STUDIES ON THE ETIOLOGY OF RABBIT POX

II. Clinical Characteristics of the Experimentally Induced Disease

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In the first paper of this series the acute and rapidly fatal type of experimental rabbit pox was described (1). The condition developed in rabbits inoculated with tissues from spontaneous cases of pox and it was characteristic also of the subsequent rabbit passage of virus with an inoculum of testicular tissue-virus and the intratesticular route of injection. In marked contrast to this fulminating type of infection was the more prolonged and less fatal disease which was observed particularly in connection with a small dosage or with routes of injection other than the intratesticular. Under these conditions, a variety of clinical manifestations developed, the most conspicuous of which was a generalized maculopapular eruption of the skin. The clinical picture of the experimental disease was a faithful reproduction of that observed in spontaneous cases of pox.

The comparisons made between the experimentally induced disease and spontaneous pox included post mortem examination of every animal inoculated with pox virus, together with microscopic examination of representative tissues. Space limitations do not permit the inclusion of this material in the present report, but it should be stated that in essential respects the pathological findings of the experimental disease and those of spontaneous pox as reported by Greene (2) were the same. The discrepancies principally concerned the degree of involvement of certain organs, but it was felt that they could be accounted for on the basis of differences in the routes of infection, in the size of infecting doses, etc.

It is the purpose of the present paper to discuss first, the clinical signs and symptoms of the experimental disease and second, certain special features of the reaction arising from different routes of inoculation.

Materials and Methods

Inoculations were in most cases carried out with tissue-virus emulsions used for the serial passages of virus maintained by the intratesticular injection of Berkefeld V filtered and of unfiltered testicular tissue emulsified in Locke's solution. The preparation of emulsions is described in the preceding paper (1). All inocula were bacteriologically controlled.

The present analysis of the clinical manifestations observed is based upon the findings on 84 male rabbits which survived for a week or longer. 61 of these animals were inoculated with the Xy171 strain of virus with which most of the experimental work was done and 23 were inoculated with 5 other strains (1). Berkefeld V filtrates were used in 72 and unfiltered emulsions in 12 cases. For 68 inoculations emulsions of testicular tissue were employed and for 16 inoculations various other tissues, namely, liver, spleen, lung, lymph nodes, defibrinized blood, heparinized blood, blood clot, and skin.

The following figures give the number of animals injected by various routes: intratesticular 38; intradermal 24; intravenous 6; intramuscular 2; intraperitoneal 2; conjunctival instillation 4; intranasal instillation 8.

The dosage varied widely. In the case of intratesticular injections, small amounts of dilutions of virus emulsions were usually employed, as for example, 0.1 cc. of a 1:1,000 dilution. Dilutions up to 1:10,000,000 were injected intradermally in 0.1 or 0.2 cc. doses. Undiluted emulsions in doses of 0.1 to 0.5 cc. were inoculated by the other routes employed.

Clinical Manifestations and Course of Disease

The analysis of the clinical manifestations of the experimental disease is based upon observations on 84 rabbits whose period of survival was a week or longer. Before taking up this analysis, however, the protocols of 2 typical cases are presented in order that the clinical picture in its entirety may be appreciated.

Both rabbits were injected intravenously with 0.5 cc. of a Berkefeld V filtrate of testicular tissue-virus emulsion of the 3rd passage of the Xy171 strain (1). An area of the body was shaved and scarified but no virus was applied. The course of the disease in both animals was similar, but whereas one succumbed, the other survived.

Rabbit A.—1st, 2nd, and 3rd day, no signs or symptoms. 4th day: profuse maculopapular eruption on shaved area not limited to scarified lines. Nodular

indurated areas in both testicles. Rectal temperature 103.8°F. 5th day: cutaneous eruption distinctly papular and hemorrhagic; size and number of papules greatly increased in shaved area (Fig. 1) and many found elsewhere, including the genito-anal regions. Pronounced orchitis with moderate scrotal edema. Popliteal adenitis. Temperature 105.7°F. 6th day: cutaneous eruption umbilicated with crust formation. Fresh papules on tip of left ear, scrota, and perineal region (Fig. 2), and on the mucocutaneous border of the lower lip and the right nostril. Thin but lively. Temperature 104.7°F. 7th day: fresh papules on the right upper lip (Fig. 3) and right upper eyelid. Blepharitis with fine scales on lids; mild conjunctivitis with a watery discharge. Cutaneous lesions markedly hemorrhagic with large necrotic centers. Very thin and weak. Temperature 103.8°F. 8th day: found dead.

Rabbit B.—1st and 2nd day: no signs or symptoms. 3rd day: area of nodular induration in left testicle. Rectal temperature 104.4°F. 4th day: widespread papular eruption on shaved area not limited to scarified lines. Temperature 105.3°F. 5th day: increase in size and number of papules on shaved areas and a similar profuse cutaneous eruption widely distributed over the body. Marked bilateral orchitis. Temperature 105.3°F. 6th day: cutaneous lesions becoming hemorrhagic and necrotic. Fresh papular eruption about the anus and on the prepuce. Popliteal adenitis. Extremely marked orchitis with scrotal edema. Temperature 105°F. 7th day: papules increasing in size and number; fresh lesions on lips. Slight watery nasal discharge. General condition good. Temperature 104.6°F. 9th day: Lesions regressing. Umbilication and crusting of papules. Temperature 102.3°F. 2 weeks: continued healing of lesions with crusted cutaneous pustules. Temperature 103.4°F. 4 weeks: negative.

With the general information derived from these protocols as a background, the clinical manifestations observed in 84 rabbits which survived a week or longer will now be discussed (Table I).

Incubation Period.—The attempt was not made to determine the exact incubation period of the disease because of the large number of rabbits required. In the case of intratesticular inoculation, some degree of local reaction was usually detected a day or so before the development of other signs.

Of the 38 rabbits injected intratesticularly with material from a variety of tissues, 33 or 94 per cent developed a definite orchitis in a mean time of 4.9 days. The earliest signs were detected on the 2nd and the latest on the 11th days. With other routes of inoculation other criteria of the incubation period were employed. The available information on the total group of animals shows that the mean times of the occurrence of fever and of a cutaneous eruption were 5.4 and 7.3 days respectively after inoculation (Table I).

