

tion is one to three weeks while that of the dog hookworm is from several weeks to months. We also have the impression that the larvae of the cattle hookworm do not penetrate in as large numbers as those of the dog hookworm. In the instances coming under our observation there was no secondary infection while in the case of the dog hook-

worm larvae this may develop as a result of the prolonged scratching. There is much similarity to the schistosome dermatitis which has been so extensively studied in recent years by Cort,⁵ and his associates. There is, however, no migration in schistosome dermatitis.

⁵ Cort, W. W., *Am. J. Hyg.*, 1936, **23**, 349.

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Histochemistry XIX. Localization of Alkaline Phosphatase in Normal and Pathological Human Skin.*

ISADORE FISHER AND DAVID GLICK.

From the Division of Dermatology and the Department of Physiological Chemistry, University of Minnesota, Minneapolis.

The present study was undertaken in the hope of establishing the histo- and cytologic distribution of alkaline and acid phosphatase and lipase in normal and pathologic human skin. Our results were essentially negative for the acid phosphatase and lipase, so that this report deals with alkaline phosphatase alone. The methods of Gomori¹ for the demonstration of the enzymes were employed throughout. All tissue sections were placed in 0.1 M citrate buffer, pH 4.5-5.0, for 30 minutes before the enzyme staining reaction was applied in order to remove preformed phosphates and other substances which might give a positive reaction. Inasmuch as control experiments on these sections demonstrated no staining whatsoever in the absence of substrate, any staining obtained could be ascribed to enzyme action alone. An incubation period of 12-24 hours in the substrate medium was used. In this study 32 specimens of normal skin, collected from 27 individuals, were selected from the scalp, face, breast, axilla, chest, palm, back, abdomen, penis, scrotum and hip. This selection was made because of the differences in histologic

structure of skin from those sources. The 33 pathological specimens were biopsied from 8 cases of eczema, 2 cases of lupus erythematosus, 2 cases of psoriasis, 1 case of papular urticaria, 2 cases of pyogenic granuloma, 15 cases of healing wounds and scars, and 3 cases of acne vulgaris.

In normal skin, alkaline phosphatase activity was demonstrated in all specimens. There was slight staining in the stratum granulosum in agreement with the findings of Bourne and MacKinnon² who investigated guinea pig skin. The endothelial walls of the capillaries showed the most uniform and clear cut staining effects as previously observed by Gomori³ and others. The reaction was most pronounced in the nuclei of the endothelial cells and the nucleoli showed a stronger reaction than the other parts of the nuclei. The hairs and hair follicles, especially the papillae, gave an intense reaction in much the same manner as found for guinea pig skin by Johnson *et al.*,⁴ and Bourne and MacKinnon.² However, in the present investigation, a

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¹ Gomori, G., *Am. J. Clin. Path.*, 1946, **16**, 347.

² Bourne, G., and MacKinnon, M., *Quart. J. Exp. Physiol.*, 1943, **32**, 1.

³ Gomori, G., *J. Cell. and Comp. Physiol.*, 1941, **17**, 71.

⁴ Johnson, P. L., and Bevelander, G., *Anat. Rec.*, 1946, **95**, 193.

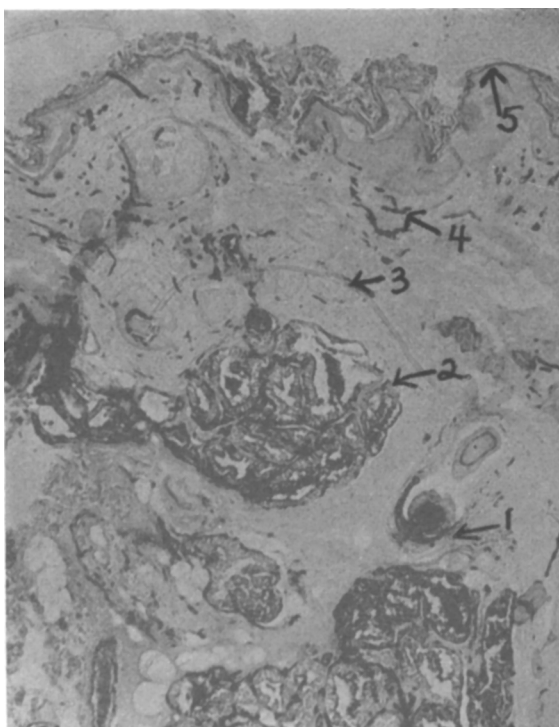


Fig. 1.

Section of axillary skin $\times 75$. Showing slight enzyme activity in stratum granulosum and intense reaction in follicular papilla, capillaries and apocrine glands. (1) Follicular papilla, (2) apocrine glands, (3) sebaceous glands and surrounding vascular bed, (4) capillaries, (5) stratum granulosum.

greater intensity of staining was observed at the base of the follicular papillae which is the site of the nutrient capillary (Fig. 1). This was not previously described in the work on guinea pig skin. The only evidence of alkaline phosphatase activity we were able to demonstrate associated with the sebaceous glands was in the rich capillary bed surrounding them (Fig. 1). This is in contrast to the reports dealing with the work on guinea pigs² in which definite staining of sebaceous glands was reported.

