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HISTOCHEMISTRY OF SWEAT GLANDS*

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The differences in enzyme activity between eccrine and apocrine glands have been often used in investigations of the origin and differentiation of skin tumors (10, 11, 12, 13, 17, 22, 23, 24).

Result reported in the literature regarding the amount and distribution of the enzymes in sweat glands (1, 3, 4, 5, 6, 7, 8, 9, 12, 14, 15, 18, 19, 24, 25) are often controversial because of its regional, developmental and physiological variations. Besides, different histochemical methods used as well as different interpretations of obtained reactions make the problem even more difficult.

In the present study, adult axillary skin was investigated particularly toward enzymes which are claimed to be a reliable basis for sweat gland differentiation.

MATERIAL AND METHODS

Seven volunteers were selected for this study (three Negro males, three Caucasian males, one Negro Female) ranging from 17—53 years of age.

Skin biopsies were taken from axillary regions, immediately frozen in carbon dioxide and stored at —20°C until further treatment. The samples contained epidermis and dermis with hair follicles, eccrine, apocrine and sebaceous glands. The tissues were sectioned in a cryostat at 10—20 microns and stained by the following methods:

1. Phosphorylase (Takeuchi 1956 (19).
2. Branching enzyme (Takeuchi 1958 (20).

For demonstration of the phosphorylase and the branching enzy-

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me activity we used the same incubation solution (Glucose- l-phosphate, adenosin-5-phosphoryc acid,- glycogen and few drops of insulin, acetate buffer pH 5,7). By adding ethanol the activity of branching enzyme was inhibited and only phosphorylase developed. Newly formed polysaccharides were detected by iodine reaction. The sections were mounted in iodine-glycerol, the reactions were reestablished by placing the slides into Gram's iodine solution.

3. Beta glucuronidase (after Seligman 1954 (2)

Cryostat sections postfixed in cold neutral formaline were incubated in a substrate solution at 37°C (6 bromo-2-naphthyl-beta-D-glucuronide dissolved in methanol, phosphate-citrate buffer pH 4,95) and coupled with Fast blue B in 0,02 phosphate buffer pH 7,5.

4. Esterase (Gomori 1952) Naphthol AS acetate method (16).

Gryostat sections postfixed in cold acetone were incubated at room temperature in a solution (1% solution of naphthol AS acetate and propylene glycol, 0,2 M phosphate buffer pH 6,5 and Fast Garnet GBC diazonium salt).

5. Aminopeptidase (Nachlas et all. 1957 (2, 19)

Cryostat sections, postfixed in cold acetone, incubated at 37°C in a solution (L-leucyl-beta-naphthyl amide hydrochloride, 0,1 M acetate buffer pH 6,5; 0,90% sodium chloride and Fast Garnet GBC diazo dye. After incubation sections were washed in saline, placed into 0,5 copper sulphate and rinsed in saline.

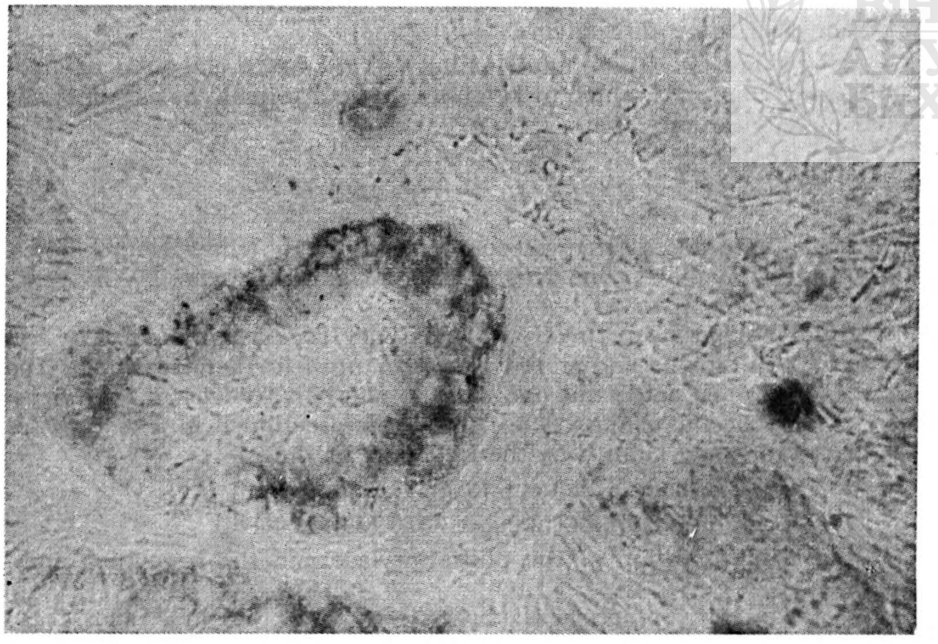

Fig. 1.

