STUDIES ON THE ETIOLOGY OF RABBIT POX

V. Studies on Species Susceptibility to Rabbit Pox Virus

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The isolation and serial transmission of a filterable agent from spontaneous cases of rabbit pox, together with a description of certain of its pathogenic properties and of the clinical manifestations of the experimentally induced infection, were reported in the first two papers of this series (1, 2). The experiments on the immunological aspects of the reaction to pox infection have also been reported (3, 4). In the latter work, the relation of pox virus to other viruses was taken up primarily from the standpoint of identification. The results of a large number of crossed inoculation and immune serum-virus neutralization tests led to the opinion that while pox virus was closely related to vaccine virus, its complete identity with two specimens of dermovaccine and one of neurovaccine virus employed could not be established.

In connection with the identification of the etiological agent of pox, information on the susceptibility of other animal species was obviously important. In addition, such information had a practical bearing from the standpoint of the possible spread of the spontaneous infection and of potential carriers and reservoirs of the virus. Although it was not possible to investigate the susceptibility of many species nor to carry out as complete a study on any one of them as was desired, enough was accomplished to show that the mouse, the guinea pig, the calf, and probably the rat were susceptible to inoculation with pox virus.

The results of these experiments are reported in the present paper,

together with a general discussion of the investigations on experimental pox, particularly with reference to the relation of pox virus to other viruses and of rabbit pox infection to other pock diseases.

Materials and Methods

Pox Virus.—The pox virus tissues used for inoculation were obtained from rabbits of the consecutive transmission series of the Xy171 strain (1). In this series Berkefeld V filtered or unfiltered emulsions of testicular tissue derived from an acute orchitis were injected intratesticularly and the inocula employed in the present experiments were portions of these emulsions. The tissue was ground with alundum and Locke's solution to concentrations of 10 to 15 per cent by weight. Each emulsion was examined for the presence of ordinary aerobic and anaerobic organisms. The bacterial sterility of filtered emulsions was tested in the usual manner against *B. prodigiosus* (1).

Animals.—Albino mice from the Institute breeding stock and albino rats, guinea pigs, and male rabbits from outside sources were used. The rabbits were of hybrid stock and averaged 4 to 6 months of age.

A Jersey Holstein calf 3½ months of age and 75 kilos in weight was used in one experiment.

Routes of Inoculation and Dosage.—Mice were injected intraperitoneally, subcutaneously, intracerebrally, and by nasal instillation. In the case of certain intraperitoneal injections, 0.05 cc. of a 2 per cent sterile starch emulsion was also injected intracerebrally. Rats and guinea pigs were injected by the intratesticular, intraperitoneal, and nasal routes, and in addition, the intradermal route was used for certain guinea pigs. Inoculation of a calf was accomplished by rubbing 1.0 cc. of unfiltered tissue-virus emulsion on scarified shaved skin areas of the sides of the body.

The dosage for the small animals ranged from 0.01 to 1.0 cc., the latter amount being used for intraperitoneal injections in guinea pigs.

Experiments on Mice

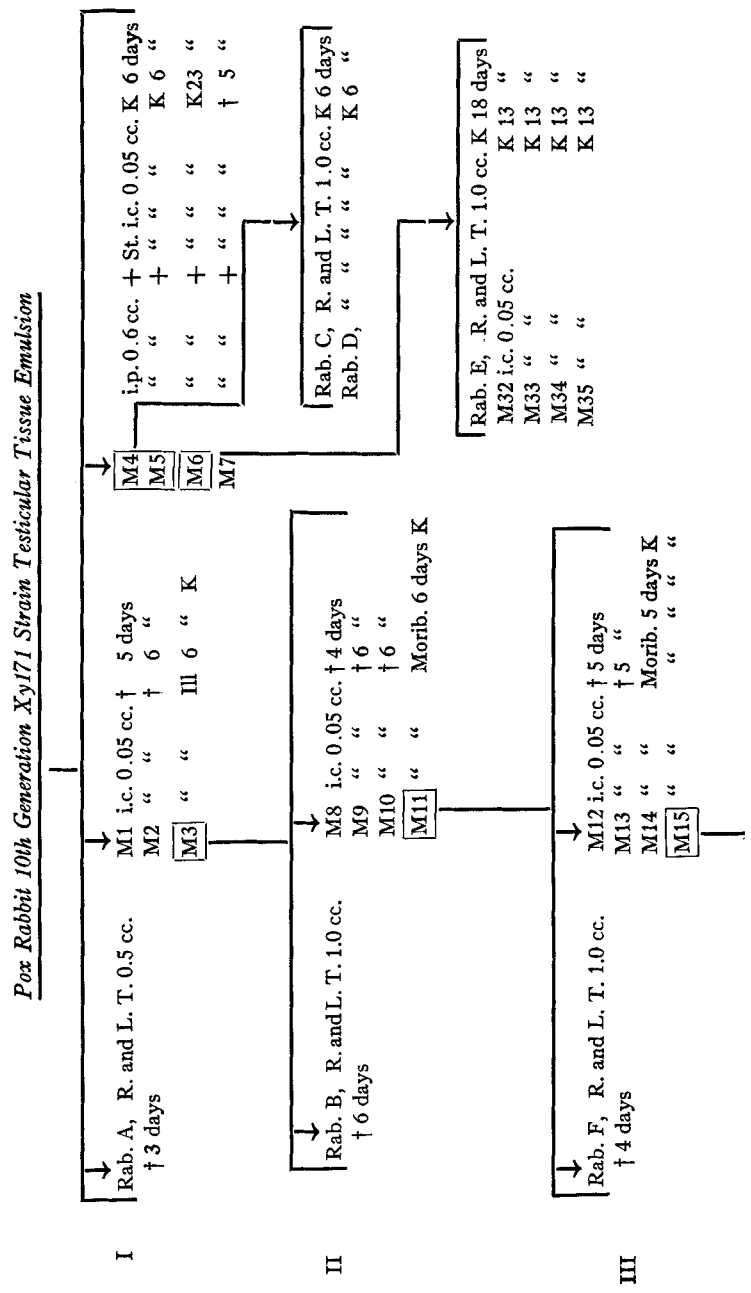
The first experiments on the inoculation with pox virus of species other than the rabbit were carried out early in the course of the work when Berkefeld V filtrates were employed for the routine passage of the virus.

