HISTOCHEMICAL AND ELECTRON MICROSCOPIC STUDY OF EPIDERMAL NEOPLASMS

By
SATYAVATI M. SIRSAT, ARNAVAZ H. VANIREWALLA
and
V. R. KHANOLKAR
Indian Cancer Research Centre, Bombay 12.

Robb-Smith[3] has very aptly called connective tissue the biological moderator of cellular energy. During the last few years its role in primarily cellular diseases is being increasingly understood. This study on benign and malignant skin neoplasms was undertaken to evaluate the role of the extracellular dermis in the presence of neoplasia of different layers of skin. The material studied was 2 samples of normal skin, 8 ulcers biopsied for exclusion of malignancy, 1 papilloma, 1 intraepidermal carcinoma, 2 basal and 13 squamous carcinomas and 1 melanoma. Serial sections were stained with Haematoxylin and Eosin, the usual differential stains for connective tissue. Periodic Acid-Schiff (Mcmorris) and Tolvidine Blue metachromasia at pH 4.5 collagen, isolated from areas in the sections which appeared abnormal with the connective tissue stains was observed in an RCA-EHV-2D electron microscope with an objective aperture of internal diameter 25 mu.

RESULTS

Alteration of collagen from normal was assessed as early, moderate or advanced according to the response of the fibres to mallarys Trichrome, Mallarys Phosphotungstic Acid Haematoxylin (PTAH) and Weigerts Resorcin Fuchsin stain for elastica. Normal collagen stained on even blue and even orange with the two Mallary techniques and the sections showed none or normal quantities of resorcin positive thin anastomasing strands. Altered collagen stained unevenly or pale with the two Mallary methods (Fig, 1, 3). Coarse individual fibres stained orange/purple or deep purple with PTAH (Fig. 3) were strongly resorcin positive (Fig. 2, 4, 5). Electron micrographs of abnormal collagen (Fig. 6) show the degradation of normal collagen structure (640 A° repeating macroperiod) into ill defined filaments and amorphous material. The table shows the state of collagen in all the material studied. In the benign hyperplastic papilloma, intraepidermal carcinoma and all the malignant tumours the collagen shows moderate to advanced change. The increase in PAS positive and metachromatic substance is also considerable in the biopsies from malignant skin lesions.

DISCUSSION

In his classic treatise on histopathology of the skin, Uma[5] has classified connective tissue fibres in aging and injured skin as (1) elastin (2) elacin (3) collacin or collastin, on the basis of tinctorial reactions to the connective tissue stains.

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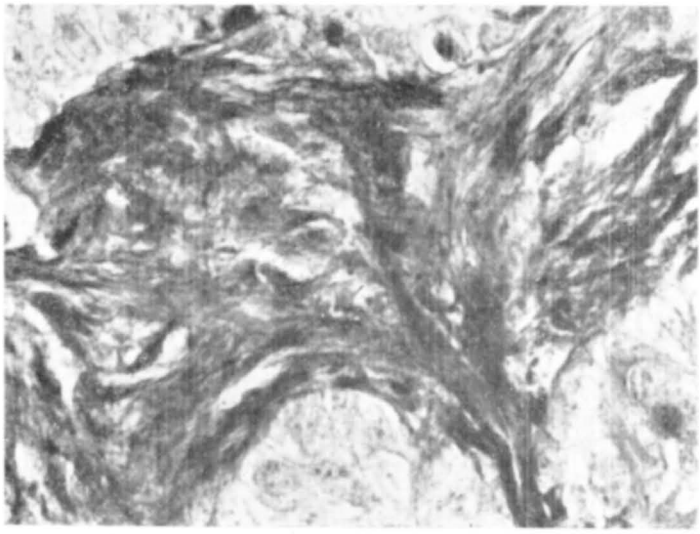

Fig. 1

Section from basal cell carcinoma showing unevenly stained connective tissue. There are areas of pale staining as well as intensely stained fibre bundles.

—Mallary's Tinchrome stain X 500.