Phosphorylase activity in apocrine secretory segment

All slides with developed histochemical reactions were stored at 4°C during the time of analyses (several days). Skin specimens obtained by authopsy or surgery when frozen after several hours showed maked loss of enzyme activity. Immediately frozen fresh specimens preserved enzyme activit for a weak at —20°C. Cryostat sections, however, showed loss of enzyme activity after several days at — 20° C.

RESULTS

Eccrine sweat glands

The secretory segment as a whole showed intense phosphorylase reaction without differences between dark and clear cells activity. However, different parts of the gland, even in the same section, displayed different activities in so far as basal part of the secretory segment evidenced the most intense phosphorylase reaction. The secretory segment developed also beta-glucuronidase, esterase and aminopeptidase activity.

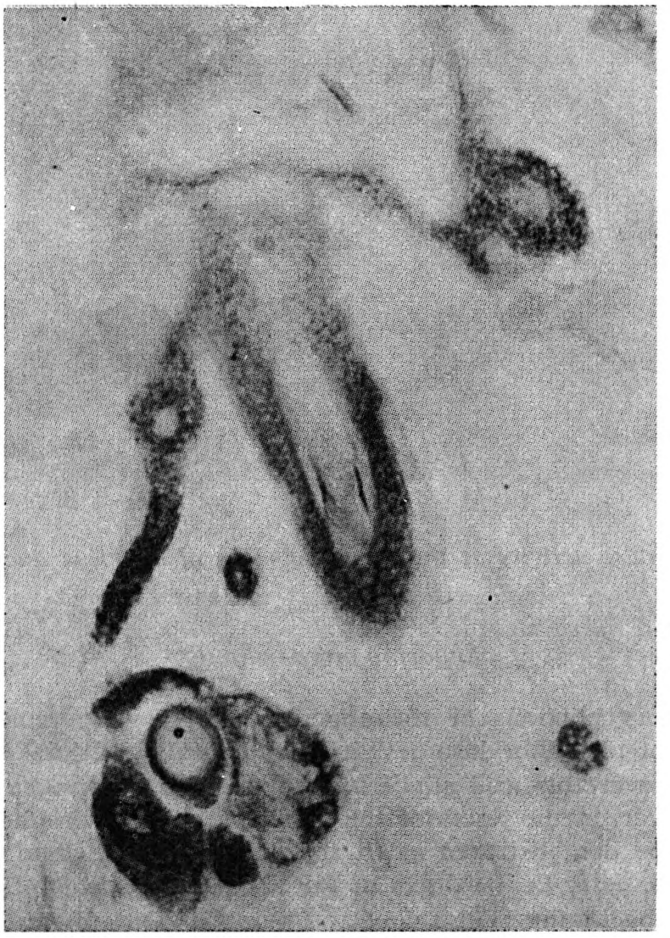

Fig. 2.
Pholphorylase activity in hair follicle and apocrine duct

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The eccrine sweat duct demonstrated also a strong phosphorylase activity in all parts (basal coiled portion, straight segment and in one part of the terminal intraepidermal segment), but could not be followed up to the level of the stratum granulosum. Whereas in the upper part of the intraepidermal duct branching enzyme activity was found. Also beta-glucuronidase activity was well developed, but less intensive than in the secretory segment. The reaction diminished along the duct, toward the epithelial surface. In the duct a moderate nonspecific esterase and slightly positive aminopeptidase activity could also be found.