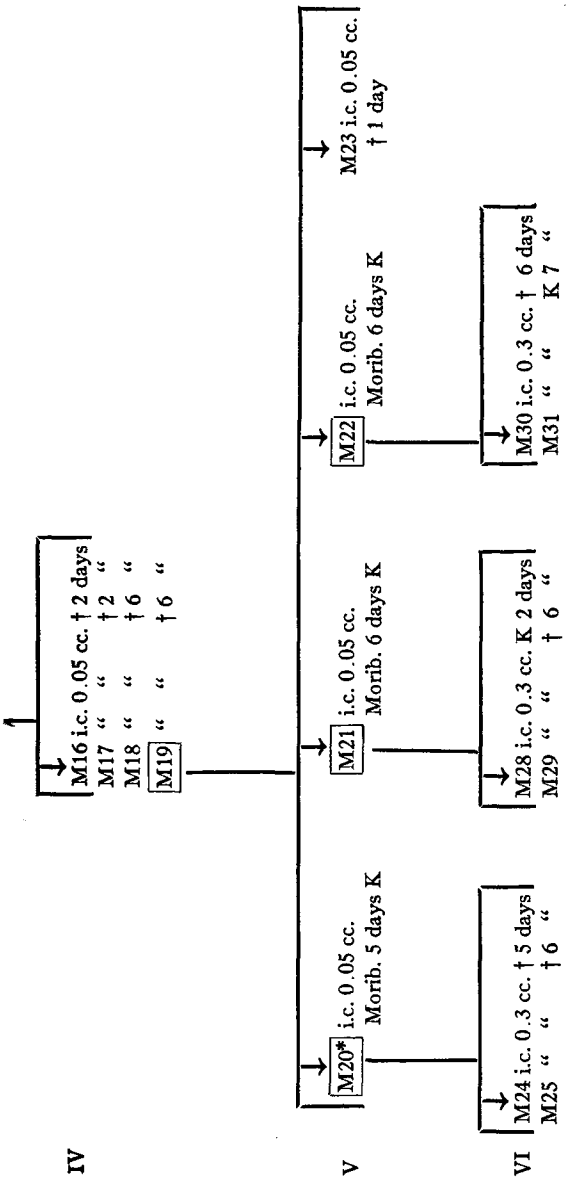
In the first group of 10 mice, a filtrate of testicular tissue from the 2nd serial rabbit passage of the Xy171 strain of virus (1) was injected by the intraperitoneal, subcutaneous, or nasal routes; the dosage ranged from 0.01 to 0.05 cc. A second group of 4 mice were injected intracerebrally with 0.05 cc. doses of a testicular tissue filtrate of the 4th rabbit passage. A third group of 6 mice were injected intraperitoneally with 0.6 cc. of the filtrate of the 6th rabbit passage and intracerebrally with 0.05 cc. of a 2 per cent starch emulsion.

The results of all 3 experiments were negative clinically and at post mortem examination carried out 1 to 4 weeks after injection. Evidence of a successful inoculation in the case of the 3rd experiment, however, was furnished by the results obtained in a rabbit injected with mouse brain.

