AN OUTBREAK OF SWINE POX-LIKE DISEASE IN TOTTORI PREFECTURE, JAPAN

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It was believed that the viruses of sheep pox^{4,20}), cowpox²⁰), and variola^{15,20}) were responsible for pox-like disease of swine.

YOSHIKAWA³²⁻³⁴) indicated that pox disease in swine was caused by vaccinia or cowpox virus. Outbreaks of pox disease of swine caused by vaccinia virus were reported by AKAZAWA and MATSUMURA¹) and PEIPER²⁶).

Swine pox virus isolated by SHOPE³⁰) in Iowa, U.S.A., was innocuous to rabbits, and no lapinization of the isolate successful.

MANNINGER et al.¹⁹) isolated a virus which was proved to be immunologically different from cowpox virus and infectious for swine, but not for other animals (rabbits, sheep, guinea pigs, or chick embryos).

At present, only two organisms, vaccinia virus and swine pox virus, may be responsible for the disease of swine^{7,8,16,21,22}). According to BLAKEMORE and ABDUSSALAM³), vaccinal infection is severe and occurs among swine of all ages, but swine pox viral infection is considerably mild and is generally restricted to young pigs.

KASZA et al.¹³) isolated a swine pox virus. KASZA and GRIESMER¹⁴) demonstrated that the disease produced by vaccinia virus in swine had an incubation period of two days and a course of six or seven days, and was milder than that by swine pox virus.

According to DAT⁷), pox infection in pigs presents a rather complicated picture of etiology. In his comparative tests, vaccinia virus was shown to infect a very wide range of animals, although its pathogenicity for pigs was proved to be of lower order than "pigpox" virus. Furthermore, no infection with vaccinia virus could be passaged in series, which suggested a failure of this virus to multiply.

Recently, MAYR²¹) and MAYR et al.²²) reported that vaccinia virus caused a feverish, severe but relatively transient disease without producing secondary pox, and that the "original swine pox virus" caused a disease which was slightly feverish and rather chronic at the beginning, took a longer and severer course, and gave rise to secondary pox and pyrexia about 16 days after the onset of disease.

On the other hand, BLAKEMORE and ABDUSSALAM³) pointed out the presence of empty spaces or a poorly stained structure in the nuclei of cells infected with swine pox virus. After that, the occurrence of some nuclear changes (appearance of a nuclear vacuole or empty structure) was mentioned emphatically by many workers^{7,13,14,16,21,22}).

Numerous reports have been presented on the histopathology of pox disease of swine^{2,3,7,11,12,14,16,19,25,29,30,34}).

LÜBKE¹⁶) studied comparatively such cutaneous pox as induced after inoculation with

the viruses of "original swine pox", vaccinia, and cow pox. Cow pox virus caused but insignificant wart-like inoculation-pox without any microscopic specificity. In "original swine pox", specific lesions are predominant in the epithelium (characteristic brightening of nuclei in the stratum spinosum and appearance of intraprotoplasmatic inclusion bodies). In the pox caused by vaccinia virus, lesions are predominant in the corium and no inclusion bodies were found.

DATT⁷⁾ observed granular acidophilic inclusion bodies in the cytoplasm in cells of the stratum spinosum infected with "pigpox" virus. In some sections, these inclusion bodies and the nuclei overlapped. On the other hand, in vaccinia infection there was a slight suggestion of small granular inclusion bodies. When administered to pigs by the cutaneous route, cowpox virus induced a milder reaction than vaccinia virus.

There have been several papers dealing with electron-microscopic studies on skin lesions in swine pox^{5,6,28)}.

RECZKO²⁸⁾ compared skin lesions between "original swine pox" and vaccinia viruses. She emphasized that a lamellar body running parallel with a crystal-like lattice structure was observed in the cytoplasm in "original swine pox" virus infection, but not in vaccinia virus infection. Furthermore, she pointed out that the structure of the virus of swine pox was identical with that of the vaccinia virus which was schematically performed by PETERS²⁷⁾.