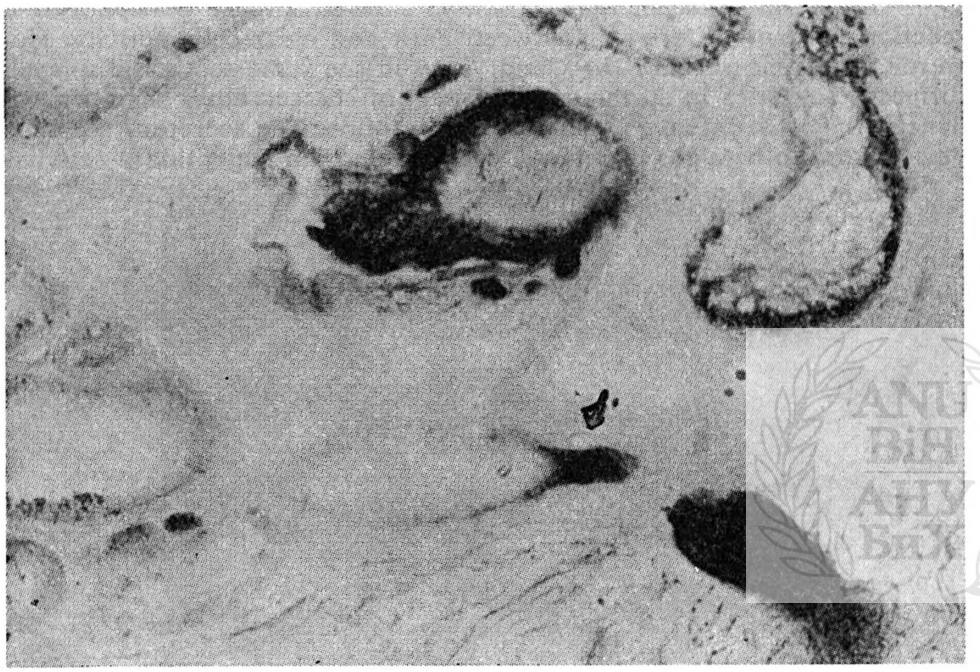

Fig. 3.
Phosphorylase activity at the very beginning of apocrine duct (arrow)

Apocrine sweat glands

Secretory portions of the apocrine glands were mostly without any traces of phosphorylase activity, but in some sections in the basal part of the secretory coil slight to moderate patchy reaction could be seen. However, in the ducts of the apocrine glands phosphorylase activity could be demonstrated as in the eccrine sweat gland ducts. Phosphorylase activity in the ducts of the apocrine swat glands was found at the very beginning of the duct and along its way toward the epidermis as far as the opening into the pilary canal above the level of the sebaceous glands.

The apocrine duct could easily be distinguished from the secretory parts by smaller diameter, cuboidal epithelium and sharply outlined lumen.