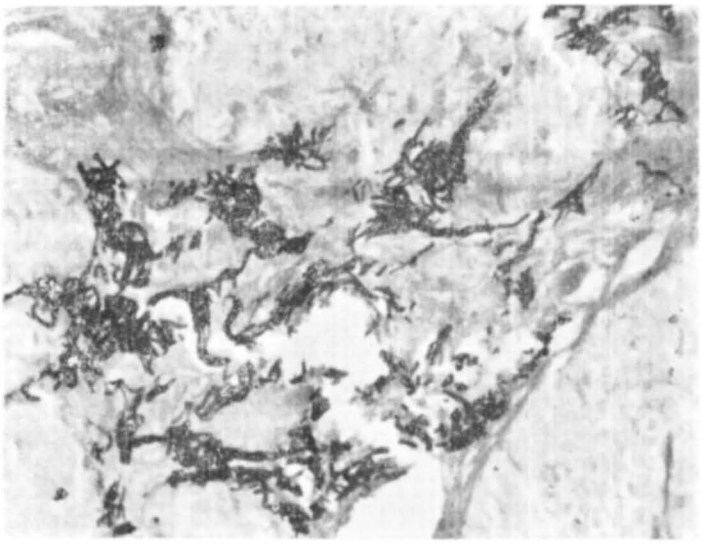

Fig. 2

Serial section of Fig. 1. Picture shows thick fragments, bits and heads of resorcin positive material in the intercellular region.

—Weigerts resorcin fuchsin stain for elastica X 250.

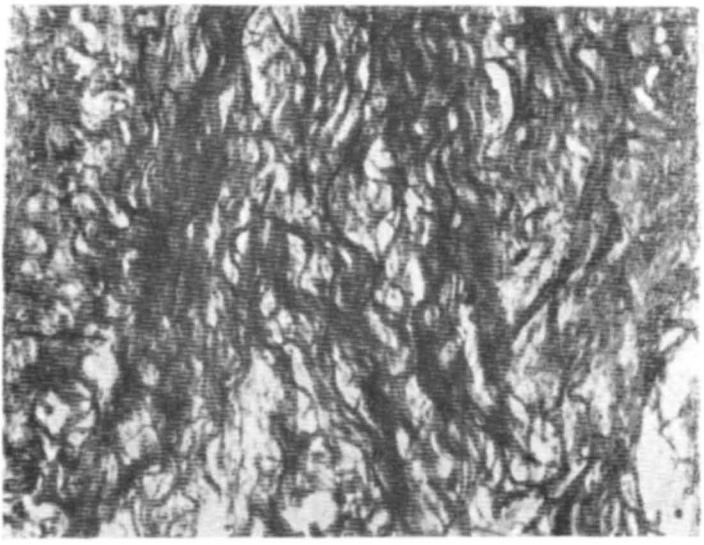

Fig. 3

Section from squamous carcinoma. Picture ahows unevenly stâined, coarse purple collagen fibres (block in picture).

—Mallarys PTAH X 250.

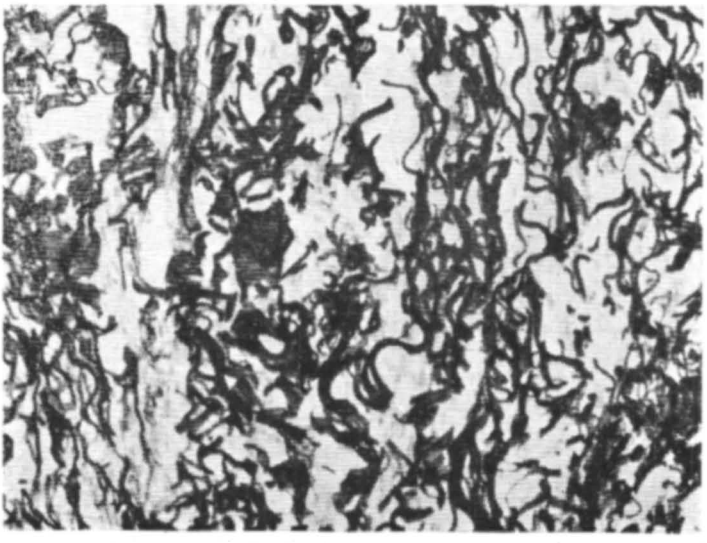

Fig. 4

Serial section of Fig. 3. The whole area is packed with thick bundles and fragments of resorcin positive fibres,

—Weigerts resorcin fuchsin stain for elastica X 250.