TABLE I

Clinical Results in 84 Rabbits Surviving 8 or More Days. Various Inocula, Dosage, and Routes of Injection

Strain	No. of rabbits	Inoculum	Route of inoculation	Fever		Cutaneous eruption		Nasal involvement		Eye involvement		Popliteal adenitis		Orchitis		Diarrhea		Dead		Killed		Recovered
				No.	*days*	*No.*	*days*	*No.*	*days*	*No.*	*days*	*No.*	*days*	*No.*	*days*	*No.*	*days*	*No.*	*days*	*No.*	*days*	
Xy171	8	7 test. filt. 1 " not filt.	I. T.	8	4.6	6	7.8	4	6.3	5	8.2	4	5.5			1	14	6	12.0	1	9.0	1
	7	Various filt.*	"	7	7.1	4	8.0	4	9.5	1	11	4	7.3			2	6	3	14.0	—	—	4
	17	Test. "	I. D.	17	5.8	14	6.9	9	7.2	9	8.7	10	8.1	13	7.4	3	6.3	5	16.2	—	—	12
	7	" not filt.	"	7	3.0	7	5.3	6	9.5	6	9.5	1	6	3	10.7	—	—	5	9.4	2	14.0	—
	6	" filt.	I. V.	6	4.5	6	5.7	2	7.5	1	6	5	6.4	5	4.8	1	12	2	10.5	1	8.0	3
	2	" "	I. M.	2	4.0	2	6.5	1	4	1	10	2	4.5	2	6.0	—	—	—	—	—	—	2
	2	" "	I. P.	2	3.5	2	5.5	1	10	1	11	2	4.5	2	5.5	1	17	1	10.0	—	—	1
	4	" "	Conj.	4	5.3	4	6.8	—	—	—	—	3	7.3	2	7.5	—	—	—	—	—	—	4
	5	" "	Nose	5	5.6	3	7.7	—	—	—	—	3	5.3	2	10	—	—	1	10.0	—	—	4
	3	" not filt.	"	3	3	3	7	—	—	—	—	3	6.3	3	6.3	—	—	—	—	3	8.3	—
AB18	11	" " "	I. T.	11	5.8	8	8.4	4	7.0	6	7.3	6	6.8			1	11	6	9.3	1	8.0	4
P 2	4	Various filt.†	"	4	6.8	3	12	4	8.5	3	11.7	2	8.0			3	14.7	4	18.8	—	—	—
HA46	2	" " ‡	"	2	7.0	1	7	1	9	—	—	1	7			—	—	—	—	2	11.0	—
8415	2	1 test. " 1 " not filt.	"	2	6.5	—	—	1	10	—	—	2	7.5			2	7.5	1	9.0	—	—	1
X667	4	Various filt.§	"	4	7.0	4	8.5	1	10	1	10	2	10			1	12	1	14.0	1	9.0	2
Total..	84	70 filt. 14 not filt.		84	5.4	67	7.3	38	8.0	34	8.9	50	6.9	32	7.2	15	10.4	35	12.5	11	9.9	38
Incidence, *per cent*.....				100.0		79.8		52.8		47.2		59.5		69.6		17.8		41.7		13.1		45.2

I. T. = intratesticular; I. D. = intradermal; I. V. = intravenous; I. M. = intramuscular; I. P. = intraperitoneal; Conj. = conjunctival instillation.

* 2 lung, 3 heparinized blood, 1 defibrinated blood, 1 liver.

† 1 spleen, 1 lung, 1 heparinized blood, 1 blood clot.

‡ 1 testicle, 1 popliteal lymph node.

§ 2 liver, 2 skin.

Mortality.—The actual mortality rate of the group was 42 per cent and the mean time of death was 12.5 days after inoculation (Table I). Of the 35 fatalities 27, or 77 per cent, occurred on the 8th to the 14th days. The earliest deaths were on the 8th and the latest on the 28th days respectively.

Eleven rabbits were killed because of their general condition at a mean time of 9.9 days after inoculation, the earliest being on the 8th and the last on the 14th day respectively. With the inclusion of these animals, the mortality rate is raised to 55 per cent.

Recovery.—Practically half the animals of the group, that is, 38 or 45 per cent, recovered from the infection and survived (Table I).

Fever.—A rectal temperature higher than 102°F. was recorded at some period of the disease in each of the 84 rabbits in this assembled group (Table I).

The mean time of the first record of fever was 5.4 days after inoculation; the earliest instance was on the 2nd and the latest on the 9th day. Both the onset and the decline of fever were abrupt, but since readings were made only at 24 hour intervals, this feature may have been more apparent than real. The duration of fever varied considerably. In animals which developed an extensive cutaneous eruption or those with marked testicular or respiratory involvement, it was frequently present for several days. In less severe conditions, it was usually noted for 2 or 3 days.

Cutaneous Eruption.—The most conspicuous objective manifestation of the experimental disease was a maculopapular or papular eruption on the skin and mucocutaneous borders.

The eruption was observed in 80 per cent of the group at a mean time of occurrence of 7.3 days after inoculation (Table I). The earliest example noted developed 3 and the latest 14 days after inoculation. The condition began as small pinkish macules or maculopapules distributed most frequently over the back and sides of the body (Figs. 1, 5, and 6), the nape of the neck, the ears (Fig. 4), the eyelids, the muzzle, the mucocutaneous borders of the nose and lips (Figs. 3 and 11), the anus and the sheath and scrotum of male animals (Fig. 2). The number of lesions in the beginning varied from 1 or 2 to a dozen or more, but within a day or so there was frequently a countless number. The earliest lesions were very small pink or red spots which were just palpable or indurated points which were almost colorless. The size of the papules increased to a diameter of 2 or 3 mm. and in 2 or 3 days many of them became pustules with an umbilicated center and a thin yellowish crust (Figs. 4 and 6); occasionally, an intervening vesicular stage was seen. In other instances the papules became purpuric in appearance and in a

few hours were hemorrhagic and edematous with necrotic centers (Fig. 5). As a rule, individual lesions continued to be discrete but occasionally, and notably in the case of the hemorrhagic type of lesion, contiguous papules coalesced and later these areas might become secondarily infected. Regression of the eruption was usually well under way within a week of its appearance and in 2 or 3 weeks, healing was completed. In certain instances this was accompanied by scar formation; in many cases, there was no scar visible in the gross.

A striking feature of the eruption in certain animals was the development of fresh macules and papules during the evolution of the earlier lesions (Figs. 5 and 6). The new lesions appeared not only in previously uninvolved parts of the body, but also in areas already similarly affected. The number was variable, few or numerous lesions being observed. As a rule, they did not exhibit to the same degree the tendency toward umbilication and pustulation shown by the earlier lesions, and their healing consequently often occurred at the same time or might even antedate the healing of older lesions. The picture presented by a severe case at the height of the cutaneous eruption was that of macules, maculopapules, papules, and pustules, all present at one and the same time.

Nasal and Respiratory Involvement.—A nasal discharge was a prominent feature of the disease. It developed in 53 per cent of the 72 rabbits of the group inoculated by routes other than those of conjunctival or nasal instillation (Table I).

The mean time of onset was 9.5 days after inoculation. Since a number of animals were killed at this period, the true incidence rate may have been higher. The first sign was a scanty serous discharge which occurred in some animals as early as 3 days after inoculation. The condition usually increased in severity and at a fairly rapid rate so that within 3 or 4 days the discharge was profuse. By this time it was generally of a seropurulent or mucopurulent character and frequently blood stained, and about the nares there was an accumulation of reddish or brownish yellow adherent crusts (Figs. 11 and 12). The muzzle was often swollen and the skin and underlying tissue were thickened and boggy. In severe cases the respiration was labored and rapid and instances of outspoken dyspnea were not uncommon. The stance of these rabbits in which the head was held high and extended backward was very characteristic. Cases which terminated fatally had frequently shown marked respiratory involvement.