Two mice were killed 7 days after virus had been injected intraperitoneally and starch emulsion intracerebrally, and a 10 per cent emulsion of the pooled brain tissue was prepared by grinding with alundum in Locke's solution. 1.0 cc. was injected in the testicles of a rabbit; 5 mice were injected intracerebrally with 0.05 cc. doses; 2 mice were injected intraperitoneally with 0.6 cc.; and 2 mice were given 0.6 cc. intraperitoneally and 0.05 cc. starch suspension intracerebrally. The clinical and post mortem results on the mice were negative, but the rabbit developed fever and a marked hemorrhagic orchitis with scrotal edema and was found dead on the 6th day. A Berkefeld V filtrate of testicular tissue from this animal was used to inoculate 2 rabbits with bilateral intratesticular injections of 1.0 and 0.5 cc. respectively. Fever and a pronounced orchitis with scrotal edema developed in both rabbits and in addition, a typical generalized papular cutaneous rash developed on the 8th and 10th days respectively; one rabbit was found dead on the 10th and the other on the 13th day. The clinical results in these rabbits were characteristic of experimental rabbit pox (1, 2) while the post mortem findings were also typical.

Uncertain results were obtained from the injection of 2 rabbits with the brains of 2 other mice of the 3rd series killed at a longer interval after inoculation, that is, a week later than the first 2 mice or 14 days after inoculation. A similar experiment with the brains of mice of the 2nd series killed 7 and 12 days after inoculation had also been inconclusive. The situation was complicated at this stage of the experiments by the fact that after inoculation of certain emulsions of pooled brains had been made, it was found that the emulsions were contaminated. At the time of these particular experiments the retention of virulence of the virus under conditions of refrigeration was not known. In the next experiment, therefore, in which it was planned to carry out a series of brain to brain passages, it was decided to make the inoculations within a week of each other and to use only one brain for each group of animals. In the event that the inoculum was found to be contaminated, it was possible to repeat the experiment with another fresh brain emulsion. A further modification of procedure was the use of an unfiltered emulsion of rabbit tissue virus for the


Pox Rabbit 10th Generation Xyl71 Strain Testicular Tissue Emulsion
I
Rab. A, R. and L. T. 0.5 cc.
† 3 days
M1 i.c. 0.05 cc. † 5 days
M2 " " † 6 "
M3 " " Ill 6 " K
M4
M5
M6
M7
i.p. 0.6 cc. + St. i.c. 0.05 cc. K 6 days
" " + " " " " K 6 "
" " + " " " " K23 "
" " + " " " " † 5 "
II
Rab. B, R. and L. T. 1.0 cc.
† 6 days
M8 i.c. 0.05 cc. † 4 days
M9 " " † 6 "
M10 " " † 6 "
M11 " " Morib. 6 days K
Rab. C, R. and L. T. 1.0 cc. K 6 days
Rab. D, " " " " " K 6 "
III
Rab. F, R. and L. T. 1.0 cc.
† 4 days
M12 i.c. 0.05 cc. † 5 days
M13 " " † 5 "
M14 " " Morib. 5 days K
M15 " " " " "
Rab. E, R. and L. T. 1.0 cc. K 18 days
M32 i.c. 0.05 cc. K 13 "
M33 " " K 13 "
M34 " " K 13 "
M35 " " K 13 "



TEXT-FIG. 1. Results of the serial passage of pox virus in mice. Intracerebral inoculation of unfiltered brain emulsions. i.c. = intracerebral; i.p. = intraperitoneal; T. = intratesticular routes of injection; St. = 2 per cent sterile starch emulsion. † = found dead; K = killed.

* Bacterial contamination of brain of mouse 20. The brain of mouse 27 was sterile.

initial mouse injection instead of the less potent filtrates previously employed.

The results of this experiment were striking. It was found in the first group of mice that the intracerebral injection of unfiltered rabbit tissue virus caused death in from 4 to 6 days, and in addition that this result was repeated in 5 serial passages of unfiltered mouse brain injected intracerebrally (Text-fig. 1).

The series was begun with testicular tissue from a rabbit of the 10th consecutive rabbit passage of the Xy171 strain (1). 3 mice were injected intracerebrally with doses of 0.05 cc.; 4 mice received 0.05 cc. of starch emulsion intracerebrally and 0.6 cc. of virus emulsion intraperitoneally; and a rabbit was inoculated in each testicle with 0.5 cc. As will be seen from the scheme of the experiment given in Text-fig. 1, 2 of the mice inoculated directly into the brain were found dead on the 5th and 6th days; the 3rd mouse was killed on the 6th day and the brain used for the 2nd passage to 4 mice. The results in these latter animals were identical with those in the first group and similar findings were likewise obtained in 4 additional mouse brain passages, that is, a total of 6 mouse generations. In the 6th passage comprising 8 mice the inoculating dose was reduced to 0.03 cc.