The authors¹¹⁾ have recently reported two spontaneous cases of pox disease of swine found in Tottori, Japan, and suggested swine pox virus as one of the possible causative agents of the outbreak, since histopathological examination revealed nuclear vacuoles and eosinophilic inclusion bodies in these cases.

This paper deals with the results of investigation of another outbreak of pox disease of swine which the authors again encountered.

MATERIALS AND METHODS

1. Materials

A total of 6 pigs were employed. They consisted of 3 cases of natural infection (Cases No. 1: Pr. 1245, No. 2: Pr. 1246, and No. 3: R. 673), 2 cases of the first experimental infection (Cases No. 4: Pr. 1251 and No. 5: Pr. 1252), and a case of the second experimental infection (Case No. 6: Pr. 1259). In addition, 2 rabbits and 5 two-day-old mice were used.

2. First experimental infection

A skin lesion was collected from a naturally infected pig (No. 1), emulsified, and inoculated into 2 pigs (Nos. 4 and 5), a rabbit, and 5 mice.

3. Second experimental infection

A skin lesion was collected from one of the 2 experimentally infected pigs (No. 4), emulsified, and inoculated into a pig (No. 6) and a rabbit.

Sometimes, skin lesions were collected by biopsy from the pigs of the first experimental infection; that is, R. 688 and R. 690 from one pig (No. 4), and R. 689 and R. 691 from the other (No. 5).

4. Methods for histopathological examination

Specimens were collected from skin pieces fixed in 10% formalin solution or Bouin's fluid and embedded in paraffin. Sections were stained with hematoxylin and eosin. When necessary, Giemsa stain, Azan stain, PAS reaction, and Feulgen reaction were used.

RESULTS

1. Clinical and macroscopical examination of natural cases

The 3 pigs naturally infected were of the same litter, about 3.5 months old, and kept in the Tottori district. On October 6, 1966, they were observed to have already many papules all over the body. These papules were of bean size and measured up to 3 cm in diameter. They were surrounded by a well-defined zone of hyperemia. Some of them had already become covered with a scab.

History of the pigs mentioned by the owner: The three pigs were born on June 19, 1966. Small reddish papules were observed in the middle of September of the same year. In a neighboring pen, small papules began to be seen on a sow which had been free from such lesions until that time.

On October 6 and 7, 1966, pig No. 1 and pig No. 2 were sacrificed, respectively. Skin lesions were collected from them for pathological examination. Some of them were stored at -20°C and used as inoculum.

The remaining pig (No. 3), however, was kept continuously. On October 31, 1966, when most of the scabs sloughed off and the lesions were almost healed, the pig was also sacrificed and the skin lesions were collected for examination.

2. Histopathological examination of natural cases

Case No. 1: Marked crust formation was observed. The epidermis was regenerated already and irregular in width, having stretched into the corium. In some areas of it ulcer formation occurred.

In the epidermis, infiltration with a few neutrophils was observed. Prickle cells were generally well stained, some of them being enlarged to some extent. No inclusion body was present. Generally, basal cells were irregular.

In the corium, edema, and activation and proliferation of mesenchymal, histiocytic, and connective-tissue cells were conspicuous. In the blood vessels, mild enlargement of endothelia was seen. Furthermore, marked infiltration with eosinophils, mild infiltration with neutrophils, and hemorrhages were observed. In a section, a foreign body giant cell was present. These changes had occurred mainly near the ulceric lesion. These inflammatory reactions were shown diffusely in the superficial layer of the corium, but not in the deeper layer or perivascular subcutaneous tissues.

Case No. 2: This case manifested changes similar to those described in case No. 1.

Case No. 3:

Lesions where crust was still observed macroscopically: Crust was observed, although it was separated from the regenerated epidermal layer. The regenerated epidermis was almost normal in appearance.

The corium presented formation of perivascular slight edema, mild proliferation of histiocytic elements, and infiltration with eosinophils mainly in the perivascular areas. In addition, ulceration accompanied by hemorrhage and necrosis was seen in a few lesions.