Eye Involvement.—The eyes and lids were affected in 47 per cent of 72 rabbits not inoculated by the conjunctival or nasal route (Table I).

As in the case of nasal signs and symptoms, the real incidence was probably higher since the mean time of development of clinical signs was 8.9 days after inoculation at which time many animals in the group were killed. The first evidence of eye involvement was usually photophobia and this was accompanied

or immediately followed by thickening and reddening of the lid margins, a mild conjunctivitis, a slight watery discharge, and a few tiny crusts or scales about the lids. Not infrequently the severity of the condition increased very rapidly and a keratitis with or without pannus formation, and an iritis developed. In these circumstances the discharge became seropurulent and profuse and the swollen lids became closed. Corneal ulcers and perforations were occasionally seen.

Once the cornea and iris became involved, there was little tendency toward a clearing of the condition until other manifestations of the disease were regressing. A diffuse or patchy clouding of the cornea was a frequent residual lesion long after all acute manifestations had healed.

Orchitis.—In male rabbits inoculated by routes other than the intratesticular, an orchitis was a common symptom.

Orchitis developed in 70 per cent of 46 rabbits at a mean time of 7.2 days after inoculation (Table I). The first signs of the condition were small areas of thickenings or of nodular induration which rapidly increased in size so that within 2 or 3 days the testicle was diffusely indurated or resistant and considerably enlarged (Fig. 2). In some of these cases the parenchyma became hemorrhagic and this change might be detected during life. In other cases, the initial lesions tended to remain discrete and multinodular in type. Edema of the scrotum usually accompanied the orchitis. In cases which recovered the condition regressed fairly rapidly and eventually the testicles seemed normal clinically. Not infrequently, however, small fibrous or nodular areas persisted for some time after other clinical manifestations of the disease had healed.

Lymph Adenitis.—Enlargement and increased resistance or induration of the superficial lymph nodes and particularly those of the popliteal group was a usual occurrence during the course of the experimental disease.

A popliteal adenitis was recorded in 60 per cent of 84 rabbits with a mean time of occurrence of 6.9 days after inoculation (Table I). In a considerable proportion of cases the condition was subject to fluctuations, that is, the nodes were enlarged and resistant for several days and then became much smaller and indurated only to return to their former enlarged state. From this finding, together with the post mortem results on rabbits which died or were killed at the height of the infection, it is probable than an adenitis is a constant feature of the disease at some time or other. In recovered cases, examined 3 or 4 weeks after inoculation the popliteal nodes were recorded as normal or negative.

Gastro-Intestinal Involvement.—Evidence of involvement of the gastro-intestinal tract was furnished by the character of the feces.

In the first days of the disease the stools were frequently soft and unformed and this might be the only abnormality in mild or moderately severe cases. In a considerable proportion of animals mucus was also present and formed and otherwise normal looking feces were coated with mucus. The discharge of large amounts of unstained mucus of a soft or gelatinous consistency was not uncommonly seen (Fig. 13). In some instances the mucus was firmer and more tenacious so that it had the appearance of a cast (1).[1] Very soft moist stools or an outspoken diarrhea was a fairly common occurrence in the more severe conditions and particularly in the fatal cases. In the group of 84 rabbits (Table I) a diarrhea was noted in 15 animals or 18 per cent, with a mean time of occurrence of 10.4 days after inoculation. Of the 15 rabbits with this symptom, all but one were fatal cases.

It should be mentioned for the sake of completeness that papular lesions of the mucosa of the mouth and tongue were relatively frequent. These could sometimes be detected clinically.

General Symptomology.—In addition to the clinical manifestations just described, there were others of a more general character. Obvious signs of illness were practically always observed in rabbits which developed widespread lesions.

A listless, apathetic appearance and a disinclination to move about were usual features. Not infrequently the eyelids were droopy and partly closed even in the absence of gross eye involvement. The fur became rough and unkempt. The appetite was impaired and a loss of weight was common. In the more severe cases, the rabbits rapidly became very thin, weak, and prostrated. The general appearance of many animals was one of profound intoxication. On the other hand, there were instances in which the rabbit was comparatively lively and active despite an extensive cutaneous eruption and other signs of the disease. Such cases usually recovered if respiratory involvement was not present or was not severe.

Blood Cytology.—Observations on the blood cytology showed certain well marked variations from normal values (3). After the examination of a number of rabbits at various stages of the disease had shown that the blood picture was abnormal, two groups of rabbits were studied systematically with daily counts.

In the first group, 5 rabbits were inoculated in each testicle and intradermally at two sites; all were dead on the 3rd day. In the second group, 7 rabbits inoculated in each testicle were all dead by the 5th day. The results on the two groups were sufficiently similar to be considered together.

The mean red cell count on the 1st day after inoculation was depressed from a

[1] Pearce, Rosahn, and Hu (1), Fig. 3.

preinoculation level of 5,320,000 to 4,891,000 cells, but rapidly rose to 5,230,000 on the 2nd day and on the 3rd day to 5,593,000. The mean value for the last count before death was 5,683,000. It is possible that this rise was relative and due to dehydration although the presence of numerous nucleated red cells in the peripheral blood gave evidence of bone marrow activity. The changes in the hemoglobin level paralleled those of the red cell count. A profound depression in the platelet count was observed from a mean preinoculation level of 619,000 to 495,000 on the 3rd day after inoculation. In animals that survived longer than 3 days after inoculation, the platelet count rapidly rose again to a mean value of 837,000 for 4 animals on the 4th day and a value of 1,040,000 for the single animal that survived 5 days. The total white blood cell count increased sharply on the 3rd day after inoculation from a preinoculation mean level of 6,971 to 11,765. In animals which survived the 3 day period after inoculation, the total white count rose to 15,000 and 20,000 on the 4th and 5th days. Neutrophils increased from a preinoculation mean level of 3,610 to mean values of 4,839, 6,132, 9,269 on the 1st, 2nd, and 3rd days respectively. In relative per cent this represented an increase from a preinoculation value of 52.1 per cent to 77.0 per cent. Basophils and eosinophils decreased in number, both relatively and absolutely.

The changes in the lymphocyte and monocyte values were most striking. Lymphocytes gradually fell from a preinoculation mean level of 2,473 cells comprising 35.1 per cent of the total count to 1,501 (21.2 per cent), 1,023 (13.7 per cent), and 805 (8.1 per cent) on the 1st, 2nd, and 3rd days after inoculation. Monocytes, however, after a slight decrease on the 1st and 2nd days from a preinoculation level of 374 (5.4 per cent) rose precipitously on the 3rd day to 1,382 (12.2 per cent). The mean monocyte count for 4 animals on the 4th day after inoculation was 2,026.