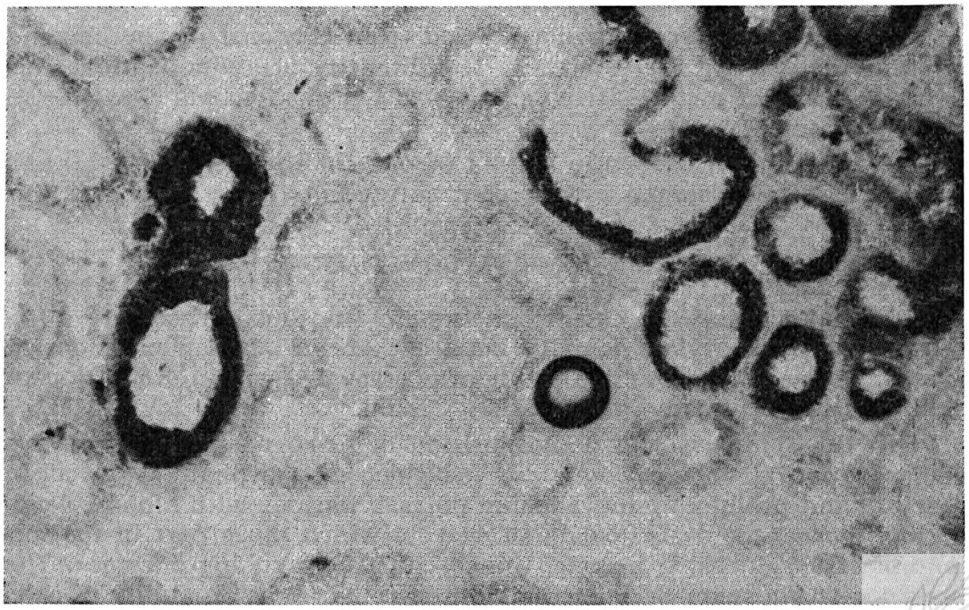

Fig. 4.
Non-specific esterase activity in apocrine secretory segment

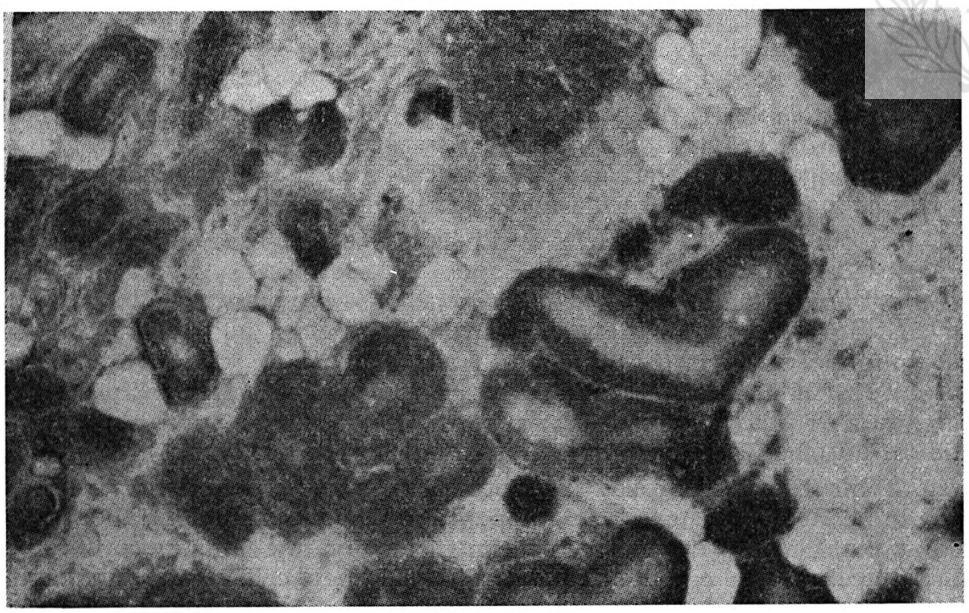

Fig. 5.
Aminopeptidase activity in apocrine gland

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The most intense blue color of the beta- glucuronidase activity was seen in the secretory parts of the apocrine glands with occasional red droplets on the inner surface or in the lumen of the gland itself. In the duct the reaction was less marked and lessened toward the surface.

Nonspecific esterase activity varied from one part to another and in some the activity was very low. The intensity of aminopeptidase activity also varied and positive reaction was probably bound to the secretory granula.

Table I. summarizes the results of our findings and table II. and III. the results presented by different authors.

DISCUSSION AND CONCLUSIONS

Investigations were performed first to find out if there are differences in phosphorylase activity between eccrine and apocrine sweat glands. These differences are frequently suggested in differentiation of skin appendage tumors (10, 11, 12, 21, 22, 23).

In the synthesis and decomposition of glycogen in epidermis beside phosphorylase which catalyse the glucose-l-phosphate — glycogen reaction and produces nonbranching polysaccharides with alpha-l, 4-glucoside lincage, there also exists an enzyme which takes part in the formation of 1,6-glucoside lincage, the so called »branching enzyme«. The presence of phosphorylase has been interpreted (4) as an ability of cells to produce glycogen sythesis, but the distribution of this enzyme is not always accompanied by the presence of glycogen e.g. the basal layer of epidermis and cells in basal cell epitheliomas. Probable the many controversial information regarding the phosphorylase distribution are caused by the fact that the branching enzyme frequent is masking the phosphorylase activity in histochemical reactions. In our investigations we could develope both enzymes separately and parallely.

In the Table II., are summarized the findings of the phosphorylase and branching enzyme distribution in sweat glands structures by different investigators. The greatest differences are existing in regard to the secretory parts of the eccrine and apocrine sweat glands.

In our investigations we could demonstrate some phosphorylase activity also in adult skin in the apocrine secretory segment although in small amounts which never has reached the intensity usually present in eccrine secretory portions. However there was no differences in phosphorylase activity in the ductal parts between apocrine and eccrine structures. If ductal structures, play any role in genesis of skin appendage tumors, phosphorylase according to our results, cannot be used as indication of one or the other (apocrine or eccrine) origin.

