PATHO-HISTOCHEMICAL STUDIES ON THE SWINE PARAKERATOSIS

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by

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Introduction

Parakeratosis in swine was first reported by KERNKAMP and FERRIN(1) in 1953. Since then numerous on the clinical syndrome, pathology, etiology, treatment, and prevention have been published (1—6). No clear identification in pathology has been done in Japan until now, but the outbreak of this disease has called the attention on the animal feeding. In 1961 and 1963 a kind of skin disease was found in rapidly growing pigs under good management practices and well-balanced nutrition; these were used for the experiments in the Miyagi Prefectural Agricultural Experimental Station in Sendai.

The purpose of our study was to attempt to clarify the outbreak of swine parakeratosis in Japan and to find the histochemical natures of lesions as the pathological diagnosis.

KERNKAMP (1) reported the following symptoms of parakeratosis in swine: the over-growth of the dermal papillae, acanthosis with hyperemia, enlargement of cornified keratin layer, alopecia, absence of the granular layers of the epidermis, retention of nuclei in the thickened parakeratic layers and mixture of cellular debris. Owing to the histological examination, the skin lesions which occurred in the pigs in the Miyagi Prefectural Agricultural Experimental Station were similar to KERNKAMP's findings. The histochemical observations of which there was no report up to now, indicated the following findings: the retention of desoxyribose nucleic acid (DNA) in the cells of the parakeratic layers, existence of parakeratic materials with glucoprotein, incomplete disappearance of glycogen in the keratogenic layers, and degeneration of the sebaceous glands.

Materials and Methods

Four experiments were carried out to study the effects of feeds on the growth of pigs (Yorkshire piglets) and 16 piglets were used each year. The feeds were administered with high protein-moderate energic group which were mixed with barley produced in Miyagi Prefecture. The ratio of the mixture in the basic rations was different from in the former and later periods. Chemicai analysis of the used feeds was made by the Miyagi Prefectural Agricultural Experimental Station, and the results obtained to date are indicated in Table 1. Pigs in each lot were fed the above-mentioned ration with free choice under the drylot-feeding conditions. They were given free excercise for

Table 1. Rations, especially the Ratio of Basic Concentrates.

Basal Ratians			1961 C′	1961 A′	1961 H′	1961 E′	1963 C′	1963 A′	1963 H′	1963 E′
Year / Concentrate : Barley / Lot			7 : 3	7 : 3	6 : 4	6 : 4	7 : 3	7 : 3	6 : 4	6 : 4
Former term	Ratio of basal ration	Yellow corn	18	25	32	36	15	16	32	36
		Bran	19	11	3	6	21	20	3	6
		Defatted Rice-bran	13	20	12	7	8	6	2	2
		Soybean oil meal	9	5	5	3	10	4	12	7
		Fish meal	5	3	3	3	5	7	5	3
		Starch meal					5	11		
		Others	6	6	6	6	6	6	6	6
	Barley		30	30	40	40	30	30	40	40
Later term	Concentrate : Barley		7 : 3	7 : 3	6 : 4	6 : 4	7 : 3	7 : 3	6 : 4	6 : 4
		Yellow corn	22	29	36	40	19	20	36	40
		Bran	21	13	5	8	23	22	5	8
		Defatted Rice-bran	13	20	2	2	8	7	2	2
		Soobean oil meal	5	1	8	3	6	2	8	3
		Fish meal	3	1	3	1	3	2	3	1
		Starch meal					5	11		
		Others	6	6	6	6	6	6	6	6
	Barley		30	30	40	40	30	30	40	40

two hours per day, boars were castrated at the age of 23 or 24 days after birth, and the hogs and sows were used as follows. These animals were investigated continually for 123 days. Rations in 1961, especially on the A′ and C′ lots, were lower in administered amounts of corn and barley than that on the H′ and E′ lots, and the rations in 1963 were similar to that in 1961.

According to the literature about the experimental occurrence of para-

keratosis, the disease might be caused by essential fatty acid deficiency (KERNKAMP) (1), by a decrease of the digestibility of the lipids in the gastrointestinal tract under the high calcium levels in the rations (LEUCK) (2) and by zinc deficiency (TUCKER) (3). KERNKAMP (4) noticed that parakeratosis and greasy pig disease were similar with each other in a number of ways, and that both diseases are characterized by skin lesions, reduced rates of growth, and evidence of skin infection. In the corn belt states of U. S. A. in the last decade, these diseases have been encountered as distinct clinical entities. High calcium was known to be associated with decreased lipid digestibility in the gastro-intestinal tract, and the necessity of the fat and unsaturated fatty acids in the rations had been given attention for the rapidly growing pigs.

Essential fatty acid deficiencies have been reproduced in the rat by BURR *et al* and in the dog by HANSEN (6). Some of the specific changes have been seen in the skin of the essential fatty acid and by a decrease of the digestibility of the fat in the ration under high levels of calcium. On the one hand he found that the prevention of parakeratosis by administering with a ration with 10 percent of soybean oil containing 54 percent of the linoleic acid, the treatment of parakeratosis by supplementing with zinc sulfate at 200 p. p. m., the treatment of parakeratosis by administering with a ration of 10 percent of alfalfa meal, and the depression of the calcium level at 0.5 to 0.6 percent had considerable therapeutic value for herd treatment.

There were found no clear relationships between parakeratosis and the causes because of no measurement of specific chemical components such as linoleic acid, essential fatty acid, and calcium. The chemical composition in the mixtures with the basal ration and barley was analysed as shown in Table 2. Comparing with the ration in the former period, there was a decrease of raw

Table 2. Chemical Composition of Ration (Analysis of mixtures with basal ration and barley)

Exper. Year	Period / Chemical Component / Lots	Former term				Later term			
		Basal ration : Barley	Raw Protein	Raw Fat	Raw ash	Basal ration : Barley	Raw Protein	Raw Fat	Raw ash
1961	C′	7 : 3	17.31	3.19	6.29	7 : 3	not analysed		
	A′	7 : 3	14.52	3.51	4.66	7 : 3			
	H′	6 : 4	18.94	2.73	5.53	6 : 4	16.12	2.07	5.27
	E′	6 : 4	15.20	2.80	9.00	6 : 4	13.40	2.34	4.78
1963	C′	7 : 3	19.62	1.99	7.15	7 : 3	17.66	2.16	7.03
	A′	7 : 3	17.87	1.81	8.62	7 : 3	14.40	2.02	7.87
	H′	6 : 4	18.94	2.76	5.46	6 : 4	16.20	2.10	5.21
	E′	6 : 4	16.30	2.46	4.97	6 : 4	12.82	2.37	4.72