The changes observed in these two groups of rabbits systematically studied were duplicated in isolated examinations on other animals. It was found that the severity of the disease could be gauged with remarkable accuracy by the blood cytology findings. An increase in neutrophils, platelets, and monocytes, together with a fall in lymphocytes, were characteristic features of severe infections and in general were of bad prognostic import. At the height of the infection lymphocyte values lower than 1,000, platelet values higher than 1,000,000, and monocyte counts above 3,000 were not uncommon. On the other hand, rabbits which were recovering from the disease were observed to have decreasing values for the total white count, platelets, and monocytes, and increasing numbers of lymphocytes. Animals that recovered from severe infections usually presented a mild degree of secondary anemia, followed by a gradual return to normal values.

Miscellaneous Routes of Inoculation

The foregoing discussion on the clinical manifestations and course of disease in experimental pox was based upon an assembled group of 84 rabbits which survived for a week or more. It was pointed out that this longer survival period was associated with a small dosage and with routes of inoculation other than the intratesticular. The local and general results of particular routes of injection have hitherto not been referred to except in the case of the intratesticular route which was discussed in the preceding paper (1). Certain of these findings which present particular points of interest will now be taken up.

Both Berkefeld V filtered and unfiltered testicular tissue-virus emulsions in variable dosage were employed, the material being obtained from serial passage rabbits inoculated intratesticularly and in most instances with the Xy171 strain (1).

Intradermal Inoculation.—The intradermal injection of pox virus even in high dilutions regularly resulted in the production of marked local inflammatory lesions of the skin in which congestion, hemorrhage, edema, and necrosis were conspicuous and characteristic features. Generalized manifestations frequently developed and fever at some time or other was usually recorded. In most cases 4 or 6 virus dilutions were used, ranging from 1:10 to 1:1,000,000; amounts of 0.1 or 0.2 cc. were injected.

The character and course of the local lesion including the incubation period, rate of development, and ultimate size were directly related to dosage. The photographs of Figs. 7 to 10 inclusive illustrate typical cutaneous reactions to 4 dilutions of a *filtered* testicular tissue-virus emulsion 3, 5, 8, and 13 days respectively after intradermal injection. Doses of 0.2 cc. of the dilutions 1:10, 1:100, 1:1,000, and 1:10,000 were employed. On the 3rd day a positive reaction had developed at the site of the two largest doses (Fig. 7). On the 5th day (Fig. 8) the cutaneous lesions produced by the three largest doses were pronounced, hemorrhage and edema were present, and necrosis was beginning; in the case of the smallest dose, the lesion comprised a small slightly congested swelling. On the 8th day (Fig. 9) the lesions were very much larger, hemorrhage and necrosis had markedly increased, the edema had extended to dependent portions of the skin and subcutaneous tissues far beyond the limits of the hemorrhagic necrotic areas, and the surfaces of the three largest lesions were covered by a firm tenacious blackish red crust. The two largest lesions had coalesced. By the 10th day this area was very large and included the lesion produced by the third virus dilution of 1:1,000. At certain points the crust was beginning to break down with oozing of

a thin blood stained fluid. Along the upper margin of the area, the acute manifestations of inflammation were beginning to subside as was also the case with the lesion produced by the highest dilution of virus (1:10,000). On the 13th day the upper margin of the large crust was becoming detached and a small amount of a thick yellowish white material was exuded (Fig. 10). The lower and by far the larger portion of the lesion, however, was still a massive edematous swelling covered by a tenacious black crust and the edematous involvement of the skin and subcutaneous tissues adjacent to the lower border was more pronounced. Fever was noted on the 3rd day (105.3°F.) and on the following 5 days. A maculopapular cutaneous rash was first seen on the 4th day and by the 8th day was very profuse (Fig. 9). During the following week the eruption was more marked, individual lesions increased in size, some became hemorrhagic and umbilicated and others pustular with crust formation (Fig. 10). A nasal discharge developed on the 4th day; blepharitis and conjunctivitis, a popliteal adenitis, and a nodular orchitis were present on the 7th day. A fortnight after inoculation the animal was seriously ill and death occurred on the 20th day.

In animals which recovered the initiation of regression of the local cutaneous lesion and the duration of the process of healing depended in large measure upon the size and character of the lesion. Lesions with extensive necrosis and secondary infection were slow to heal, but healing was usually complete within a month of inoculation.

Unfiltered virus emulsions injected intradermally produced extremely marked local reactions which developed earlier and were much more extensive than those produced by corresponding doses of filtered inocula. The general character and course of the disease was likewise more severe.

The incidence and character of the principal clinical manifestations observed in 24 intradermally inoculated rabbits which survived a week or longer is given in Table I. Fever was recorded in all cases, a generalized cutaneous eruption in 21 (88 per cent), nasal and eye involvement in 15 (63 per cent), an orchitis in 16 (67 per cent), and a popliteal adenitis in 11 animals (46 per cent) respectively. 10 of these rabbits died and 2 seriously ill ones were killed, giving a mortality rate of 50 per cent. In the circumstances of the very variable dosage employed, it is not possible to say whether this value truly represents the incidence of death from intradermal inoculation. But in any event there was a sufficient number of rabbits inoculated intradermally and intratesticularly with a comparable dosage to enable one to say that the effects of intradermal injection were less severe as far as the mortality rate and the survival period were concerned.

The limits of potency of testicular tissue-virus based upon the local cutaneous reaction were not determined in a sufficient number of experiments to enable one to make more than approximate valuations of potency. With Berkefeld V filtrates in 0.2 cc. doses, lesions were produced by dilutions as high as 1:10,000 and in some instances 1:100,000. In the case of unfiltered virus, positive reactions were observed with dilutions of 1:1,000,000 and in a few instances with dilutions of 1:10,000,000.

Cutaneous Scarification.—Scarification of shaved body skin areas was carried out in many rabbits inoculated by various routes and in some cases virus was also rubbed into the scarified areas. The striking result of these procedures in which Berkefeld V filtrates were employed was the comparatively minor tendency for lesions to develop in the lines of injury despite the development of a profuse cutaneous eruption.

Typical examples of cases in which the skin was scarified but no virus was applied to the area are shown in the photograph of a rabbit taken 5 days after intravenous inoculation (Fig. 1); of another taken 9 days after intratesticular inoculation (Fig. 5); and of a 3rd case taken 8 days after intranasal instillation (Fig. 6). In a few cases lesions did develop in traumatized areas, as for example, in a small cut incurred during shaving or in lines of scarification. But such localization appeared to be a chance occurrence for the lesions were neither confined to traumatized areas nor were they more numerous there than elsewhere, while their development did not precede or exceed that of lesions in other sites. In rabbits in which filtered virus was also applied to scarified cutaneous areas, the skin along the scarified lines was reddened and thickened within 24 to 48 hours, but this change rarely persisted for more than a day or so and no other was observed.