Lesions where only cicatrization was observed macroscopically: The epidermis was normal in most lesions, and stretched into the corium in some lesions. In some prickle cells, vacuolation occurred in the perinuclear area and some nuclei exhibited atrophy and chromatolysis.

In the corium, perivascular mild reaction was seen. In addition, hemorrhage was still observed in some lesions.

The reaction was milder in the subcutis than in the corium.

3. First experimental inoculation

a. Materials and methods

The materials used were papules stored at -20°C for a month after collection from a naturally infected case (No. 1). One part of them and two parts of physiological saline were put in a homogenizer and emulsified for about 30 minutes while cooling with ice lumps. To the resultant emulsion was added 1,000 units of penicillin and $500\text{ }\mu\text{g}$ of streptomycin per ml. The emulsion was allowed to stand at room temperature for 30 minutes. After that, an equal volume of glycerol was added to it, and inoculation performed immediately.

Two apparently healthy piglets about 2 months old, weighing 12.4 kg, were used for inoculation. They were born on September 4, 1966, weaned on October 26, vaccinated against hog cholera on October 27, and inoculated with the above-mentioned emulsion on November 7, 1966.

The two piglets (Nos. 4 and 5) were prepared for infection by shaving the sites of inoculation on both sides of the abdomen and the neck. The shaven areas were washed with soap and brush, and disinfected with alcohol. The alcohol was rinsed away with a large quantity of sterile distilled water and wiped dry with sterile gauze. At the cleansed shaven areas, shallow scratches were made with a surgical knife. Each inoculum was rubbed into the scratches with the aid of the index finger which had been covered with a sterilized rubber sack.

Furthermore, another piglet (No. 5) was injected intraperitoneally with about 60 ml of an papular emulsion which had been prepared by adding 4 parts of sterile physiological saline. In addition, it was injected intramuscularly with 200,000 units of penicillin and 1 g of streptomycin.

b. Clinical and macroscopical examination

The sites of inoculation appeared to have been healed. They showed, however, redness after about one week in both cases. After that, samples were sometimes collected from the lesions by biopsy and some of them examined histopathologically without delay.

On the 8th day after inoculation, small papules developed in the lesions. On the 10th day, the papules became remarkable, and samples were collected from some of the lesions by biopsy (R. 688) in Case No. 4. Samples were collected also from some of the lesions by biopsy in Case No. 5 on the 11th day. Twelve days later, the papules grew into lesions of red bean size. On the following day, they became confluent with one another. Fourteen days later, samples were collected from some of the lesions in Case No. 4 by biopsy (R. 690). Fifteen days later, when the central portion of the papules became dry and yellowish, a piglet (No. 4; Pr. 1251) was sacrificed. Lesions were collected from it for pathological examination, isolation and preservation of the virus. The other piglet (No. 5) was held continuously under clinical observation. Sixteen days later, the papules became brownish, and the central portion of the lesions exhibited a crust-like appearance. Nineteen days later, a crust was formed and some of the lesions were examined by biopsy (R. 691). Twenty-four days later, the crust began to fall. Twenty-five days later, the animal was sacrificed and lesions were collected from it for investigation (Pr. 1252).

The fever ranged from 38.5 to 40.2°C in all the animals, except in Case No. 4 which showed 41.0°C on a day before the occurrence of redness.

The rabbit and five mice developed no papules at all.

c. Histopathological examination of cutaneous lesions of the pigs

R. 688 (sample collected 10 days after inoculation)

This sample showed marked thickening and swelling of the epidermis. The border between the keratinous layer and the prickle cell layer became indistinct. The granular

cell layer disappeared. Parakeratosis was observed.

The prickle cell layer presented conspicuous vacuolar and reticular degeneration. The prickle cells had markedly vacuolar or pale nuclei.

Furthermore, swollen prickle cells contained inclusion bodies.

Basal cells were swollen and stained poorly.

The corium exhibited congestion, hyperemia, edema formation, perivascular focal histiocytic proliferation, and cellular infiltration with a few neutrophils, but with no eosinophils.