Table 3. Clinical Finding of the Affected Pigs in Parakeratosis

Exper. Year	Exper. Lot	♀, ♂ Sex (Cases)	Age after birth: Beginning (dys.)	Age after birth: Affection (dys.)	Age after birth: Finishing (dys.)	Body-weight (kg): Beginning period	Body-weight (kg): Middle period	Body-weight (kg): Terminal period	Body-weight (kg): Increase +	Uptake of feed (all periods) kg: Beginning period	Uptake of feed (all periods) kg: Middle period	Uptake of feed (all periods) kg: Terminal period	Localization of lesions: Shoulder	Back	Lumbar	Thigh, femur	Blank	Abdomen	Pastern
1961	C′	♀ 2	71	140	194	20.0	50.5	65.5	+45.5	179.1	60.8	239.9 (479.8)	—	—	—	0	0	0	0
		♀ 2	70	97	—	14.0	20.2	20.2	+6.2				—	—	—	0	0	0	0
		♂ 2	71	—	194	23.9	63.9	89.9	+66.0	335.4	262.8	598.1 (1196.2)	—	—	—	—	—	—	—
	A′	♀ 2	71	114	—	21.6	34.4	34.4	+12.8	161.9	67.1	229.0 (458.0)	—	—	—	0	0	0	0
		♀ 2	70	114	193	15.2	45.1	49.4	+34.2				—	—	—	0	0	0	0
		♂ 2	71	—	194	20.3	61.3	77.9	+57.6	293.5	197.3	490.8 (981.6)	—	—	—	—	—	—	—
	H′	♀ 2	71	—	194	21.5	51.1	88.6	+67.1	199.4	297.6	477.0 (1359.0)	—	—	—	—	—	—	—
		♂ 2	71	194	194	18.6	48.4	98.1	+79.5	160.7	418.6	579.3 (1158.6)	—	—	—	—	—	—	—
	E′	♀ 2	71	—	194	21.4	55.8	101.6	+80.2	220.9	405.3	626.1 (1152.3)	—	—	—	—	—	—	—
		♂ 2	71	—	194	18.1	46.7	92.1	+73.9	175.2	374.1	549.4 (1098.7)	—	—	—	—	—	—	—
1963	C′	♀ 2	70	—	196	18.2	69.0	117.4	+98.2	185.4	205.5	390.9 (781.8)	—	—	—	—	—	—	—
		♂ 2	70	—	196	19.0	61.9	108.5	+89.5	267.9	383.5	651.4 (1302.8)	—	—	—	—	—	—	—
	A′	♀ 2	70	—	196	18.2	59.3	103.9	+85.7	248.2	368.0	616.2 (1232.4)	—	—	—	—	—	—	—
		♂ 2	70	—	196	17.9	64.0	103.9	+86.0	307.5	352.3	659.8 (1319.6)	—	—	—	—	—	—	—
	H′	♀ 2	70	—	196	17.9	63.0	98.8	80.9	251.4	312.0	563.4 (1126.8)	—	—	—	—	—	—	—
		♂ 2	70	—	196	18.2	71.6	118.4	+100.2	335.3	367.3	702.6 (1405.2)	—	—	—	—	—	—	—
	E′	♀ 2	70	114	196	17.0	52.8	82.6	+65.6	265.9	190.5	456.4 (912.8)	—	—	—	0	0	0	0
		♀ 2	70	117	196	15.7	46.4	80.4	+64.7				—	—	—	0	0	0	0
		♂ 2	70	—	196	18.0	68.0	112.2	+94.2	327.4	352.1	679.5 (1357.0)	—	—	—	—	—	—	—

Remarks : In the affected lot were shown the results of every individual cases.

protein and raw fat at the ration in the later period, but no difference was found between the affected lot and non-affected lot.

1. Clinical Findings

The skin deseases occurred in 25 percent of 16 pigs in 1961 and 13 percent of 16 pigs in 1963. The periods of occurrence, body-weight, appetite from the point of feed-uptake and dermal lesions are indicated in Table 3.

Generally, sows took less uptake of feeds than the boar after the middle period. The pigs affected with dermal disease during 97 to 140 days after the beginning of the experiment, and parakeratosis appeared at the time of a half to three fourths of the all experimental periods. The difference of occurrence of parakeratosis was that the sows were affected whereas the boar was healthy.

The earliest detectable lesion of parakeratosis was the presence of red papules on the underside of the abdomen and flank areas of the pigs but the tips of the papules might not be covered by keratin. Thereafter, keratin began to build up in the regions of the flank pastern, fetlock, and hock. The nasal, periorbital, and aural regions of the head showed similar lesions in the disease. Parakeratosis was characterized by hard, dry, and crusted proliferations of the superficial layer of the epidermis. The regions of the shoulder, withers, saccrum, thigh, flank, and abdomen were vulnerable areas and many times were the first to show the crusted proliferations. This disease did not seem to cause the patients much discomfort or distress. They did not scratch or rub the body, indicating that the lesions did not cause an irritation. Except in severe cases, the appetite and food intake were not impaired. Some, however, lost weight during the course of the disease. The presence of keratinous crusts on the surface of the skin constitutes the chief symptom. In regions of the body where the hairs were short, the crusts were generally compact and their surface was granular. The crusts sometimes became 5 to 7 mm thick and, as a rule, they were not firmly attached to the underlying cutaneous structures. The crusts were separated by clefts or crevices which often contain a moist and somewhat sticky brownish to black-colored substance. This was an admixture of a secretion from cutaneous glands and particles and their debris.

2. Patho-histological changes

The lesions were due to unsaturated fatty acid deficiency in the rat and dog which received as extensive reference to the lesions of swine parakeratosis. According to SINCLAIR (7), the skin of the rat showed the following changes: increase of the MALPIGHI's and granular layers in the epidermis and pronounced hyperkeratosis, globule-formation of keratin, direct contact of the keratotic lamellae with stratum granulosum, acanthosis and hyperkeratosis in the lining epithelium of the opening of hair follicles which became plugged, dilation of

capillaries in the dermis, hypertrophy of sebaceous glands, and dryness of the skin due to an obstruction of the outflow of sebum.