In a group of 20 rabbits inoculated by various routes other than the intratesticular with virus filtrates and in which cutaneous areas on one or both sides of the body were scarified, a generalized maculopapular rash developed in 14 animals or 70 per cent at a mean time of 5.9 days. 9 rabbits recovered and in 8 of them a cutaneous eruption developed at a mean time of 6.6 days; 11 rabbits died at a mean time of 7.3 days and in 6 of them a papular rash developed at a mean time of 5 days. In a group of 12 rabbits inoculated intratesticularly with various amounts of filtrates and in which virus was also applied to scarified skin areas, the average time of death of 11 animals was 5.7 days; there were 4 cases of a generalized cutaneous eruption developing at a mean time of 4.8 days. In the single recovered animal, a rash was seen on the 7th day. In none of these 18 rabbits with a generalized cutaneous eruption did there appear to be any predilection for lesions to develop in the lines of scarification. From the above figures it appears that in the fatal cases as well as in those which recovered there was sufficient time for cutaneous lesions to develop in lines of scarification had there been any outspoken tendency for them to do so.

The use of *unfiltered* tissue-virus emulsion in experiments of this kind was comparatively limited. The results on animals inoculated by various routes and in which the skin was scarified but no local application of virus was made, were similar to those just described in which filtrates had been used. In the cases in which unfiltered emulsions were rubbed into scarified cutaneous areas, there was a very pronounced local reaction along the lines of scarification with swelling, edema, congestion, hemorrhage, and necrosis. The lesions developed rapidly and within a

week adjacent skin areas might be included with the formation of boggy necrotic masses resembling the lesions produced by intradermal inoculation (Fig. 14). In certain instances, some discrete lesions did develop in the lines of scarification, particularly at their ends, but on the whole this result was overshadowed or masked by the other more general lesions.

Considering the high incidence of a generalized cutaneous eruption in cases of the experimentally induced disease, the fact that similar lesions failed to develop to any extent in scarified skin areas seems curiously inconsistent. Moreover, as will presently be described, a similar failure occurred in connection with scarification of the cornea. Is it possible that the slight injury incurred by these structures from superficial scarification led to the production *in situ* or to the localization of immune principles which were sufficient to prevent the development of visible discrete lesions? Whatever the explanation may be, the effect was not only rapidly attained but was also prolonged. In so far as clinical observation was concerned, the result obtained was contrary to that observed in other conditions in which the localization of lesions at points of injury characteristically occurs.

Intranasal Instillation.—Infection was regularly accomplished by dropping testicular tissue-virus emulsion in one nostril; 14 rabbits were inoculated in this manner.

For 5[2] animals Berkefeld V filtrates were used; 4 animals which received 0.1 or 0.3 cc. recovered while 1 inoculated with 0.5 cc. was found dead on the 10th day. Unfiltered emulsions in 0.5 cc. and 0.6 cc. amounts were used for 9[3] inoculations; 3 animals were dead 3 and 6 days later and 6 were killed from 3 to 9 days after inoculation. Some of the latter rabbits might have recovered. The available though scant evidence suggests that with a comparable dosage the mortality rate after intranasal inoculation would be lower than after intratesticular inoculation.

The local clinical reaction resulting from intranasal inoculation was similar to that observed in instances of nasal involvement occurring in the course of the disease produced by other routes of injection (Figs. 11 and 12). In a typical case inoculated with 0.1 cc. of filtered virus in the right nostril, there was, 10 days later, a profuse bilateral serosanguinous discharge, more marked on the right side, a few thin crusts had formed about the nares, and the tissues of the lips and lower nose were swollen and indurated. The nasal discharge was first noted on the 4th day, fever on the 6th, eye involvement, a few cutaneous papules, and popliteal adenitis

[2] The results on these rabbits are summarized in Table I.

[3] The results on 3 of these 9 rabbits are summarized in Table I.

on the 7th, and a marked nodular orchitis on the 12th day respectively. Regression of all lesions was well under way a fortnight after inoculation and a week later the animal was practically negative.

In 13 of the 14 rabbits inoculated intranasally, fever was recorded at some time or other; it was first noted on the 5th or 6th day in the case of filtrates and on the 2nd or 3rd day in the case of unfiltered inocula. The one exception was an animal with a subnormal temperature on the 2nd day which was found dead on the 3rd day. A generalized cutaneous rash was observed in 3 of the 5 rabbits inoculated with filtrates and in 4 of the 9 inoculated with unfiltered material; the mean time of development was 7.6 days after inoculation. Omitting the 4 animals of the latter group which died or were killed 3 to 6 days after inoculation, the incidence rate of a cutaneous eruption was 70 per cent. An example of the rash in these rabbits is shown in Fig. 6; the photograph was taken 8 days after the nasal instillation of 0.3 cc. of filtered virus. Other clinical manifestations of the disease were also observed (Table I), but little can be said about their incidence or general character because of the limited number of animals.

Conjunctival Instillation.—This route of inoculation was employed in 4 rabbits; 0.1 or 0.15 cc. filtered virus emulsions were used for 5 eyes, 0.5 cc. for 1 and 0.01 cc. of a 1:10 virus dilution for another; 1 eye was not inoculated.

A well marked local reaction developed in 4 and a minor response in 2 eyes; none was observed in connection with the diluted virus. All 4 rabbits developed generalized disease manifestations, including fever which was first noted on the 3rd to the 9th day, and a maculopapular cutaneous eruption at a mean time of 6.8 days (Table I). There were no fatalities.

The first indication of a local reaction was seen on the 3rd and 4th days and comprised swelling and reddening of the lids, lachrymation, and photophobia. The condition rapidly became intensified and within a few days the lids were tightly glued together by a thick purulent secretion and about the eye was an accumulation of yellowish tenacious crusts. Separation of the lids revealed a marked conjunctivitis and diffuse keratitis. Regression of the lesion was usually well under way by the end of the 2nd week.

An acute edema of the lid conjunctiva was also seen, and in some cases it developed in the absence of other marked eye lesions. In the case of an animal in which 0.15 cc. of virus filtrate had been dropped in the conjunctival sac, edema of the lower lid conjunctiva and of the 3rd eyelid was well marked on the 4th day, but there was only slight congestion of the conjunctival vessels and slight lachrymation. On the 7th day (Fig. 16) the lesion included marked edema of the upper lid conjunctiva, marked swelling and reddening of both lids, and a mucopurulent secretion.

Various degrees of corneal involvement were usually noted after conjunctival instillation of virus, and the keratitis sometimes included pannus formation.

Corneal ulceration and perforation might subsequently occur. Examples of these lesions are illustrated by the photographs (Figs. 17 and 18) of a rabbit taken 11 and 23 days after the conjunctival instillation of 0.3 cc. of a 1:10 dilution of a virus filtrate.