Table III. summarizes the findings by various authors in respect of the activity of esterase, aminopeptidase and beta glucuronidase in skin sweat glands. In our investigations we could not find any conclusive differences in the activity of these enzymes between eccrine and apocrine sweat gland structures.

The differences found were actually only quantitative in nature without strong criteria which could be easily influenced by subjective

estimation. Consequently the histochemical findings alone can not be decisive in differentiation or histogenesis of the skin appendageal tumors.

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Table I

HISTOCHEMICAL REACTIONS IN ECCRINE AND APOCRINE GLANDS

Enzyme	Eccrine		Apocrine	
	secretory segment	duct	secretory segment	duct
Phosphorylase	+	+	±	+
Branching enzyme	+	+	—	+
Aminopeptidase	+	+	+	+
Esterase	+	+	+	+
beta-glucuronidae	+	+	+	+

Table II

PHOSPHORYLASE AND BRANCHING ENZYME IN ECCRINE AND APOCRINE GLANDS (LITERATURE DATA)

Structure	enzyme		Authors
Eccrine			
secretory coil	phosphorylase	+	Braun-Falco 1956, Ellis and Montagna 1958, Yasuda et all. 1958, Hashimoto and Lever 1968.
intradermal duct	phosphorylase	+	
intraepidermal duct	phosphorylase	+	
Apocrine			
Secretory segment	phosphorylase	—	Ellis and Montagna 1958, Yasuda, Furusawa and Ogata 1958, Montagna 1962, Hashimoto and Lever, 1968
Secretory segment in children	phosphorylase	+	Montagna 1959
secretory segment	Branching enzyme	—	Ellis and Montagna 1958 Hashimoto and Lever, 1968
Apocrine duct	phosphorylase	+	Ellis and Montagna 1958, Montagna 1962, Hashimoto and Lever, 1968

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Table III

ESTERASE, AMINOPEPTIDASE AND BETA GLUCURONIDASE ACTIVITY IN ECCRINE AND APOCRINE GLANDS (LITERATURE DATA)

Structure	esterase	aminopeptidase	beta-glucuronidase
Eccrine secreetory segment	+ Steigleder and Shultis 1957	+ Montagna 1962	+ Braun-Falco 1956 + Montagna 1957
	+ Montagna and Ellis 1958	Hashimoto and + Lever 1968	— Hashimoto and Lever 1968
	Hashimoto and Lever 1968 (indoxyl esterase)		
eccrine duct	+ Steigleder and Shultis 1957	+ Montagna 1962	— Hashimoto and Lever 1968
	Montagna and + Ellis 1958	Hashimoto and Lever 1968	+ Montagna 1957 + Braun-Falco 1956
	Hashimoto and Lever 1968		
Apocrine secretory coil	+ Montagna and Elis 1963	+ Adachi and Montagna 1961	+ Hashimoto and Lever 1968
apocrine duct	+ Montagna and Ellis 1963	— Adachi and Montagna 1961	+ Hashimoto and Lever 1968
		+ Braun-Falco and Rupec, 1968	

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HISTOKEMIJA ZNOJNIH ŽLIJEZDA

KRATAK SADRŽAJ

Ispitivanja su posvećena prvenstveno enzimima ekrinih i apokrinih znojnih žlijezda koji se koriste u diferenciranju kožnih tumora.

Ispitane su ekrine i apokrine znojne žlijezde u odnosu na prisustvo fosforilaze, aminopeptidaze, beta-glukuronidaze i nespecifične esteraze.

Rezultati ispitivanja pokazuju da su razlike između ekrinih i apokrinih žlijezda u odnosu na ispitivane enzime samo kvantitativne prirode. Najveće su razlike u fosforilazi u sekretornim dijelovima ekrinih i apokrinih žlijezda, dok su kanali ovih žlijezda histokemijski ne razlikuju.

Ova ispitivanja su pokazala da su histokemijski rezultati sami nedovoljni za donošenje zaključaka o diferenciranju ili histogenezi tumora kože.