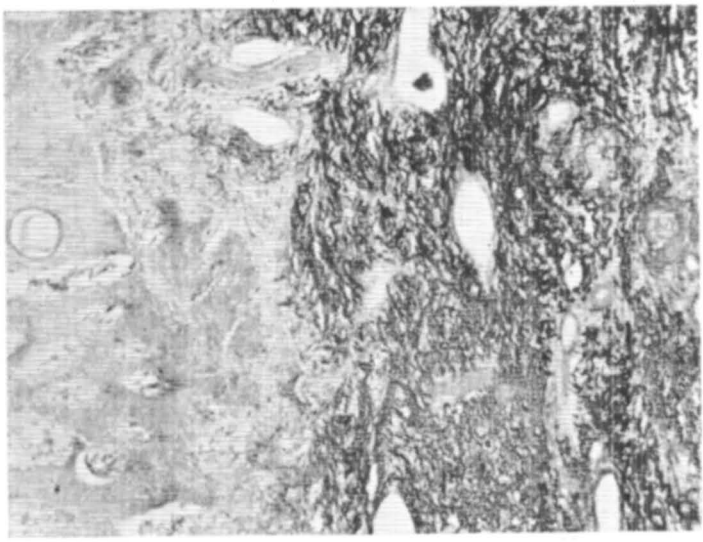

Fig. 5

Section from intraepidermal carcinoma. The dermis is full of resorcin positive material.

—Weigerts resorcin fuchsin stain for elastica X 250.

TYPE OF TISSUE	NO. OF CASES	ALTERATION OF COLLAGEN			PAS			TOL. BLUE MET.			
		Normal	V. Early	Moderate	Advanced	+	++	+++	+	++	+++	++++
NORMAL SKIN	2	2	–	–	–		2		2			
BENIGN ULCERS NO EVID. MALIG.	8	2	6	–	–	4	3	1	7	1		
PAPILLOMA	1	–	–	–	1	–	–	1	–	1		
INTRA EPID. CARCINOMA	1	–	–	–				1				
BASAL CELL CARCINOMA	2	–	–	1	1			2		1		1
SQ. CARCINOMA	13	–		6	7	1	3	9	3	4	5	1
MELANOMA	1	–	–	1	–	1				1		

Fig. 6

According to his description of staining reactions collagen in precancerous dermis and skin cancers appears to be altered to collastin. This alteration is striking and widespread in all the tissues studied making it quite evident that while epithelial overgrowth may be the primary feature of epidermal cancer, the constant presence of connective tissue alteration also seems significant. Vasilev[6], Vernoni G.[7] and Orr[2] postulate changed dermis as a necessary antecedent to epidermal malignancy. It is not possible to recount the sequential order of tissue change in this study. One important observation made by Gillman and his group in cutaneous and vascular dycrasias (1) and by us in an extensive study of cutaneous and mucosal disorders (4) is that the presence of abnormal exogeneous collagen provokes profound hyperplasia and even invasiveness of the overlying epithelium. There is a considerable increase in the acid mucopolysaccharides in the papilloma and all the malignant tumours. This is probably secondary to the basic degeneration of collagen, being released during the degradation of the fibrils themselves.

Electron microscopy of altered collagen shows a degradation of the fibrils into ill defined fiilaments and sheets, similar in structure to elastics. Treatment of the section with elastase removes all resorcin positive abnormal collagen. The altered tinctorial properties, submicroscopic morphology, and enzyme sensitivity of the abnormal collagen suggests that in human epidermal carcinogenesis, the dermal collagen undergoes a hydrogen bond dissociation and a molecular rearrangement. The altered orientation would then be represented in tinctorial and electron optical properties similar to elastica and susceptibility to the enzyme most specific to elastin–elastase.

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