According to HANSEN's report on the canine parakeratosis, the skin was seen to be subjected to the following changes: the thickening of the epidermis with palisade and peg formation, extensive edema of the dermis with fragmented collagen and swollen endothelial cells, hyperkeratinization of the hair follicles plugged of the hair shafts and enlargement or shrinkage of sebaceous glands.

KERNKAMP (1953, 1955) (1) stated that parakeratosis was characterized by hard, dry, and crushed proliferations of the superficial layer of the epidermis. According to his studies the histological pictures were as follows: crusted masses composed of keratin and debris, large numbers of nuclei scattered through the stratum corneum, rete-pegs formation and increase of basal cell layer. Also he studied on biopsied skin specimens as upon which the following histological changes were observed: crusted masses composed of cornified epithelium, collection of paraeleiden (keratin) and debris, thickening of parakeratotic layers, piles of cornified layers, desquamation of stratum corneum, scattered distrubution of nuclei throughout the proliferated and keratinized substance, atrophy of MALPIG's layer, and collections of granulocytes and masses of amorphous material at the base of clefts. He stated that the inspection of a cadaver for significant tissue changes in the digestive, respiratory, and urogenital organs was limited, and sections from the various organs were taken for histological examination but no significant changes were observed.

As the conclusion by KERNKAMP(1), it was a primary disease of the skin, and was marked by tissue alterations such as characterized parakeratosis. Parakeratosis, according to LEVER (8), signified an imperfect keratinization of the skin with retention of nuclei in the horny layers. It was different from hyperkeratosis characterized by an increased amount of keratinized substance in the epidermis and devoid of nuclei.

Histopathological views of six cases of swine parakeratosis which occurred in the Miyagi Prefectural Agricultural Experimental Station showed the following indications of skin lesions: as shown in Table 4, there were characterized parakeratotic globules in the stratum corneum, and irregular arangement of cornified layers.

There were found a decrease or disappearance of stratum granulosa and that of keratohyalin granules and the appearance of eosinophilic inclusion in the granular layer. Dyskeratotic cells or corpus ronds found in dyskeratosis, were similar to the disappearance of keratohyalin granules or eosinophilic inclusions in the parakeratosis. Acanthosis in the stratum spinosum was hyperkeratosis of papillary layers and reticular bifurcation of epithelial folding like rete-peg. This acanthosis was found in large amount in this desease, and also we found the hyperkeratosis of the hair follicle, and parakeratosis in the

Table 4. Histopathological Changes in the Skin of Swine Parakeratosis.

Skin layers / Histo-Pathological Changes	Feeding	Medium caloric medium protein diet		Medium caloric high protein diet		Medium caloric high protein diet	
	Period	Autumn 1961		Autumn 1961		Autumn 1963	
	Name of pig	A′—5	A′—16	C′—6	C′—18	E′—4	E′—5
Stratum corneum	Palisade formation	++	+++	++	+++	++	++
	Globules with parakeratosis	++	+++	++	+++	++	++
	Keratotic lamella	+	+	++	+	++	++
	Increase of rete peg	++	+++	++	++	++	++
	Retention of nuclei	+++	+++	+++	+++	+++	++
Stratum granulosum	Disappearance or decrease	++	+++	++	++	++	+
	Disappearance of keratohyalin	++	+++	++	++	++	+
	Inclusion	++	+++	−	++	+	−
Str. germinativum	Thickening	++	+	+	+	+	−
Stratum spinosum	Acanthosis	++	+++	++	+++	++	+
Papillary and hair follicle	Hyperkeratosis of hair follicles	++	+++	++	++	++	+
	Elongation or folding in papillary layers	++	+++	++	+++	+++	+
	Parakeratosis in orifice of hair follicle	+	++	+	+	+	+
Dermis.	Increase of capillaries	++	+	+	+	++	+
	Degeneration of sebaceous glands	+++	+++	++	++	+	−
	Abscess formation	+	++	−	−	+	−
	Thrombosis in the orifice of hair follicles	+	++	+	+	+	+
	Perivascular cell infiltration	++	++	++	++	++	+

orifice of the hair follicle with the thrombus, degenerative atrophy of the sebaceous glands. In the dermis there were shown proliferation of the capillary vessels and cell-infiltration around the blood vessels. In some cases the elongation or overgrowth of the papillary layers and abscess at the bottom of remarkable dentine process were observed.

3. Histochemical findings

As the previous description in the part of histopathological findings, parakeratosis was characterized by the retention of nuclei in the cornified layers, parakeratotic globules in the palisade formation, disappearance of the keratohyalin granules and eosinophilic inclusion in the granular layers, and degenera-

tive sebaceous glands with the plugging of the hair follicles, acanthosis and rete-peg formation. For the sake of histochemical observation on these findings, the following methods were used for studies: FEULGEN-reaction for the demonstration of desoxyribose nucleic acid (DNA), BRACHET's pyronine-methyl green staining for depolymerized DNA, rinose-nucleic acid (RNA) and polymerized DNA, McMANUS's periodic acid-SCHIFF (PAS) reaction for polysaccharides, PAS reaction after saliva-digestion for glycogen, DUIJN's acrolein-SCHIFF reaction for protein, staining by acidic dyes such as eosin or picrinic acid for protein, Sudan III or black B staining for lipids, GOMORI's alkaline phosphatase staining, GOMORI's lipase staining, SELIGMAN's nitro-blue-tetrazolium staining for succinic dehydrogenase.