REFERENCES

1. Adachi K. and W. Montagna: Histology and cytochemistry of human skin. XXII Sites of leucine aminopeptidase LAP. J. Invest. Derm. 37, 145, 151, 1961.
2. Barka T. and Anderson P. J.: Histochemistry. Harper and Row eds. New York, 1965.
3. Braun-Falco O.: Zur Histotopographie des beta glucuronidase in normaler menschlicher Haut. Arch. Klin. exp. Dermatol. 203, 61—67, 1956.
4. Braun-Falco O.: Über die Fähigkeit der menschlichen Haut zur Polysaccharidsynthese, ein Beitrag zur Histotopochimie der Phopsphorylase. Arch. Klin. exp. Dermatol. 202, 163—170, 1956.
5. Braun-Falco O.: Histochemical Demonstration of Lap in normal and pathological Skin. J. Cytochem. Histochem. 5, 94, 1957.
6. Braun-Falco O. und M. Rupec: Apokrine Schweisdrüssen. In Jadassohn: Handbuch der Haut und Geschlechtskrankheiten Bd I/1, Springer Verlag, New York, 1968.
7. Ellis R. A. and Montagna W.: Histology and citochemistry of human skin. XV. Sites of phosphorylase and amylo-1, 6-glucosidase activity. J. Histochem. Cytochem. 6, 201—207, 1958.
8. Ellis R. A.: The fine structure of eccrine sweat glands. In Advances im biology of Skin (W. Montagna, R. Ellis and A. Silver eds) vol. 3, New York, Pergamon Press, 1962.
9. Ellis R. A.: Eccrine sweat glands. In Jadassohn: Handbuch der Haut und Geschlechtskrankheiten, BdB I/1. Springer Verlag, New York, 1968.
10. Grin E. I. and Švob M.: Histogenesis of coexisted cylindroma and trichoepithelioma. Radovi — XL, Akad. nauka i umjet. BiH, knjiga 15 : 155—166, 1970.
11. Hashimoto K. and Lever W. F.: Eccrine Poroma. Histochemical and electron microscopic study. J. Invest. Dermatol. 43, 4, 237—249, 1964.
12. Hashimoto K. and Lever W. F.: Appendage tumors of the skin. Charles C. Thomas Publ. Springfield, 1968.
13. Holubar K. und Wolff: Zur Histogenese des Cylindroms. Arch. Klin. exp. Derm. 229, 215—226, 1967.
14. Montagna W.: Histology and cytochemistry of human skin. XI. The distribution of beta glucuronidase. J. Byophys. Biochem. Cytol. 3, 343, 1957.
15. Montagna W. and Ellis R. A.: The histochemistry of skin and cutaneous glands. In: 1 st Int. Congr. Histochem. Cytochem. p. 447—464, Oxford Pergamon Press, 1963.
16. Pearce A. G. E.: Histochemistry. Little Brown, Boston, 1961.
17. Pinkus H. und A. H. Mehregan: Das ekkrine Porom und seine klinischen Varianten. Hautartzt, 15, 10, 561, 1964.
18. Steigleder G. K. and K. Shultis: Zur Histochemie der Esterasen der Haut. Arch. Klin. exp. Derm. 205, 196—211, 1957.
19. Steigleder G. K. und Löffler H.: Zum histochemischen Nachweis unspezifischer Esterasen und Lipasen. Arch. Klin. exp. Derm. 203, 41, 1956.
20. Takeuchi T.: Histochemical demonstration of phosphorylase. J. Histochem. Cytochem. 4, 84, 1956.
21. Takeuchi T.: Histochemical demonstration of branching enzyme in animal tissues. J. Histochem. Cytochem. 6, 208, 1958.

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22. Urbach F. and Graham J., Goldstein J. and E. B. Helwig: Dermal eccrine Cylindroma. Arch. Derm. 88, 880—894, 1963.
23. Winkelmann R. K. and V. A. Mc Leod: The dermal duct tumor. Arch. Derm. 94, 50, 1966.
24. Winkelmann R. K. and Gottlieb B. F.: Syringoma: an enzymatic study. Cancer 16: 665, 1963.
25. Yasuda K. Furusawa H. and Ogata N.: Histochemical investigation on the phosphorylase in the sweat glands of axilla. Okajima Folia Aant. Japon. 31: 161—169, 1958.

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