Slight swelling was observed in the vascular endothelial cells and slight loosening in the vascular walls. Collagenous fibers of the corium showed swelling and necrobiotic changes.

R. 689 (sample collected 11 days after inoculation)

Massive infiltration of neutrophils was observed between the parakeratotic stratum corneum and the prickle cell layer. Incrustation already began. Infiltration of neutrophils was seen in the epidermal layer.

The inflammatory reaction of the corium was severer than that in R. 688. There were marked histiocytic reaction, appearance of fibroblastic cells, swelling of blood vascular endothelial cells, marked neutrophilic infiltration, and swelling and necrotic changes of collagenous fibers.

Inclusion bodies were present in the prickle cell layer.

R. 690 (sample collected 14 days after inoculation)

The changes observed were almost the same as revealed by R. 689.

Pr. 1251 (sample collected 15 days after inoculation)

The changes exhibited were almost the same as shown by R. 690. However, crust formation was rather conspicuous. Small clumps of bacteria were found in the epidermis. Abscess formation took place in the corium.

Inclusion bodies were observed.

R. 691 (sample collected 19 days after inoculation)

Crust formation was marked. In lesions, most epidermis disappeared. Borders became indistinct between the crust, epidermis, and corium. Ulceration occurred. In and around the bordering areas, marked serous exudation and hemorrhages were observed. Remarkable infiltration of neutrophils mixed with a considerable number of eosinophils was present. Connective-tissue fibers were edematous. Some bordering areas, however, already presented pictures of irregular and thin regenerated epidermal cells which seemed to have been derived from the remaining epidermal cells or mesenchymal cells.

In the corium, activation and proliferation of histiocytic cells and infiltration of neutrophils and eosinophils were conspicuous. These cellular reactions were diffuse in the superficial layer, but perivascular in the deeper layers.

In the subcutis, changes were seen in perivascular areas.

Pr. 1252 (sample collected 25 days after inoculation)

The epidermal layer was almost completely regenerated and separated from the crust.

The granular layer was present. Cornification was almost normal. In some areas, no granular layer was present and parakeratosis still observed.

In the epidermal cell layer, no characteristic pox lesions (ballooning degeneration, nuclear vacuolation, or inclusion body) were seen. The cells were normal in size and compact in arrangement. In some areas, however, the border between the epidermis and corium was indistinct, and infiltrating cells, including a few neutrophils, were atrophic. The epidermis stretched deeply into the corium.

The corium had slight edema in the stratum papillosum. It showed histiocytic

reactions and mild infiltration of eosinophils and lymphocytes, including few neutrophils, chiefly in the perivascular areas.

Inclusion body

Inclusion bodies were observed in some sections (R. 688, R. 689, R. 690, and Pr. 1251). Most of them exhibited irregular borders with projections and clefts. Their boundaries were not always well defined from the protoplasm (irregular-type inclusion body). A few of them (observed in R. 690 and Pr. 1251), however, had smoothly oval or round borders and seemed to be homogeneous, including minute ones and ones almost as large as the nuclei (homogeneous-type inclusion body).

When tinctorial properties were compared, the former were slightly acidophilic, and sometimes neutrophilic and basophilic in hematoxylin-eosin staining, pale blue or hardly stained with Giemsa stain, orange with Azan stain, and almost negative for PAS reaction. On the contrary, the later were conspicuously acidophilic in hematoxylin-eosin staining, bright or dark blue with Giemsa stain, pale blue with Azan stain, and definitely positive for PAS reaction.

Most of the inclusion bodies were negative for Feulgen reaction, and some of them stained slightly blue in this reaction.

d. Electron-microscopic examination

Materials for ultra-thin sections were collected from the experimental pigs, fixed in MILLONIG's fluid²³), dehydrated with graded alcohol, and embedded in epoxy resin by LUFT's method¹⁷). Thin sections were cut with glass knives on a UM-3 type (Hitachi) ultra-microtome. They were examined by an HS-7 type (Hitachi) electron microscope.