The results histochemically studied on the swine parakeratosis are shown in Table 5:

Table 5. Histochemical Changes of the Skin Lesions of the Swine Parakeratosis

Histochemical reaction			Skin layers / Pathological changes	Stratum corneum: Palisade formation	Parakerat. globules	Keratinic lamellae	Rete-peg formation	Retention of nuclei	Mucin production	Enzymatic activity	Keratin formation	Glycogen deposition	stratum granulosum: Keratohyalin granule.	Mitochondria, DHGase	Inclusion	Glycogen-deposition	Enzymatic activity	Str. spinosum: Acanthosis	str. basale: Enzymatic activity	Glycogen deposition	Mitosis	Plug in orifice of hair follicle	Dermis: Degen. Sebaceous glds	Perivascular cell infiltration
High-polymer of DNA	FEULGEN reaction	Nuclei		+		+	+	+											+			+			+
	Methyl-green -phylic			+		+	+	+											+			+		+	+
Depolym. DNA	Pyroninophylic			+			+	+											+					+	
RNA																			+			+			+
Poly-saccharide	PAS-reaction	cytopl-asms		+	+	+		+				—	+		+	+				+			+		
	PAS-reaction after saliva digestion			+	+	+		+				—	+		+	—				—			+		
Protein	Acrolein-Schiff reaction			+	+	+	+	+		+			+		+			+		+		+	+	+	+
	Van GIESON Staining (picrinic acid-phylic.)			+	+	+																			
	H-E. Staining (Eosinophylic)			+	+	+		+		+			+		+										
Lipid	Sudan III or Black B-Stain.																						+	+	
Dehydro-genase	SELIGMAN's Nitro-B T. stain									—			+			+		+		+					
Phospha-tase	GOMOLI's alkaline P-ase			+	+	+	+			+							+		+		+				
	GOMOLI's acid P-ase			+	+	+	+			+							—		—		—				
Lipase	GOMOLI's method									+									—						

Owing to ROTHMAN's (9) "Physiology and Biochemistry of the skin", 1) the cellular components, especially complete decomposition of phospholipids, 2) disappearance of a large amount of water, and 3) disappearance of nuclei and decomposition of nucleoproteid in the parakeratosis were noticed. BERNARD (10) found that there was an immense quantity of glycogen in the soft cornified layers and glycogen disappeared by the keratinization process. Recently SMITH and PARKHURST (11), and UNNA (12) reconfirmed these facts, but the relationship between the keratinization and glycogen-synthesis has remained unknown. ATP of high energy bond might correspond to glycogen synthesis and the degradation of glycogen in the keratinization might be supplied from the energy to the chemical reaction which need keratinization. According to BULLOUGH(13) the retardance of mitosis by the disturbance of glycogen-synthesis such as the muscular movement or coldness and remarkable acceleration of mitosis by the glycogen-synthesis such as the sleep and rest were found. BRADFIELD (14) stated that glycogen was used to supply energy to the keratinization and all the glycogen has been used up by the time that keratinization was complete.

The stratum granulosum and the stratum lucidum could be regarded as "transitional" layers between non cornified and cornified epithelium. The cells of these layers contained microscopically visible corpuscular elements such as the keratohyalin granules and eleidin droplets. The histochemical and staining reactions of these corpuscles have been summarized recently by SMITH and PARKHURST(11). In recent times three observations have been reported which might be significant, although they were controversial. Firstly SMITH and PARKHURST(11) reported that keratohyalin and eleidin lost their tingibility and metachromatic stain when the sections were pretreated with ribonuclease, but LANSING and OPDYKE (15) could not demonstrate their digestibility with ribonuclease. Thus it remained doubtful whether the particulate elements contained ribonucleic acid. Secondly it was shown that keratohyalin stained deeply with unoxidized hematoxylin and that this findings suggested the presence of a bivalent "metal mordant" in the granules (SMITH and PARKHURST) (11). SMITH and PARKHURST suspected that the metal salt might be iron or copper, but neither one could be conclusively demonstrated histochemically. Thirdly LANSING and OPDYKE(15), on the basis of microincination experiments, were of the opinion that keratohyalin granules are rich in calcium, but GANS (16) found no conspicuous accumulation of calcium, in the transitional layers. Fourthly SMITH *et al* (11) and LANSING *et al* (15) reported that there were present alkaline phosphatase in the transitional layers, clearly outside the granules, together with glycogen. According to SMITH and PARKHURST both glycogen and alkaline phosphatase were absent in the horn layer. These findings again pointed to the possible role of glycogen degradation as a source of energy in keratinization.

An accelerated rats of keratinization was caused by accelerated epithelial proliferation. ROTHMAN(17) described that such accelerated keratinization was incomplete, as evidenced by remnants of nuclear structures, by skipping of granule formation (parakeratosis), and by increased less of water vapor. MONTAGNA (18) described in detail in the BRACHET's "The Cells" (Vol. 5) as follows: strong succinic dehydrogenase activity upper layers and extinct activity in the stratum granulosum; very large quantities of acid phosphatase presented throughout the epidermis, up to and including the stratum granulosum. The presence of small amounts of alkaline phosphatase and lipases abounded in the epidermis. Lipase was distributed from the basal layer to the stratum of cells immediately above the stratum granulosum and the reaction stoped suddenly above this band (MONTAGNA) (19). These enzymes might serve to hydrolyze the lipids during keratinization and splited off from the free fatty acids known to be abundant in the surface lipids. The epidermis abounded in β-glucuronidase, and the reaction was concentrated in the stratum of cells immediately above the stratum granulosum. A concentration of thiol groups was found in a narrow band of cells above the stratum granulosum, which corresponds to the cells rich in acid phosphatase, esterases, and β-glucuronidase. FISHMAN (21—26) investigated the relationship between β-glucuronidase and the activation of sex hormone. If we would consider the existence of β-glucuronidase and the enzymatic activation by the sex hormone, it might be necessary to study the participation of sex hormone in the keratinization.

According to LOBITZ and HOLYOKE (20) when glycogen was stored in the basal cells, mitotic activity always stoped and when mitotic activity was restored, glycogen disappeared. The epidermis contained traces of mucopolysaccharides that stained metachromatically and were stainable with PAS reaction (MONTAGNA) (19). All of the cells of the MALPIGHIAN layer contained perinuclear lipid granucles. The stratum corneum contained abundant lipids released by the cells in the final process of keratinization. Cholesterol could always be demonstrated in the stratum corneum.