In the 2nd and 3rd mouse passages, a rabbit was injected intratesticularly with the mouse brain emulsions. The condition which developed was indistinguishable from the acute fulminating type of infection associated with the intratesticular injection of testicular tissue-virus regularly observed in the rabbit passage series of the virus (1). Fever and a marked hemorrhagic orchitis with scrotal edema developed and the rabbits were found dead on the 4th and 6th days respectively.

Bacteriologic examinations of the mouse brain inocula used for the first 4 serial transfers were negative, but the 5th passage emulsion was contaminated, and consequently two additional sets of inoculations were made with two other 5th generation brains. Cultures of these emulsions were sterile. The results on all three 6th generation groups, however, were similar and furthermore, in the case of the brain of one mouse inoculated with contaminated material and killed 8 days after injection, bacteriologic examination was negative.

The first lot of mice in this experiment inoculated with rabbit tissue-virus included 4 mice which were inoculated intraperitoneally with virus and intracerebrally with starch emulsion (Text-fig. 1). 1 mouse was found dead on the 5th day; 2 were killed on the 6th day and the pooled brains were injected intratesticularly in 2 rabbits; the 4th mouse was killed on the 23rd day and the brain was used for the intratesticular injection of 1 rabbit and the intracerebral injection of 4 mice. Both inocula were bacteriologically sterile. The 2 rabbits injected with the brains inoculated 6 days previously developed fever and an acute hemorrhagic orchitis and scrotal edema typical of experimental fulminating pox infection (1). In the case of the rabbit and the mice injected with mouse brain ob-

tained 23 days after inoculation, the clinical and post mortem results were negative. Although the number of animals is small, the findings suggest that the condition in mice resulting from the intraperitoneal inoculation of pox virus, together with the intracerebral injection of starch, was less severe than that which developed from the intracerebral inoculation of virus alone. In the present experiment, there was only 1 fatality among 4 mice given the double injection while there were 2 deaths among 3 mice injected only with virus and the 3rd mouse was seriously ill when killed. The negative results following the subinoculation of mouse brain 23 days after its injection confirm the previous findings on the uncertainty of recovering virus from this source later than approximately a week after inoculation.

There seems to be no reason to doubt that rabbit pox virus was successfully transmitted to the mouse and was continued in this species by the use of brain emulsions injected intracerebrally, although confirmatory evidence in the form of subinoculations of rabbits is available for only the first 2 passages. The results on the 6 consecutive series of mice were consistent in that a fatal outcome occurred in the great majority of cases and within quite a constant time range. Of 27 mice, 20 were found dead or were critically ill and were killed 5 or 6 days after inoculation; 2 were ill and were killed on the 7th and 8th days respectively; 1 died on the 4th day, 3 on the 2nd, and 1 on the 1st day after inoculation.

In these various experiments none of the inoculated mice developed a disease picture comparable to that of the rabbit with its wide diversity of clinical manifestations. On the other hand, the fatal condition which did develop might be compared to the acute fulminating and rapidly fatal infection of the rabbit associated with a large dosage and the intratesticular route of inoculation such as obtained in the routine serial passage of the virus (1). It is not known how long the virus remains in an active state in the mouse brain, but the results obtained indicate a period of approximately a week after inoculation. Whether the mouse can be infected by routes other than the intracerebral was not sufficiently investigated for any opinion to be formed.

Experiments on Rats

The work on the inoculation of rats with pox virus was limited to 3 experiments, one of which gave results which indicate the susceptibility of this species.

In the first experiment a Berkefeld V filtrate of testicular tissue from a rabbit of the 2nd generation of the Xy171 strain of pox virus was used (1); 2 rats were

injected intratesticularly, 2 intraperitoneally, and 2 by nasal instillation. Doses of 0.1 to 0.2 cc. were employed. In the second experiment unfiltered testicular tissue emulsions of the 10th rabbit generation of the same strain of virus were used; 2 rats were injected intraperitoneally and 2 subcutaneously with doses of 0.5 cc. The results of both experiments were negative in that no clinical evidence of infection developed in any of the animals and the post mortem examinations made 9 to 14 days after injection showed nothing of note.

The third experiment was carried out with testicular tissue from a rabbit of the 15th consecutive passage of the Xy171 strain transmitted by Berkefeld V filtrates. 2 male rats were injected in both testicles with 0.5 cc. of an unfiltered emulsion. There was a slight transitory swelling of the testicles, but no other clinical change was observed. 8 days after inoculation both rats were killed and one testicle of each animal was used for the subinoculation of a rabbit. 10 per cent tissue emulsions were made in the usual manner. Bacteriologic examinations of the emulsion were negative. Each emulsion, unfiltered, was injected intratesticularly in 0.5 cc. doses. Similar results were obtained in both subinoculated animals. Fever and a marked acute hemorrhagic orchitis with accompanying scrotal edema developed and the animals were found dead on the 5th and 7th days respectively. The findings were typical of the acute fulminating type of pox infection regularly produced by the serial transmission of virus in rabbits by means of intratesticular injections of testicular tissue emulsions (1).