Photomicrographs were taken on materials from typical pox lesions harvested 10 days (R. 688) and 15 days (Pr. 1251) after inoculation.

The central area of the nucleus was replaced by a delicate fibrillar strand of lower density (Fig. 7). The cytoplasm contained many mature and developmental forms of viral particles¹⁰) (Fig. 18). The mature virus particles had rounded quadrangular forms, as described by FENNER and BURNET⁹), and a longer axis 200–330 m μ in length (Fig. 19). Furthermore, the cytoplasm contained a parallel running lamellar body with crystal-like lattice structure (Fig. 18).

4. Second experimental inoculation of a pig and a rabbit

The materials used for inoculation had been collected from some skin lesions of a pig (No. 4). They were inoculated to a pig about 7 weeks old and a rabbit. The experimental period was from March 11 to March 22, 1967. Inoculation was carried out on March 13 by the same method as employed in the first experimental inoculation.

As a result, the pig exhibited redness 3 days after inoculation, forming a small eruption. Conspicuous papules were formed 9 days later, when materials were collected.

Clinically, the fever ranged from 39.2 to 40.6°C.

Histopathologically examination revealed the same conspicuous pox changes as demonstrated in the first experimental inoculation.

DISCUSSION

In the present investigation, the virus contained in the material from a naturally infected case of pox disease in swine caused typical pox lesions in piglets for 2 generations, but not in rabbits or mice.

Clinically, the period of incubation was 8 days in the first experiment and 5 days in the second. No marked elevation was observed in body temperature. It took about 18

days from the appearance of redness to the beginning of falling of scabs (Case No. 5), and about 45 days to a period when a greater part of the scabs disappeared (Case No. 3).

Histologically, papules caused in the experimental piglets had massive cytoplasmic inclusion bodies and nuclear vacuoles. In the ultra-thin section, many pox-viral particles and a lamellar body with lattice structure were observed.

The above-mentioned clinical and morphological findings and that of the inoculation to the rabbits and mice of the present investigation were identical with those of the original swine pox described by some of the earlier workers^{3,5-8,13,14,16,21,22}.

Furthermore, MORITA and SOEKAWA²⁴ isolated the virus from papules (Pr. 1251) in porcine testicle monolayer cell culture. The structure characteristic of the pox group of viruses was observed in the isolate by the electron microscope. The virus did not grow on the chorioallantoic membrane of chick embryos nor was neutralized by anti-vaccinia serum.

In the present investigation, the histopathological changes of the cutaneous lesion were the same as those observed after the eruption.

The conspicuous changes of the pox diseases were shown for about one week after the occurrence of eruption, but not on the 11th day after the occurrence of eruption. On the 17th day, regeneration was seen in the epidermis. The initial changes were studied by LÜBKE¹⁶ (original swine pox, vaccinia pox, and cowpox in swine) and YOSHIKAWA³⁴ (vaccinia- or cowpox). It was impossible to examine them in the present investigation.

It was suggested from the present investigation that eosinophilic leukocytes might appear in a later stage of the disease.

The appearance of inclusion bodies was reported by many workers^{2,3,6,11,12,14,16,18,19,25,30,31} in the skin of swine infected with pox disease.

In the present investigation, the inclusion bodies were divided into two types. Most of them belonged to a type which showed irregular borders with projections and clefts, and the boundaries of which were not always well defined from the protoplasm (irregular-type inclusion). A few of them were classified into the other type smoothly oval or round in shape showing a tendency to occur in the prickle cells of the epidermis where papules usually disappeared (smooth, round, and homogeneous inclusion). When tinctorial properties were compared, the latter were markedly eosinophilic in hematoxylin and eosin staining, pale or dark blue with Giemsa stain, pale blue with Azan stain, and positive for PAS reaction. On the contrary, the former were slightly acidophilic, sometimes neutrophilic and slightly basophilic in hematoxylin and eosin staining, pale blue or hardly stained with Giemsa stain, orange with Azan stain, and almost negative for PAS reaction. They were almost negative for Feulgen reaction, to which some of them became slightly blue.