As described in Table 5 the relationships between pathological changes and histochemical findings could be shown as follows: The retention of nuclei in the stratun corneum characterized in the swine parakeratosis was shown clearly by the existence of polymerized DNA stained with FEULGEN reaction and was stainable with pyronine methyl-green staining. This nuclei also contained protein stained with acrolein-SCHIFF reaction. Accordingly the retention of nuclei shows the existence of nucleoproteid. There are found principally the polymerized DNA stained with methyl green in the nuclei and mixed depolymerized DNA stained with pyronine among some nuclei. From this point the keratinization process in some places of parakeratotic skin were indicated. The cells of stratum spinosum and stratum basalis which contained rich RNA

in the cytoplasm accelerated the activity of protein synthesis. The lesions such as the palisade formation, acidophilic bodies and rete peg in the so-called parakeratotic layers of stratum corneum contains glucoproteid reacted positively in PAS, negatively in saliva digestion and strongly in acrolein-SCHIFF reaction. The keratohyalin granules in the cells of stratum granulosum were not clear as the ones in the normal type, swollen and fused, but these granules seemed to be similar to parakeratotic bodies which contained glycoprotein. Thickening of stratum spinosum developed to the hypertrophy and to over-growth of papillary layers and reticular appearance as rete peg. The cytoplasm of these lesions contained rich RNA and protein. Alkaline phosphatase distributed in the parakeratotic layers, stratum granulosa, stratum spinosum and stratum basalis. According to SMITH and PARKHURST (11) both glycogen and alkaline phosphatase were absent in the horny layers from the points of glycogen degradation as a source of energy in keratinization and also alkaline phosphatase condensed in the immediate upper zone of the stratum granulosa. Acid phosphatases existed in the parakeratotic layers and weakly in the other parts. Lipase indicated strong activity in the parakeratotic layers in contrast to the normal views contained with strong activity in the zone of stratum granulosa just above. Lipase activity did not correspond to the existence of lipids.

The cells in the stratum granulosa and stratum basalis stored a large amount of glycogen, and there were recognized the appearance of glycogen as the retardance of keratinization following with the development of the parakeratosis. The fat storage on the surface of the skin decreased by the atrophy of sebaceous gland and the thrombus in the orifice of sebaceous glands.

Conclusion and Summary

Among the Yorkshire swines administered with the investigation of meat pigs feeding in the Miyagi Prefectural Agricultural Station, there occurred a kind of skin disease in 20 per cent (six cases) of 32 pigs during 1961 and 1963. As the result of patho-histological examination, this disease was identified to be similar to parakeratosis found clearly by KERNKAMP (1953) in U. S. A. Characteristics of parakeratosis were shown as the over-growth of dermal papillae, acanthosis and retention of nuclei in the thickend parakeratotic layers. We could appoint for the first time the occurrence of swine parakeratosis pathologically in Japan. It is easy to differentiate this lesions by the histochemical methods.

The retention of polymerized DNA, remarkable deposition of glycogen in the stratum granulosum or spinosum, the retardance of glycogenesis followed to parakeratosis were different to the lesions of hyperkeratosis. And also it was different that keratin contained protein and palisade-formation with bodies in the parakeratosis consisted of glycoproteid.

Owing to KERNKAMP's opinion this disease might be caused by linoleic acid defficiency, zinc defficiency and the retardance of fat digestion, but we could not discuss the etiology because of no analytical values of the feed. The outbreaks were concentrated to no increase in body weight and no appetite, and the hogs recovered in slight diseased process which increases the body weight with appetite. It might be necessary that the glycogenesis as a energic source needed to keratinization from the point of activation of sex hormone by β-glucronidase. When the maturation of the gonad in the sows have been developed, it might lack the sex hormone to cornify the skin. On the one hand it might be supplied by a need of sex hormone in the keratinization of the hogs by means of castration. Accordingly, these relationships might become the causal agent in the parakeratosis.

In this paper the existence of swine parakeratosis in Japan was certified pathologically and histochemically.

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Plate 1.

Explanation of Figures

Fig. 1. Parakeratosis in a 16 week-old Yorkshire sow. Typical of many cases were the crusted proliferations in the regions of the fetlock, pasterns and hock. In the regions of the body where the hairs were short, the crust was generally compact and their outer or free surface was granular. The crusts contained a moist and somewhat sticky brownish to black colored substance. From the courtesy of Miyagi Pref. Agr. Exp. Statn.

Fig. 2. Parakeratosis in a 16 week-old Yorkshire sow. This photograph taken from the posterior side showed the crusted proliferation in the fetlock, pasterns, hock, rump and thigh. From the courtesy of Miyagi Pref. Agr. Exp. Statn.

Fig. 3. Photomicrograph of the epidermal layer of the parakeratinized skin which contained the crusted masses, rete peg formation, increase of basal cells, and pronounced parakeratosis. Hematoxylin-eosin stain. ×20.

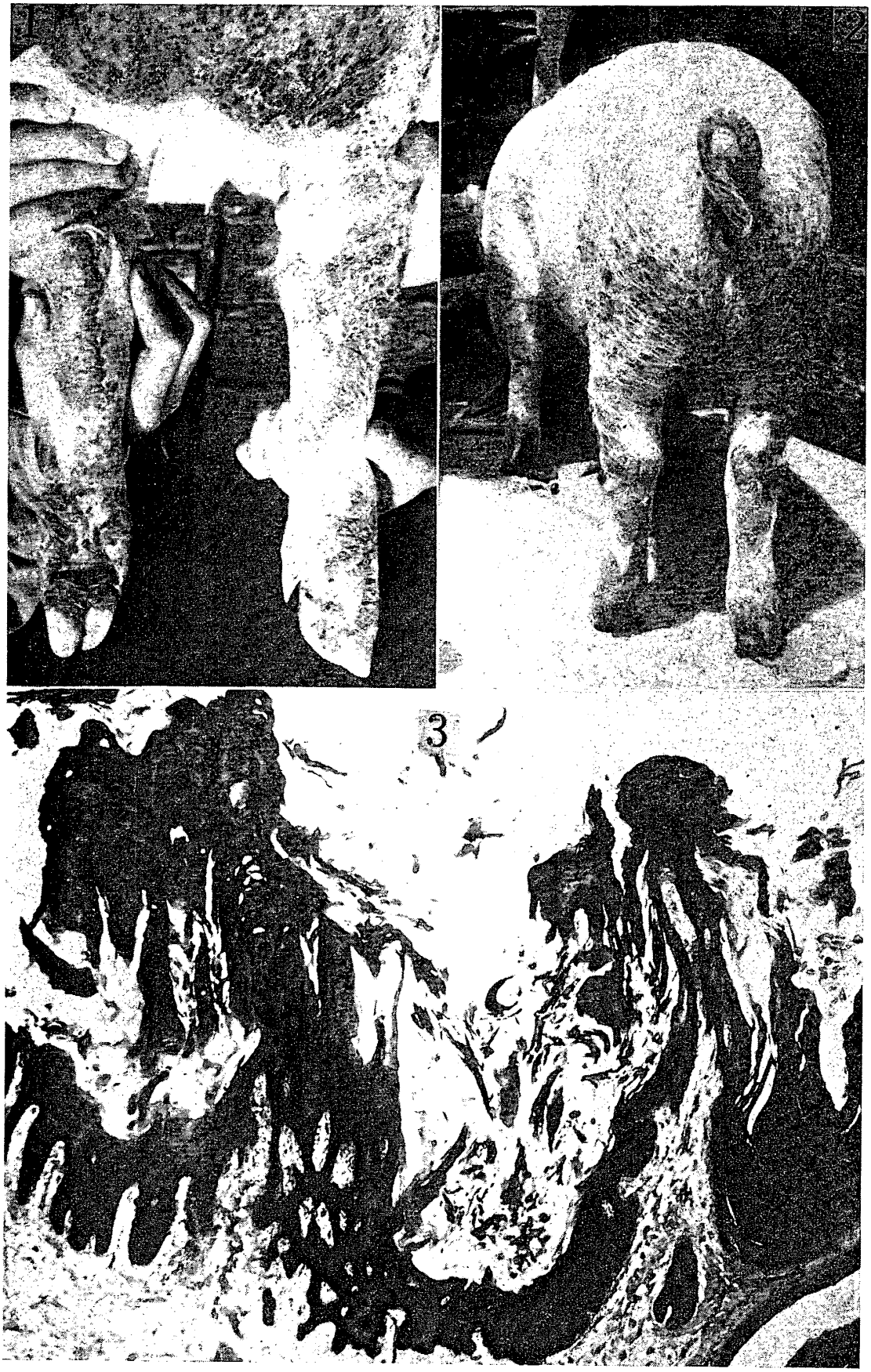
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Plate 2.