All of the eye lesions which developed after conjunctival inoculation were also observed in certain rabbits inoculated by other routes. Their general character and course was the same. Healing was accomplished without residual effects except in certain cases of persistent small areas of corneal opacity or of the depressed scar of a previous ulcer. There were also some instances of scarring and distortion of the eyelids.

Scarification of Cornea and Conjunctival Instillation of Virus

Attempts to produce lesions of the cornea in lines of scarification were unsuccessful.

The cornea was anesthetized by the instillation of a 2 per cent solution of cocaine and then scarified with the point of a corneal knife. Of 4 rabbits so prepared and inoculated with virus filtrates intravenously, intramuscularly, or intraperitoneally, no gross lesions of the 8 eyes were detected; all 4 animals developed fever and a generalized maculopapular eruption, and 1 was found dead on the 10th day. In 2 other rabbits 2 drops of full strength virus filtrates were dropped on the scarified cornea of both eyes; a minor conjunctivitis developed but no corneal lesions were observed. The same procedure but with the use of unfiltered virus was carried out on 4 rabbits. A blepharitis and conjunctivitis developed on the 2nd and 3rd days, and there was a faint diffuse clouding of 2 corneas but no focal lesions confined to the lines of scarification could be made out.

Inclusion Bodies

The question of inclusion bodies in rabbit pox was first investigated in cases of the spontaneous disease (2). Microscopic examination of tissues in different stages of the infection failed to reveal any cytoplasmic or nuclear changes which were sufficiently characteristic to be called inclusion bodies. A similar negative result was obtained on tissues from experimental cases until inoculated corneas were studied. These specimens were obtained 1 to 5 days after conjunctival instillation of unfiltered virus in eyes in which corneal scarification had been carried out just prior to inoculation. The corneas themselves showed no gross change other than a faint diffuse clouding. It was found on

microscopic examination, however, that many epithelial cells contained definite cytoplasmic bodies which appeared to be identical with Guarnieri bodies (Fig. 15); they were most frequent in the deeper cells. The inclusions were found in greatest numbers in the specimens examined 48 hours after inoculation and were distinctly less numerous in older lesions.

Intravenous Inoculation.—The results of intravenous inoculation were particularly successful in the production of a pronounced clinical syndrome including an extensive maculopapular cutaneous eruption.

Berkefeld V tissue-virus filtrates were used for the 6 rabbits inoculated by this route (Table I). No intravenous injections were carried out with unfiltered emulsions. 3 rabbits which received doses of 0.2, 0.4, and 0.5 cc. respectively recovered, 2 injected with 0.4 and 0.5 cc. were found dead on the 13th and 8th days, and 1 injected with 0.4 cc. was killed on the 8th day. Each rabbit developed fever, the mean time of the first observation being 4.5 days, an extensive maculopapular cutaneous eruption (Figs. 1 and 4) at a mean time of 5.7 days, and a nodular orchitis which was detected at a mean time of 4.8 days. In 5 of the 6 rabbits a well marked popliteal adenitis was noted on an average of 6.4 days. Other less constant features were nasal involvement in 2 (Fig. 11), eye involvement in 1, and a diarrhea in 1 animal respectively.

Intramuscular Inoculation.—2 rabbits injected in the thigh muscles with 0.4 and 0.5 cc. respectively of Berkefeld V tissue-virus filtrates recovered from severe infections (Table I). There was a very marked swelling of the injected muscles. At first, the muscles felt tense, then somewhat boggy and within a few days they became very indurated. Fever was first recorded on the 3rd and 5th days and a profuse maculopapular cutaneous rash developed on the 8th and 5th days (Table I). In both animals a bilateral popliteal adenitis and orchitis with scrotal edema developed, and in one there was nasal and eye involvement including a keratitis. Regression of all lesions began in about a fortnight and healing was practically complete 3 weeks after inoculation.

Intraperitoneal Inoculation.—2 rabbits were injected intraperitoneally with 0.4 and 0.5 cc. respectively of Berkefeld V tissue-virus filtrates (Table I). Both animals developed a well marked disease from which 1 recovered while the other was found dead on the 10th day. Fever was first observed on the 3rd and 4th days, and a cutaneous rash developed on the 5th and 6th days. In both animals there was a bilateral orchitis and a popliteal adenitis. Eye involvement with keratitis, a nasal discharge, and a diarrhea were also seen in the rabbit which recovered.

SUMMARY AND CONCLUSIONS

The clinical manifestations and course of disease observed in experimental rabbit pox have been described and analyzed. The condition

differed from the acute fulminating and rapidly fatal type of experimental infection (1) in that the period of survival was longer, a variety of clinical manifestations developed and a considerable proportion of the cases recovered. The most conspicuous symptom was a generalized papular eruption on the skin and mucocutaneous borders.

The production of the disease was associated with routes of inoculation other than the intratesticular or with a small dosage. The majority of cases were inoculated with Berkefeld V filtrates of tissue-virus emulsions and not with the more potent unfiltered emulsions.

The local reactions resulting from various routes of inoculation were described. Of special interest were the pronounced cutaneous reactions induced by intradermal injection, the high instance of marked clinical manifestations after intravenous inoculation, the failure of lesions to localize in the lines of scarification of skin and cornea even in cases with a profuse cutaneous eruption, and the development of cytoplasmic inclusion bodies in the epithelial cells of the cornea following scarification and conjunctival instillation of virus.

In the character of its clinical manifestations and course of disease, experimental rabbit pox was indistinguishable from cases of the spontaneous pox.

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EXPLANATION OF PLATES

PLATE 21

FIGS. 1, 2, and 3. Intravenous inoculation of 0.5 cc. Berkefeld V filtrate of testicular tissue emulsion; 3rd generation of filtrate series of virus passage. Skin was scarified but not inoculated. Rabbit found dead on 8th day.

FIG. 1. 5 days. Profuse papular cutaneous eruption. Hemorrhage and beginning necrosis of papules.

FIG. 2. 6 days. Papules on left scrotum, prepuce, and anus. Orchitis present and scrotal edema.

FIG. 3. 7 days. Papules on right upper and lower lips and left nares.

FIG. 4. Profuse cutaneous eruption on ears. 8 days after intravenous injection of 0.4 cc. Berkefeld V filtrate of testicular tissue emulsion; 4th generation of filtrate series of virus passage. Rabbit found dead on 14th day.

FIG. 5. Recent and late cutaneous papules; older lesions hemorrhagic and umbilicated. 9 days after unilateral testicular injection of 0.2 cc. Berkefeld V filtrate of skin emulsion from spontaneous case of pox (rabbit X667-1 (1)). Rabbit found dead on 14th day.

FIG. 6. Recent and late cutaneous papules; 1 large umbilicated lesion. 8 days after intranasal instillation of 0.3 cc. Berkefeld V filtrate of testicular tissue emulsion; second generation of filtrate series of virus passage. Rabbit recovered.