The results on these rabbits indicate that pox virus may survive 8 days in the rat and apparently without an attenuation of virulence. It is not known how frequently such results would be obtained nor whether the virus can be serially transmitted from rat to rat. It would seem likely, however, that serial transmission could be accomplished by means of testicle to testicle passage carried out at approximately weekly intervals.

Experiments on Guinea Pigs

The results of experiments on guinea pigs indicate that this species is also susceptible to inoculation with pox virus.

The first experiment comprised 6 animals which were injected intratesticularly, intraperitoneally, or intranasally with 0.1 to 0.4 cc. doses of testicular tissue filtrate derived from the 2nd rabbit passage of the Xy171 strain of virus (1). In the 2 guinea pigs injected intratesticularly there was a moderate enlargement and resistance of the testicles which developed on the 2nd day, persisted for 3 or 4 days, and then subsided. The other animals showed no clinical effect of the injections. Post mortem examinations on the 9th and 15th days were negative except in the case of the liver of 1 guinea pig killed on the 9th day. The organ appeared normal except that on the surface there were several small greyish opaque

spots about a millimeter in diameter. The significance of these areas is not entirely certain, but they may have been pox lesions. The subinoculation of rabbits with this liver material gave inconclusive results.

In the second experiment, 2 guinea pigs were injected intradermally with an 0.3 cc. dose of a Berkefeld V virus filtrate derived from testicular tissue of a rabbit in the 5th rabbit passage of the Xy171 strain. The clinical and post mortem results on these animals were likewise negative.

For the third experiment an unfiltered virus emulsion prepared from the testicles of a rabbit of the 10th serial passage of the Xy171 strain of virus was used (1). 2 guinea pigs were injected intratesticularly with 0.5 cc. and 2 intraperitoneally with 1.0 cc. In addition, each animal was injected intradermally on a shaved area of the side of the body with a dose of 0.3 cc. A definite cutaneous reaction developed in each animal which was considered positive. There was some swelling and reddening of the skin on the day following injection. By the 5th day these areas measured 1.5 to 2.0 cm. in diameter, they were definitely congested and indurated with a small central crust. One of the lesions was also edematous and in this case the reaction increased in intensity during the next 2 days. Regression of all lesions was well under way 8 days after inoculation at which time the animals were killed.

The 2 guinea pigs injected intraperitoneally showed no general signs of infection and post mortem examination 8 days after inoculation was negative. In both intratesticularly injected animals swelling and induration of the testicles developed on the day after inoculation and persisted for 3 or 4 days. Post mortem examination on the 8th day was negative except for slight congestion and enlargement of the testicles. The testicles of one animal were ground with Locke's solution and alundum to make a 10 per cent emulsion. Cultures of the emulsion showed no growth. A rabbit injected intratesticularly with 1.0 cc. doses of the unfiltered emulsion developed a typical case of the fulminating type of experimental pox infection (1). The reaction was characterized by fever which on one occasion reached 106.3°F. and an acute marked hemorrhagic orchitis and scrotal edema. On the 8th day the animal was seriously ill and was killed.

In the fourth experiment 2 guinea pigs were injected intratesticularly with 1.0 and 0.5 cc. doses of an unfiltered emulsion prepared from the testicles of a rabbit of the 15th consecutive filtrate series of the Xy171 strain of virus. The emulsion was bacteriologically sterile. Both animals developed swelling and induration of the testicles and in one case there was slight scrotal edema. 1 guinea pig was killed on the 8th day; the testicles were congested and the parenchyma swollen. The 2nd animal was found dead on the 4th day; the testicles were swollen, indurated, and hemorrhagic. An emulsion of the left testicle from the latter animal was prepared in the usual manner; bacteriologic examinations were negative. The following subinoculations of unfiltered emulsion were made. A rabbit was injected intratesticularly with 1.0 cc. doses, 2 guinea pigs were injected intratesticularly with 0.5 and 1.0 cc. doses respectively, and a 3rd guinea pig was injected intradermally with 0.2 cc. In the case of the rabbit, the results were typical of the fulminating type of experimental pox infection. A hemorrhagic orchitis and

scrotal edema developed rapidly and reached massive proportions on the 4th day, fever was observed on the 3rd, 4th, and 5th days, on the latter day a generalized cutaneous papular rash developed, and on the 7th day there was a bilateral blepharitis and conjunctivitis, a profuse watery nasal secretion, and diarrhea. The animal was found dead on the 8th day.