Although previous reports have not mentioned alkaline phosphatase in the sweat glands, these glands showed marked activity with some variation in intensity in the loops. The activity in the apocrine glands was more clearly and uniformly demonstrable than in the eccrine glands. In both, the stain was

fairly diffuse but it was most prominent at the periphery of the cell and in the nucleus (Fig. 2). The heavier staining of the nucleolus as compared to the rest of the nucleus was most clearly apparent in the apocrine cell (Fig. 3).

Among the dermatologic states found to show no abnormal alkaline phosphatase activity are lupus erythematosus, psoriasis, and papular urticaria. Three specimens from different patients with acne vulgaris showed some diffuse deeper staining involving the lymphocytic infiltrate. The greatest change was seen in scar tissue or tissue showing evidence of new fibroblastic proliferation. In two biopsies obtained a few days after closure of a burn wound the nuclei of the fibroblasts gave a stronger reaction than the cellular cytoplasm. Three older scar tissues (2-8

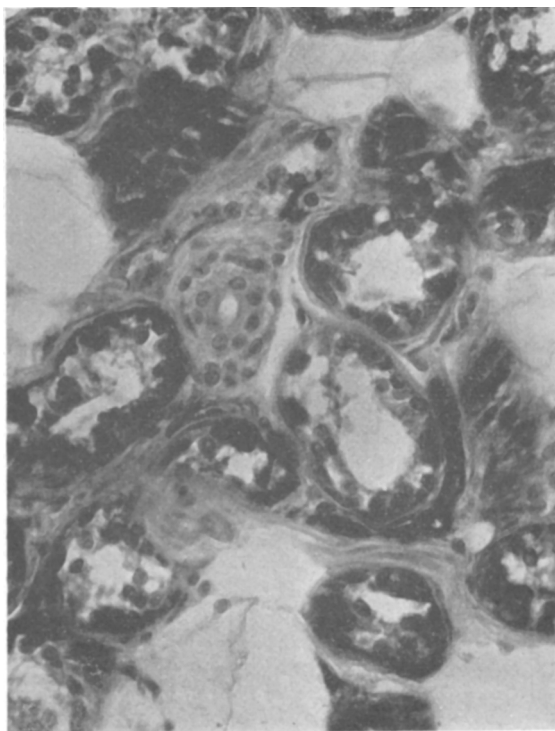


Fig. 2.
Section of eccrine glands $\times 300$. Nuclei, nucleoli and cell walls show positive reactions. The lighter staining sweat duct with its multiple layered cell wall can be seen.

months after healing of an operative wound) showed very definite, though slightly diminished, alkaline phosphatase activity in the fibroblasts (Fig. 4). Still older scars of the same type contained a few scattered groups of fibroblasts giving the positive reaction. These results are in accord with the observations of Fell and Danielli⁵ who described the distribution of alkaline phosphatase in the fibroblasts and new collagen fibers of healing experimental wounds in rats. In the same year, Gomori reported phosphatase positive fibroblasts in connective tissue subjacent to newly formed epithelium in fascial transplants of bladder epithelium.⁶ In a pyogenic granuloma the new fibroblasts reacted in the same fashion as those in scar tissue. The infiltrate, consisting of polymorphonuclear leucocytes and lymphocytes also gave

a positive phosphatase reaction.

A study of the skin in 3 cases of eczema and in 3 cases showing positive allergic skin tests was made. Sections from the sites of positive allergic reactions showed no abnormal change nor did those from relatively acute eczemas. In 3 cases of long standing eczema with infiltration and thickening of the skin the picture was quite different (Fig. 5). Here the phosphatase reaction of the perivascular infiltrate, made up of lymphocytes, monocytes and some plasma cells, was distinctly positive. There was also some diffuse staining of the connective tissue including some of the fibroblasts in the deeper cutis.

Summary. In normal skin, alkaline phosphatase activity was observed in the stratum granulosum, endothelial lining of the capillaries, in hairs, hair follicles, and sweat glands. In the hair follicles, the greatest activity was

⁵ Fell, H. B., and Danielli, J. F., *Brit. J. Exp. Path.*, 1943, **24**, 196.

⁶ Gomori, G., *Am. J. Path.*, 1943, **2**, 197.