PLATE 22

FIGS. 7 to 10. Cutaneous reaction 3, 5, 8, and 13 days after intradermal injection of Berkefeld V filtrate of testicular tissue emulsion; 5th generation of filtrate passage series of virus. 0.2 cc. doses of 1:10, 1:100, 1:1,000, and 1:10,000 dilutions. Rabbit found dead on 20th day.

PLATE 23

FIG. 11. Papular eruption on lips, nares, and muzzle. Marked nasal discharge; swollen muzzle. Same rabbit as Fig. 4, 11 days after inoculation.

FIG. 12. Blood stained crusts about nares. Nasal discharge. 13 days after bilateral testicular injection of 0.5 cc. Berkefeld V filtrate of defibrinated blood from spontaneous case (Xy171 (1)). Rabbit found dead on 18th day.

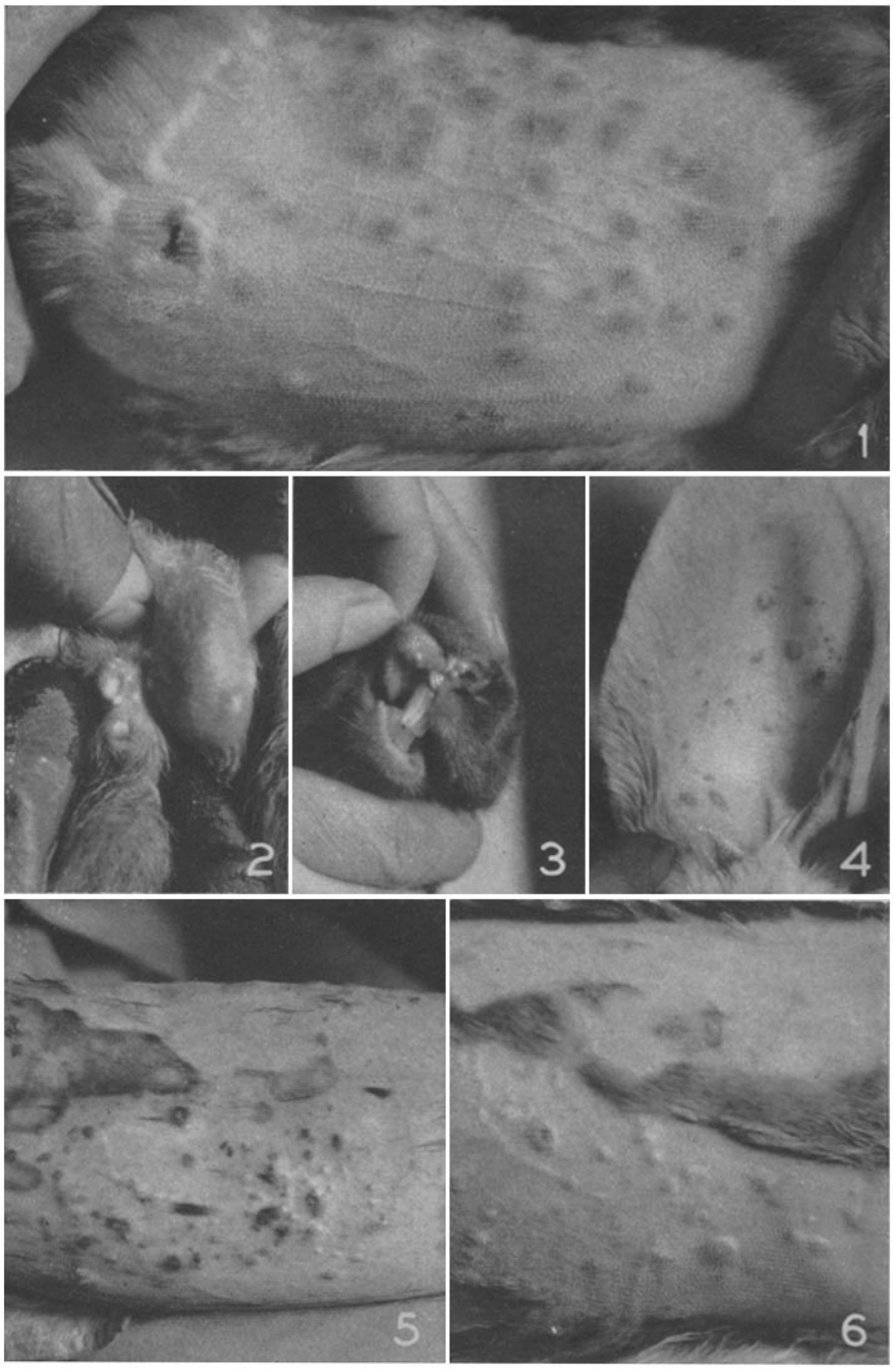
FIG. 13. Mucous rectal discharge. 5 days after bilateral testicular injection of 0.4 cc. Berkefeld V filtrate of testicular tissue emulsion; 4th generation of filtrate passage series of virus. Rabbit found dead on 7th day.

FIG. 14. Cutaneous reaction 8 days after inoculation of scarified skin with unfiltered testicular tissue-virus from 17th generation of virus passage. Rabbit recovered.

FIG. 15. Cytoplasmic inclusion bodies in epithelial cells of cornea. Giemsa stain. 48 hours after corneal scarification and conjunctival instillation of unfiltered testicular tissue-virus; 14th consecutive rabbit passage. × 1,000.