Both guinea pigs injected intratesticularly with guinea pig tissue virus developed swelling and marked induration of the testicles 2 days after inoculation. The condition continued for 4 days and then began to subside. The animals were killed on the 12th day; post mortem examinations were negative except for some congestion of the testicular parenchyma.

The cutaneous reaction of the guinea pig injected intradermally comprised a swollen congested area 1.5 mm. in diameter on the day after inoculation. On the 2nd day the area was considerably larger and more congested, edema had developed, and the central portion was necrotic. On the 4th and 5th days the entire area was quite indurated and the necrotic portion was covered with a thin scab. Resolution began on the 6th day. On the 12th day when the animal was killed, healing was almost complete and only a small crusted thickening of the skin remained.

These results, although few in number, show that it is possible to recover pox virus from the testicles of guinea pigs injected intratesticularly 4 and 8 days previously with unfiltered rabbit tissue-virus. In addition, a 2nd generation of virus in guinea pigs was obtained by the use of testicular tissue obtained from a 1st generation animal and the intratesticular route of injection. In the case of both the 1st and 2nd generations, the presence of active virus was demonstrated by rabbit inoculation, the animals developing typical examples of clinical pox. The findings indicate that the continued serial passage of virus in this animal species is probably possible. While no generalized clinical manifestations developed in inoculated guinea pigs comparable to those of the rabbit (2), the death of one animal 4 days after inoculation of guinea pig tissue-virus suggests that the serial intratesticular passage of virus might be associated with a fatal outcome, as is the case in the rabbit (1).

The Calf Experiment

The susceptibility of the calf to rabbit pox virus was tested on one animal by the scarified skin route of inoculation. In this experiment which also included a number of rabbits similarly inoculated, the cutaneous reaction to pox virus was compared with the cutaneous reactions to two specimens of dermo- and one of neurovaccine. The

results of this comparison have already been reported in the third paper of this series in which other experiments with these four viruses, and in particular crossed inoculation and exposure experiments, are taken up (3). In the present instance the cutaneous reaction of the calf to pox virus will be discussed.

The calf was a Jersey Holstein 3½ months of age and 75 kilos in weight. A large area of skin on the right side of the body was shaved and scarified and 1.0 cc. of a 10 per cent unfiltered testicular tissue pox virus emulsion was rubbed on it. The testicular tissue was derived from the 17th consecutive passage of the Xy171 strain, the first 15 generations of which had been made with Berkefeld V filtered inocula (1). Similarly prepared areas on the left side of the body were each inoculated with a similar dosage of unfiltered vaccine virus emulsions, that is, of culture dermovaccine, of the New York City Board of Health vaccine, and of neurovaccine respectively.

For the first 2 days after inoculation, little change was noted in the pox inoculated area. The lines of scarification were at first a faint pinkish color which quickly faded so that the lines were barely discernible. On the 3rd day several small pinkish papules confined to the scarified lines were observed (Fig. 1); some of them had a small semitranslucent center indicative of beginning vesicle formation. On the 4th day there were several fresh papules. On the 5th day the number of papular lesions had greatly increased and there were a few which apparently did not develop in the scarified lines (Fig. 2). Several lesions were vesicular and the overlying skin showed beginning scaling. The older papules were much larger and many of them had necrotic hemorrhagic centers. In some of them the base and surrounding tissue were edematous.

During the 6th, 7th, and 8th days, all the lesions continued to become larger, and necrosis and hemorrhage were very marked. The majority were quite uniform in size. By the 8th day umbilication was especially prominent and practically all the lesions had tenacious blackish red crusts (Fig. 3). Edema was no longer present. Most of the lesions were discrete but a few had coalesced. On the 10th day the lesions felt firmer and dryer, there was no apparent increase in size, and regression was thought to have begun (Fig. 4). The crusts had become very thick and tenacious. A few lesions, and these were the ones which had formerly been vesicular with little or no hemorrhage and necrosis, felt fibrous and dry and continued to show a fine dry scaling. During the following 5 days regression of the lesions continued as shown by a definite decrease in size, but they were still large and prominent with attached thick crusts on the 15th day when the animal was killed. Post mortem examination revealed no gross abnormalities of the viscera.

The rectal temperature was taken twice daily. Values of 102.0–102.6°F. were observed on the 6th to the 9th days inclusive, in contrast to the readings of 100.8–101.8°F. (mean value 101.3°F.) recorded prior to and after this period. On the 7th, 8th, and 9th days the animal appeared listless, there was some loss of

appetite, and a rather profuse and frequent watery diarrhea developed. Definite improvement was noted on the 10th day and by the 15th day the general condition seemed excellent. During the fortnight of the experiment there was a gain in weight of 2.2 kilos. It should be remembered, however, that the significance of these general symptoms as an indication of a positive reaction to inoculation of pox virus is complicated by the fact that the reactions to 2 strains of dermovaccine and 1 of neurovaccine inoculated on the opposite side of the body were also positive, as has already been described (3). It is of interest to note, however, that the lesions induced by these three vaccine viruses were in each case very much less pronounced and persistent than those induced by pox virus.