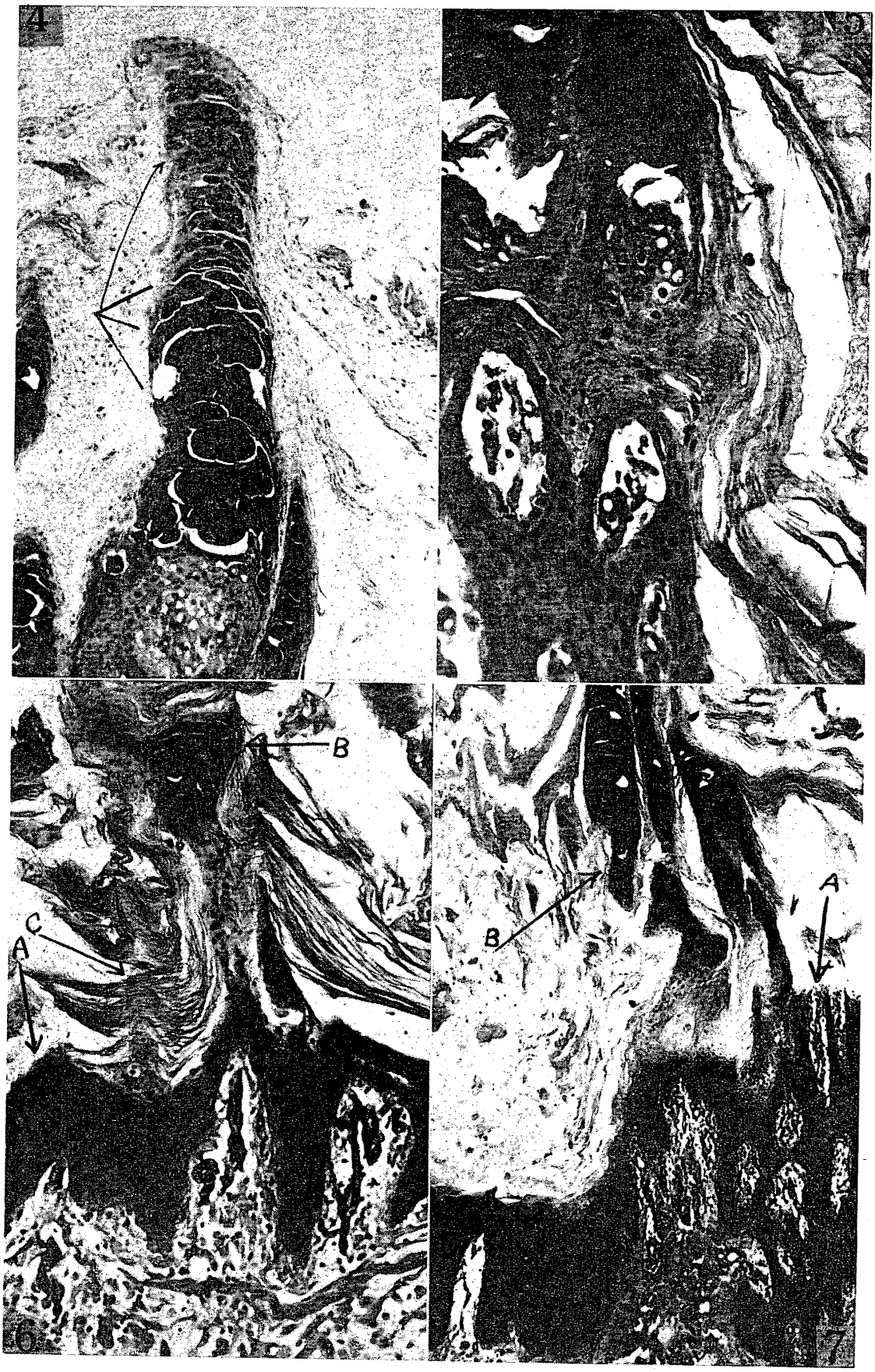
Explanation of Figures

Fig. 4. The parakeratosis lesion stained with PAS-stain. ×100.
Stratum corneum indicated the overgrowth of the papillary layer and the thickening of the epidermis with palisade and peg formation. These parakeratotic lesions contained a large amount of PAS-positive substance (arrow-mark) are showed the lammellated masses and globules.

Fig. 5. The acanthosis lesion and the appearance of corpus ronds, PAS-stain, ×100.
Folds of the epidermis, excessive lateral growth and reticulated appearance as the acanthosis are shown at the left side. The folding of a hyperkeratotic epidermis was indicated at the right side.

Fig. 6. The acanthosis, parakeratotic lesions and hyperkeratosis, PAS-stain, ×100.
The folds of the epidermis, and excessive lateral growth as the acanthosis(Mark : A) overgrowth of the papillary layer and the palisade formation as the parakeratosis (Mark : B), and the pronounced thickening of keratin layer (Mark : C) as the hyperkeratosis were indicated.