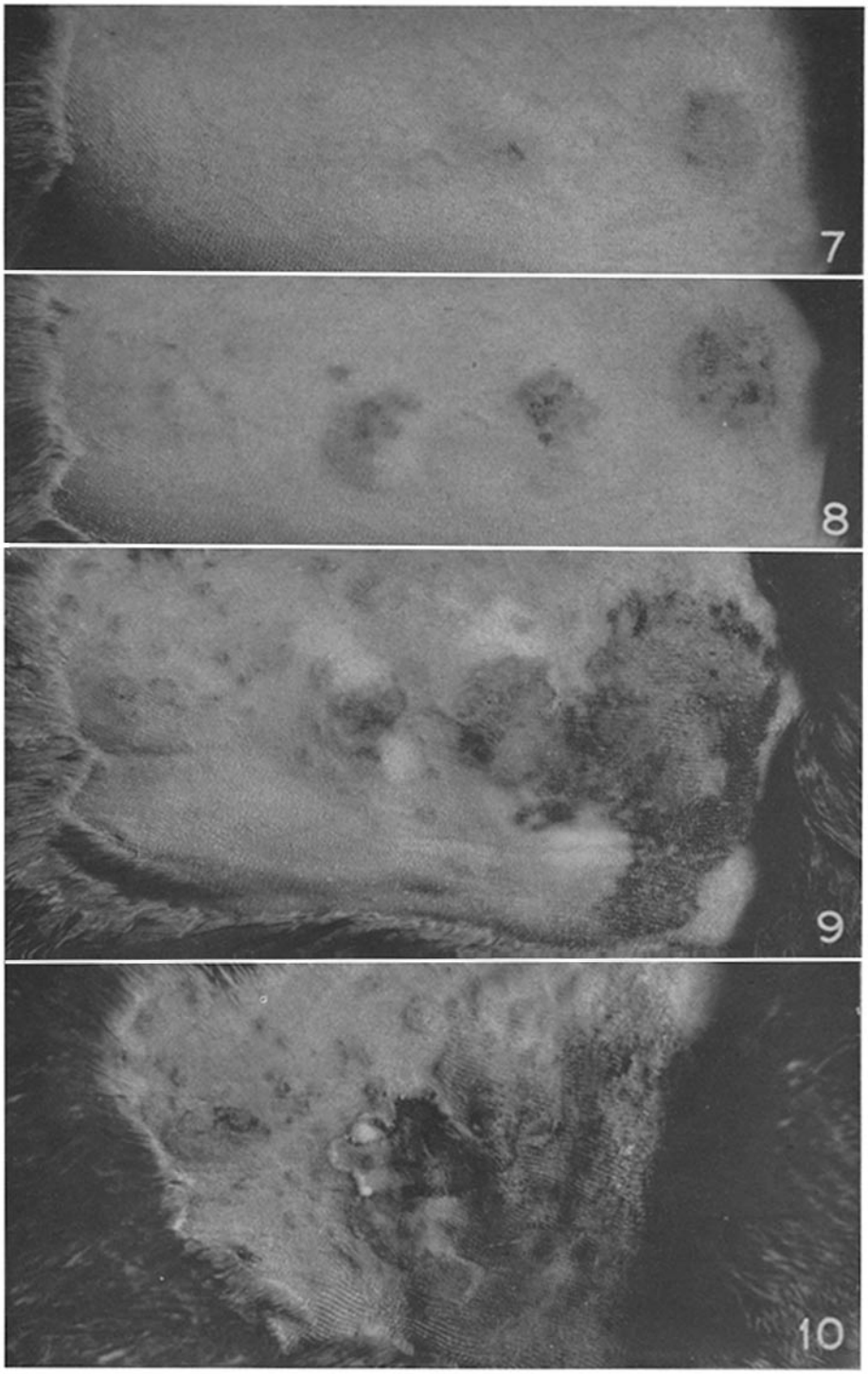
FIG. 16. Marked blepharitis, edema of upper lid conjunctiva, and purulent discharge. 7 days after conjunctival instillation of 0.15 cc. Berkefeld V filtrate of testicular tissue emulsion; 3rd passage generation of virus. Rabbit recovered.

FIGS. 17 and 18. Marked blepharitis, corneal clouding with pannus, and eventually corneal ulcerations. 11 and 23 days after conjunctival instillation of 0.3 cc. of Berkefeld V virus filtrate diluted 1:10; 3rd generation of filtrate series of virus passage. The right conjunctiva was also inoculated with a larger dose. Rabbit recovered.



Photographed by Joseph B. Haulenbeek

(Rosahn *et al.*: Etiology of rabbit pox. II)



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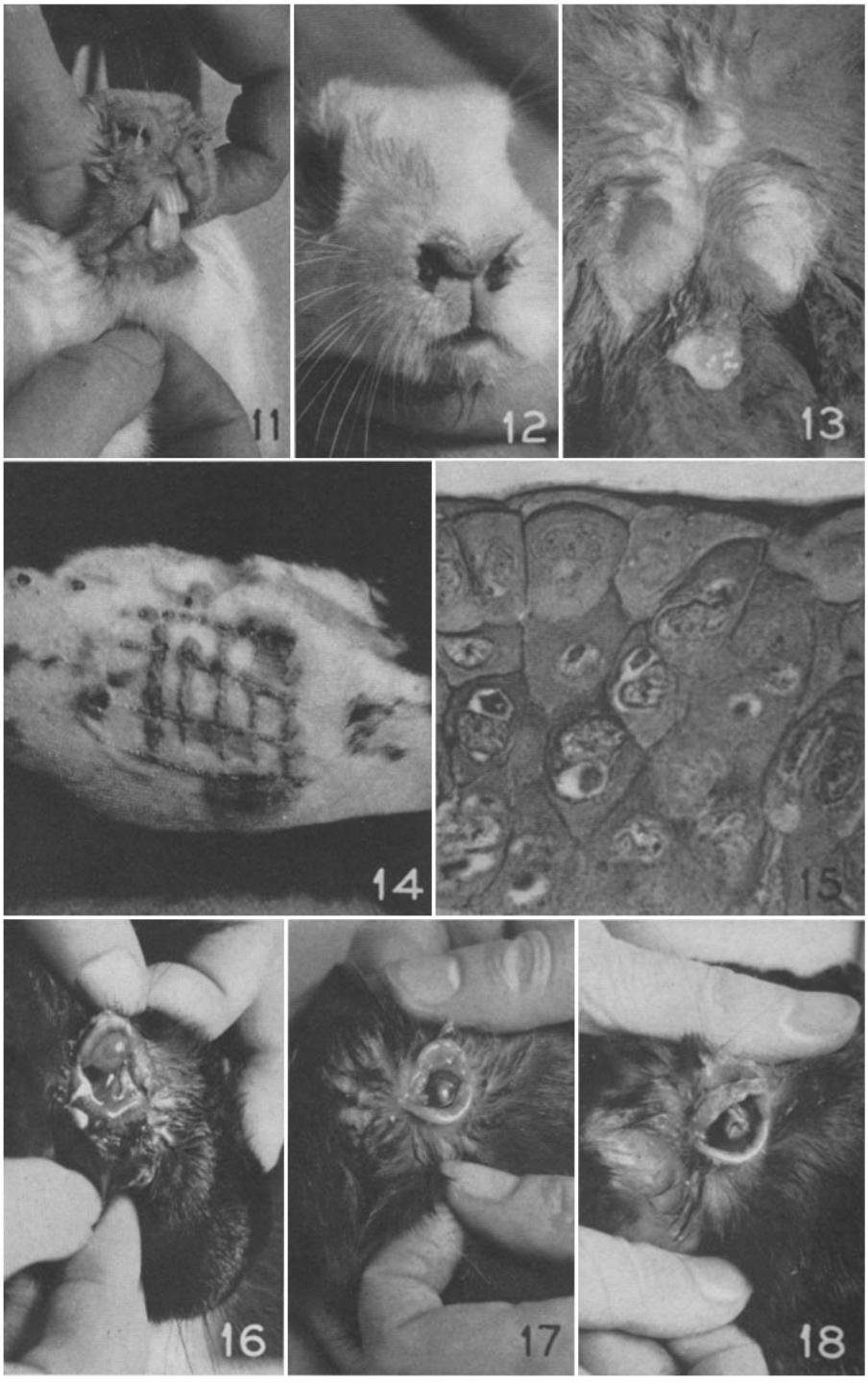

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