The results show that under the conditions of this experiment the calf is susceptible to inoculation with rabbit pox virus by the scarified skin route as far as the development of papules and vesicles in the inoculated area is concerned. The few vesicular lesions were comparatively small and typical pustule formation did not develop. The majority of lesions were large papules in which necrosis and hemorrhage and to some extent edema were very prominent features. The lesions appeared to be active up to the 10th day; they were still conspicuous although definitely regressing on the 15th day when the experiment was terminated. It should be pointed out that necrosis, hemorrhage, and edema were characteristic and conspicuous features of the reaction of the rabbit to inoculation with pox virus. They were regularly seen in the testicle after intratesticular injection of virus and in the cutaneous lesions induced by intradermal injection; they were also observed in the scarified skin inoculated with unfiltered virus emulsions, and in addition frequently occurred in the generalized papular eruption of the skin (2).

GENERAL DISCUSSION

The experiments reported in this and previous papers (1–4) were initiated by an epidemic disease of great severity which broke out in a rabbit breeding colony in December, 1932, (5). The pock-like character of the generalized cutaneous eruption was a conspicuous feature and suggested the name "rabbit pox." In the circumstances of this clinical manifestation the idea that vaccine virus might be the etiological agent was naturally considered although many features of the disease, and in particular the severe prostration and high mortality, were not those generally associated with vaccinia, especially under

conditions of spontaneous infection. In addition, no source of vaccine virus was evident at the time. There have been epidemics in rabbits, however, which have been attributed to vaccinia and particularly neurovaccinia, but satisfactory descriptions of the conditions and precise identification of the etiological agents are not available.

The experimental investigations carried out in connection with the pox epidemic were chiefly concerned with the isolation and identification of the causative agent.

A filterable agent was isolated from spontaneous cases and passed serially in rabbits without appreciable change in its pathogenic properties (1). The virulence of the virus, furthermore, was retained under conditions of ice box storage of affected tissues with or without the addition of glycerol. The clinical picture of the disease induced in normal rabbits by inoculation of tissue-virus was indistinguishable from that seen in spontaneous pox (2).

Rabbits which had recovered from the spontaneous or the experimental disease were refractory to inoculation of pox virus, nor did clinical manifestations of the infection develop under conditions of exposure to florid cases (3). Furthermore, the serum of pox recovered rabbits completely neutralized pox virus (4). The results of crossed inoculation and immune serum-virus neutralization experiments showed that there was no specific relationship between the viruses of pox and virus III disease (3, 4). A similar conclusion was drawn from the experiments on pox and infectious myxoma of rabbits (3). In the case of vaccinia, however, some relationship between pox and vaccine virus was evident although complete identity could not be demonstrated (3, 4). The relationship appeared to be closer between pox virus and neurovaccine than between pox virus and dermovaccine. It appeared probable that vaccination of rabbits with dermo- (culture) vaccine would be a satisfactory method of protection against rabbit pox.

Finally, as reported in the present paper, it was found that other animal species, namely, the mouse, the guinea pig, the rat, and the calf were susceptible to inoculation with pox virus. In mice, a fatal outcome within 5 to 6 days was regularly observed in the serial passage of virus by the method of brain to brain inoculation. In the calf, inoculation of scarified skin was followed by the development of large cutaneous papules with marked hemorrhage and necrosis. The reaction was much more pronounced than those observed with two specimens of dermovaccine and one of neurovaccine.

The epidemic of rabbit pox in the breeding colony did not spread to other animal species so far as is known and experiments were not carried out to determine whether other species could be infected with pox under conditions of room or cage exposure. It should be noted, however, that generalized clinical manifestations were not observed

in inoculated mice, rats, guinea pigs, or the calf, and consequently the possibility that these species might serve as carriers of the virus under natural conditions should be kept in mind.

In connection with the fatal infection in mice induced by intracerebral injection of rabbit tissue pox virus and subsequently seen in this species as a result of mouse brain to brain passage, the reaction of mice to intracerebral injection of vaccine virus is of particular interest.