The appearance of the two types of inclusion bodies was observed by other workers^{3,11,14}, although its description was not always satisfactory. It was also reported by the present authors in their previous paper¹¹. Since their findings were not satisfactory, the present authors re-examined the inclusion bodies. Consequently, they confirmed that the two types of inclusion bodies had the same tinctorial characters as those clarified in the present investigation.

SUMMARY

A pox disease occurred spontaneously among swine in Tottori Prefecture, Japan. Papules appeared all over the body. Histologically, the epidermis was regenerated. Ulcerous lesions with inflammatory reaction were present in some areas, but no charac-

teristic pox lesions were observed.

Inoculation with samples collected from skin lesions of infected pigs gave rise to typical pox lesions in pigs but not in a rabbit or mice.

Histological examination of the lesions revealed thickening of the epidermis, ballooning degeneration of the prickles cells, vacuolation of the nucleus, and occurrence of conspicuous cytoplasmic inclusion bodies.

The inclusion bodies observed were divided into 2 types. To one type belonged round, homogeneous and conspicuously acidophilic body (homogeneous-type inclusion body). To the other type belonged those which had irregular borders with projections and clefts, the boundaries of which were not always well defined from the protoplasm, and which were slightly eosinophilic and sometimes basophilic or neutrophilic in hematoxylin and eosin staining (irregular-type inclusion). The former type stained blue with Giemsa stain, pale blue with Azan stain, and positive for PAS reaction. The latter type stained pale blue or hardly stained with Giemsa stain, orange in Azan stain, and negative for PAS reaction. The inclusion body was negative for Feulgen reaction or hardly stained with it.

Numerous developmental and mature pox viruses and lamellar bodies with lattice structure were observed by the electron microscope. The center of the nucleus was replaced by a fine filamentous structure of low density.

The pox disease of swine investigated must have been caused by swine pox virus.

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鳥取地方に発生した豚痘様疾患について

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1966年10月初旬、発痘後約20日を経過した3.5カ月令の豚の痘瘡の自然感染例3例に遭遇した。それらは全身に著明な発痘を見、すでに痂皮を形成しているものもあった。組織学的には、すでに表皮は再生し、潰瘍の形成するものもあったが、封入体は認められなかった。真皮には、著明な組織球性反応のほか、好酸球・好中球の出現、出血・浮腫があり、異物巨細胞も認められた。

これらの皮膚病巣を乳剤として、生後1カ月の子豚2頭の皮膚に接種したところ、約1週後に発赤し、10日後に丘疹を形成した。10~15日後の組織では、表皮の肥厚、有棘細胞の風船様変化、原形質封入体の出現、豚固有の痘瘡に特徴的といわれる核内空胞の出現が目立った。16日後には、発痘部は肉眼的に褐色調を帯び、19日後の組織では、痂皮および潰瘍形成を見、表皮は消失していたが、一部にはすでに再生も認められた。25日後

には、痂皮は脱落し始め、組織では、表皮の再生が認められたが、不完全で、真皮には浮腫とともに組織球性反応、好酸球の出現があった。

細胞内封入体は、Hematoxylin-Eosin染色に著しい酸好性を呈する円形・均質性封入体と、弱酸好性で、原形質との境界やや不明瞭な、不規則な辺縁を持つ不整形封入体とに分けられた。前者はGiemsa染色で青色、Azan染色で淡青色、PAS陽性で紅色を呈した。後者はGiemsa染色で淡青または不染、Azan染色で橙色、PAS陰性を呈した。さらに後者には、Feulgen反応陰性か、わずかに淡青をなしていると思われるものもあった。電子顕微鏡的には、10~15日後の有棘細胞内に、多数のウィルス粒子が認められるとともに、豚におけるワクシニア痘との鑑別に強調されている層板状構造物が認められた。光学顕微鏡で見られた核内空胞は、電子密度の低い微細な線維状構造物

で置換されていた。

実験例における発痘部皮膚乳剤は、さらに別の子豚に痘瘡病変を起こした。

上記の自然例および実験例の皮膚乳剤は、家兎

およびマウスには発痘を来たさなかった。

以上の成績より、本例は、豚固有の痘瘡ウィルスによって起こったものと思われる。

EXPLANATION OF PLATES

PLATE I

(All figures are photographs taken from the naturally infected cases, except Fig. 5.)