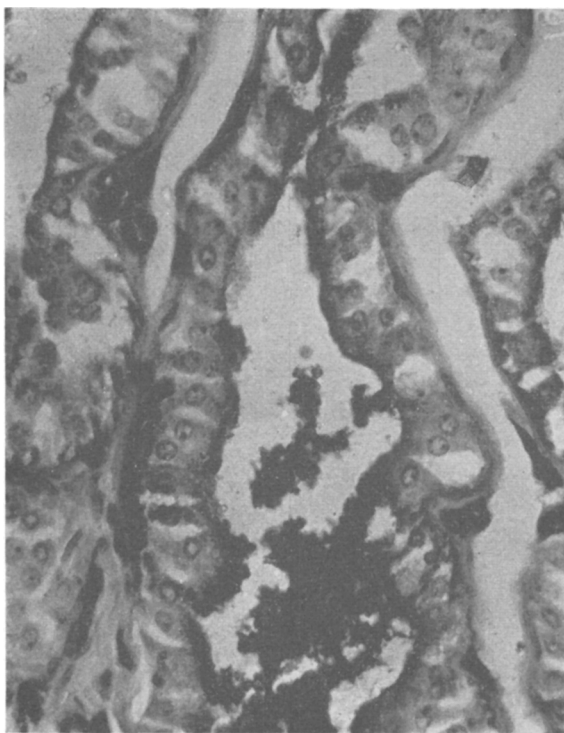


Fig. 3.
Section of apocrine glands $\times 300$. The deeper staining of the nucleoli is apparent.

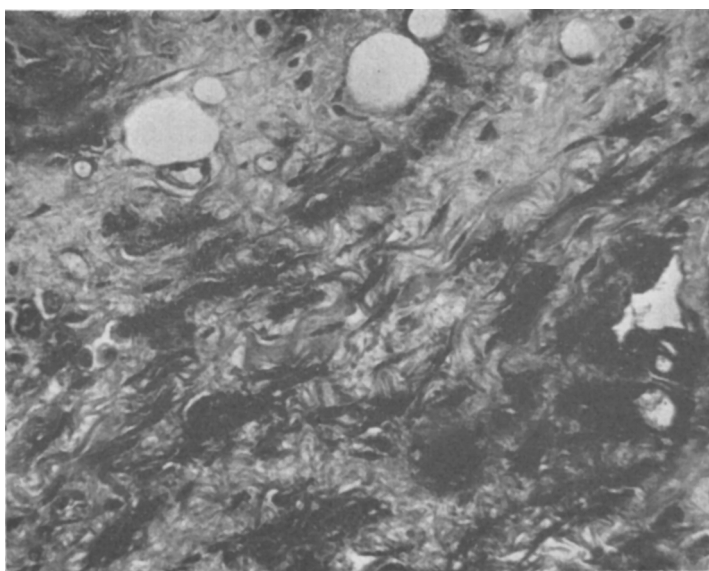


Fig. 4.
Section through a scar (8 mos.) $\times 150$. Enzyme activity pronounced in fibroblasts and connective tissue fibers.

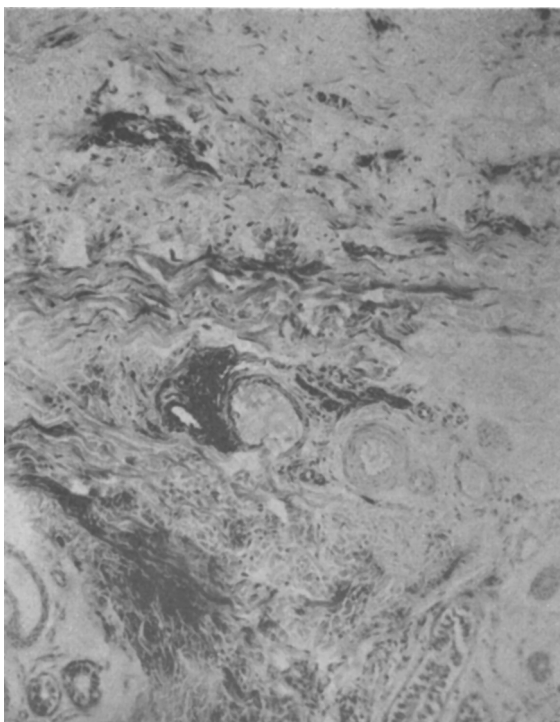


Fig. 5.
Section of chronic eczema. In the deeper cutis, proliferative activity is manifested by the presence of alkaline phosphatase in the new fibroblasts and in the connective tissue fibers. Staining of some of the infiltrate is also shown.

in the papillae. In the endothelial cells and cells of the sweat glands, the enzyme appears to be concentrated at the cell walls and in the nuclei, the nucleoli showing even greater activity than other parts of the nuclei.

Pathologic tissue from cases of lupus erythematosus, psoriasis and papular urticaria showed no significant changes in the enzyme activity.

Alkaline phosphatase activity was found in proliferating fibroblasts in scar tissue, especially in the newly formed scar, and also in the fibroblasts and infiltrate of pyogenic

granuloma. The nuclei gave a more intense reaction than the cytoplasm of the cells.

In chronic eczema of long standing, the cellular perivascular infiltrate of lymphocytes, monocytes and plasma cells, as well as the fibroblasts, showed significant alkaline phosphatase activity.

In acne vulgaris, the infiltrate exhibited the enzyme activity.

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