Fig. 7. The parakeratosis, acanthosis and hyperkeratosis, PAS-stain, ×100.
The thickening of the epidermis with palisade and peg formation as the parakeratotic lesions (mark : B), the reticulated appearance of rete pegs as the acanthosis of parakeratosis lesions (Mark : A) and the pronounced thickening of the keratin layer as the hyperkeratosis were indicated.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A
B
C
7
A
B

Plate 3

Explanation of Figures

Fig. 8. Remarkable glycogen-deposition in the cytoplams of stratum spinosum thickened in the acanthosis. PAS-hematoxylin stain, ×400. Mammalian epidermis was relatively free of histochemically demonstrable glycogen, but the epidermis under the parakeratotic or acanthosis lesions contained a large amount of glycogen.

Fig. 9. Remarkable glycogen-deposition in the cytoplasms of stratum spinosum in the acanthosis. PAS-hematoxylin stain after saliva digestion, ×400.
The intracytoplasmic glycogen disappeared.

Fig. 10. The acanthosis lesion and the appearance of corpus ronds, PAS-hematoxylin stain after saliva digestion, ×100.
The polysaccharides in the palisade formation (Mark : p) and did not disappear after saliva digestion. Corpus ronds (Mark : R) limited to the stratum granulosum, contained nuclei which were rounded and encircled by a clear cytoplasmic hallo, and the keratohyaline granules were usually absent in the corpus ronds.

Fig. 11. Polysaccharide-containing palisade formation layers and disappearance of glycogen in the rete-peg of stratatum spinosum, PAS-hematoxylin stain, ×100.
Palisade formation-layers (Mark : p) contained the polysaccharide (and also reacted to Acrolein-SCHIFF reaction, accordingly it seemed to contain glucoproteid) and the cells of stratum spinosum contained a large amount of glycogen.


8
9
P
R
10
11

Plate 4

Explanation of Figures

Fig. 12. The retention of nuclei which characterized the parakerinized lesions, BRACHET's pyronine-methyl green stain, ×100.
Almost all nuclei in the stratum corneum stained with methyl green for the demonstration of high-polymerized DNA, but some nuclei in a part staind with pyronine for the demonstration of depolymerized DNA. Homogeneous substances in the palisade formation seemed to react slightly for the existence of depolymerized DNA.

Fig. 13. The retention of nuclei in the parakeratosis, FEULGEN's nuclear reaction, ×100.
Almost all nuclei in the parakeratotic lesion stained intensively with FEULGEN reaction (HP) for high-polymerized DNA, but some nuclei stained lightly with one (Mark : DP) for the depolymerized DNA.

Fig. 14. The presence of succinic dehydrogenase in the basal layer and disappearance of succinic dehydrogenase in the parakeratotic layer., SELIGMAN's dehydrogenase reaction, ×200.
Generally in the normal skin, strong succinic dehydrogenase activity was found in the basal layers; the activity diminished gradually in the upper layers and became extinct in the stratum granulosum. The activity of the cell layers in the acanthosis became slighly extinct, and diminished gradually in the upper layers.

Fig. 15. The enlarged view of the above Fig. 14, SELIGMAN's dehydrogenase reaction, ×400.
Basal layer(Mark : B) intesively, stratum spinosum and granulosum slightly and parakeratotic lesion (p) negatively, were shown.