- Fig. 1. Ulcerous lesion on the skin. In the corium, inflammatory reaction is conspicuous. About 20 days after the appearance of eruption. Case No. 1, hematoxylin and eosin staining (HE), $\times 34$.
- Fig. 2. Regenerated epidermis stretching into the corium. About 21 days after the appearance of eruption. Case No. 2, HE, $\times 83$.
- Fig. 3. Epidermis has almost completely been regenerated. In the corium and subcutis perivascular reaction is still observed. About 45 days after the appearance of eruption. Case No. 3, HE, $\times 34$.
- Fig. 4. Giant cell is seen in the corium. Case No. 1, HE, $\times 330$.
- Fig. 5. Typical pox lesions. First experimentally infected case. 15 days post-infection (p.i.). Case No. 4 (Pr. 1251), $\times 2$.

PLATE II

(All figures are photographs taken from the first experimental cases.)

- Fig. 6. Thickening, parakeratotic change, and ballooning degeneration are observed in the epidermis. Reactionary changes are not so conspicuous in the corium as yet. 10 days p.i. Case No. 4 (R. 688), HE, $\times 170$.
- Fig. 7. Infiltration with neutrophils between the parakeratotic stratum corneum and the stratum spinosum. In the corium, inflammatory cellular infiltration is conspicuous. 11 days p.i. Case No. 5 (R. 689), HE, $\times 170$.
- Fig. 8. The border between the parakeratotic stratum corneum and the stratum spinosum is indistinct. Crust formation has begun. The superficial layer of the epidermis is devastating. In the corium, inflammatory reaction is seen. 15 days p.i. Case No. 4 (Pr. 1251), HE, $\times 99$.
- Fig. 9. Crust formation. Epidermis disappeared. The border between the crust and the corium is indistinct. In the corium, inflammatory reaction is conspicuous. 19 days p.i. Case No. 5 (R. 691), HE, $\times 60$.
- Fig. 10. Regeneration is almost complete in the epidermis layer, which is separated from the crust. In the corium, inflammatory reaction is still observed. 25 days p.i. Case No. 5 (Pr. 1252), HE, $\times 34$.

PLATE III

(All figures are photographs taken from the first experimental case, case No. 4 (Pr. 1251), 15 days post-infection.)

- Fig. 11. Homogeneous-type inclusion bodies (arrow) and conspicuous nuclear vacuoles. HE, $\times 850$.
- Fig. 12. Irregular-type inclusion bodies and conspicuous nuclear vacuoles. HE, $\times 850$.
- Fig. 13. Homogeneous-type inclusion bodies (arrow). Giemsa stain, $\times 850$.
- Fig. 14. Irregular-type inclusion bodies. Giemsa stain, $\times 850$.
- Fig. 15. Homogeneous-type inclusion bodies (arrow). Azan stain, $\times 850$.
- Fig. 16. Irregular-type inclusion bodies. Azan stain, $\times 850$.

PLATE IV

(All figures are photographs taken from the first experimental cases.)

- Fig. 17. Central area of the nucleus in a prickle cell has been replaced by delicate fibrillar strands of lower density. 10 days p.i. Case No. 4 (R. 688), $\times 8,100$.
- Fig. 18. Many mature and developing forms of viral particles and a lamellar body with

crystal-like lattice structures (arrow) are seen in the cytoplasm. Case No.4 (R.688), $\times 33,000$.

Fig. 19. Mature viruses of spindle or rounded quadrangular shape are seen. 15 days p.i. Case No.4 (Pr.1251), $\times 51,200$.