Stroma der entstehenden Geschwülste können im periolobulären Bindegewebe grosse Mengen Bluteiweisskörper nachgewiesen werden. Das sich rötlichbraun, bzw. laubbraun färbende Plasmaeiweiss durchtränkt auch die Parenchymzellen, demzufolge sich die in der Umgebung der entzündlichen Gebiete befindlichen Leber- oder Tumorzellen ebenfalls braun färben. Sonst fluoreszieren sowohl die normalen, wie auch die tumorösen Leberzellen in gelblichgrüner Farbe. Die Störung der Säfteströmung ist in der Nähe der degenerativen Herde besonders intensiv. All diese Erscheinungen spielen wahrscheinlich eine Rolle bei der Gestaltung des Verhältnisses zwischen Epithel- und Bindegewebe im Verlaufe der Karzinogenese. Die veränderte Säfteströmung ist auch an der Verteilung der wegen ihrer Tumoraffinität in den Organismus eingeführten Fluorochromvitalfarbstoffe beteiligt.

Die bösartig wuchernden Zellen verfügen auch bei dieser Färbungsmethode über keinen spezifischen Farbeneffekt auf Grund dessen man sie von den normalen Leberzellen unterscheiden könnte.

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