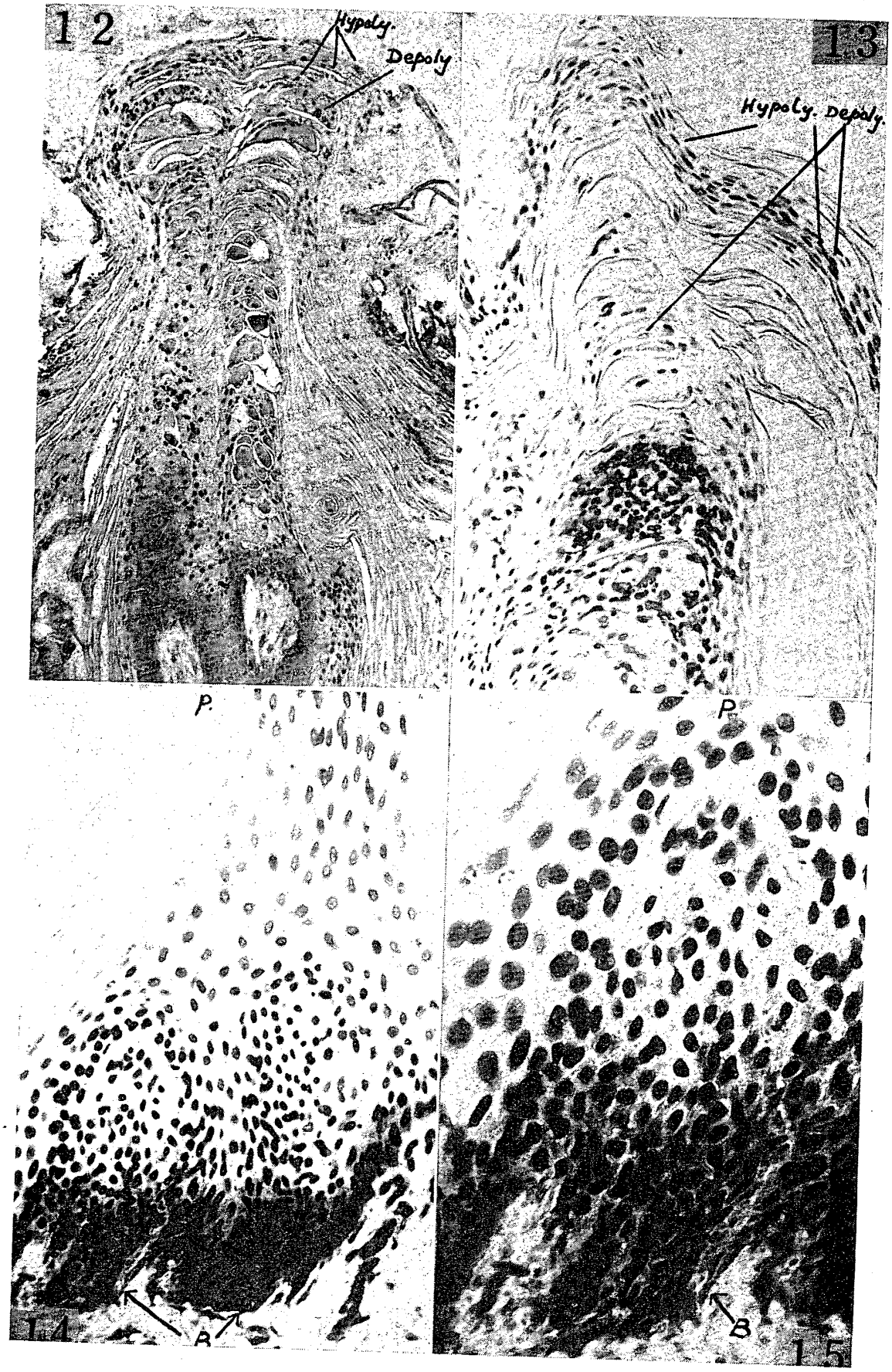

12
Hypoly.
Depoly
13
Hypoly. Depoly.
P.
P.
14
B
B
15

Plate 5

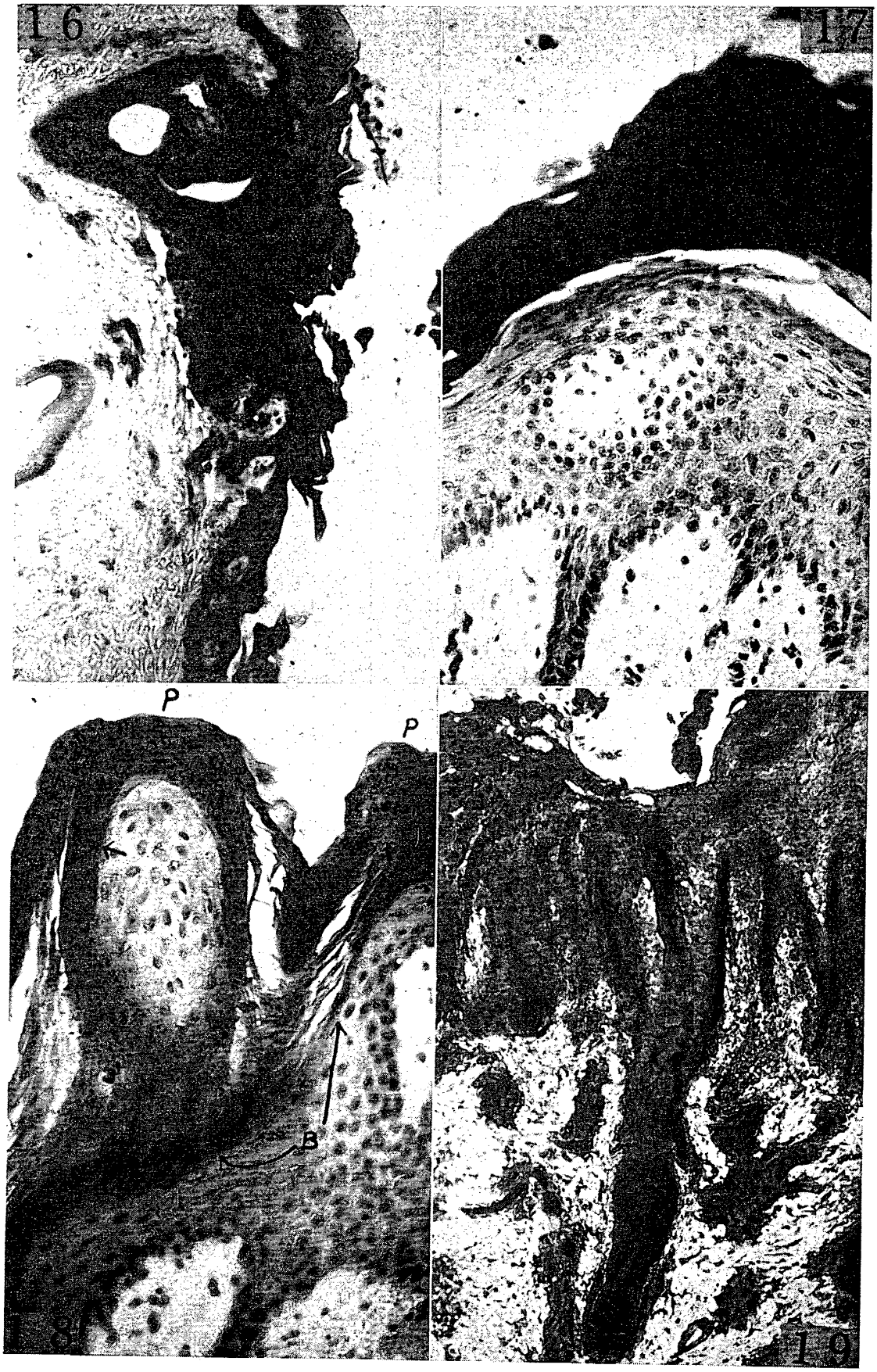
Explanation of Figures

Fig. 16. Parakeratotic lesions with alkaline phophatase. GOMORI's alkaline phosphatase stain, ×40.
According to MONTAGNA (1961), small amounts of alkaline phosphatase were present in the epidermis, and human epidermis had virtually none. Parakeratotic lesions and epidermis had this enzyme in abundance.

Fig. 17. Parakeratotic lesions with acid phosphatase. GOMORI's acid phosphatase stain, ×200.
According to MONTAGNA (1961), in an only narrow band of cells above the stratum granulosum were found rich acid phosphatase. Parakeratotic lesions above the stratum granulosum reacted intensively.

Fig. 18. Parakeratotic lesions with lipase. GOMORI's lipase stain, ×200.
According to MONTAGNA (1961), the epidermis abounded in lipases and nonspecific esterases. Lipase was evenly distributed from the basal layer to the stratum granulosum, and the esterases were mostly concentrated in a band of cells immediately above the stratum granulosum, and the reaction stopped suddenly above this band. Parakeratotic lesions(Mark : P) and the band of cells above the stratum granulosum (Mark : B) contained a large amount of lipases. Others had no lipases.

Fig. 19. Loss of lipids in the stratum corneum and thrombosis of lipids in the orifice of the hair follicle : Sudan III-stain, ×100.
In the normal skin the stratum corneum contained abundant lipids released by the cells in the final process of keratinization, but the parakeratotic region had a few lipids or none.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P
P
B
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