Rosenau and Andervont (6) confirmed Levaditi and Nicolau's (7) failure to establish Levaditi's neurovaccine within the central nervous system of mice, but they succeeded with Armstrong's (8) modified dermovaccine carried in rabbits, a virus whose virulence had been greatly enhanced by a process of continuous heat selection and propagation (7). Mice injected intracerebrally with this virus died usually on the 6th or 7th day and serial transmission in mice was carried out by means of brain to brain transfers for 28 passages. Haagen (9) has recently reported experiments in which mice were regularly killed in 5 to 9 days by the intracerebral injection of a neurovaccine, the virulence of which for mice was fixed by continued mouse brain passages. This work was carried out with the highly virulent *Reichsgesundheitsamt, Berlin* virus, originally a rabbit strain of neurovaccine virus.

The results obtained from the intracerebral injection of mice with pox virus were of the same general order as those reported by Rosenau and Andervont and Haagen with 2 strains of vaccine virus, that is, an artificially modified dermovaccine and a neurovaccine, both of which are stated to be highly virulent. Whether the property of virulence alone is responsible for the observed results in mice is not entirely clear although Haagen's virus dilution experiments suggest that it plays a major rôle. In any event, it is evident that the present results on the susceptibility of mice to intracerebral injection of pox virus do not differentiate pox virus from certain vaccine viruses.

Is rabbit pox virus a variant or a mutant of dermo- or neurovaccine, or is it an independent member of the vaccinia group of viruses? In this connection it should be pointed out that the characteristic features of pox virus persisted in unabated form during the 9 months from January to October, 1933, in which the investigations on the virus and the experimentally induced disease were carried out. That modifications of dermovaccine may occur has been shown by Armstrong (8). The greatly enhanced virulence of this vaccine which was brought about by artificial means was found to persist in successive rabbit

passages. In the case of neurovaccine and dermovaccine viruses, there seems to be no doubt that in general behavior they are at least qualitatively different and that neurovaccine is the more potent or virulent. The potency of pox virus, however, was greater than that of neurovaccine and its immunity more powerful or effective. The results of the experiments reported in this series of papers clearly showed a relationship but not complete identity of pox virus with both dermo- and neurovaccine viruses; the relationship was closer in the case of neurovaccine. In this connection the possibility that neurovaccine virus is in reality rabbit pox virus or a derivative of it should be considered. Whether pox virus as it appeared in the colony was directly derived from a passage strain of dermovaccine or neurovaccine, or whether it was originally an independent member or strain of the vaccine group of viruses cannot be stated with finality. It was thought, however, that the characteristics of pox virus were such as to merit, at least for the present, an appropriate separate designation in the general group of vaccine viruses.

The manifestations of the spontaneous pox infection and of the experimentally induced condition were sufficiently individual to warrant the tentative consideration of a disease entity. The condition differed in many respects, and markedly so in severity, from vaccinia as generally reported and from vaccinia as recently observed in this laboratory (10). With respect to neurovaccinia, the differentiation is not as definite. From the available literature, comparative estimates of the character and severity of spontaneous neurovaccinia infections are difficult to arrive at and our own experience with induced neurovaccinia is much less extensive than with pox. But from the observations which have been made, rabbit pox as it occurred in 1932–33 was a much more severe disease. Taking into consideration the spontaneous infection and its epidemic features, together with the characteristics of the etiological agent and of the experimentally induced infection, should this disease be considered a pox disease of the rabbit in the sense of vaccinia of the calf, grease of the horse, pox disease of sheep, and small pox of man? A search of the medical and veterinary literature has failed to reveal any reference to rabbit pox as such. On the continent of Europe, however, a disease of the rabbit is recognized which is called *Pocken* and which greatly resembles

or is identical with pox as it appeared in our colony.[1] The present evidence is clearly not sufficient to speak with certainty, but the fact that there are two such similar, if not identical, diseases of apparently independent existence, one in Europe and the other in America, supports the idea of rabbit pox as a disease entity.

SUMMARY

The white mouse, the guinea pig, the calf, and probably the rat, were found to be susceptible to infection with the virus of rabbit pox.

Serial transmission of the virus in mice by brain to brain passage was characterized by a fatal outcome usually on the 5th or 6th day after inoculation.

Infection of the guinea pig was accomplished by intratesticular injection and the virus was continued to the 2nd passage in this species. Guinea pigs developed a well marked cutaneous reaction from the intradermal injection of both rabbit and guinea pig tissue virus.

Active virus was demonstrated in the testicles of rats 8 days after intratesticular injection by rabbit subinoculation.

In the calf inoculation of the scarified skin was followed by the development of large papular lesions with marked hemorrhage and necrosis.

The results of the investigations on the etiology of rabbit pox and of the experimentally induced infection reported in this and the four preceding papers (1–4) are discussed with special reference to the relation of pox virus to other viruses and of rabbit pox to other pock diseases.

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[1] Mahlich's (11) handbook for rabbit breeders and fanciers contains the following description (translation) of this condition:

"*Pox.*—The disease fortunately occurs very rarely in our rabbit population. It is characterized by a rash which involves the entire body. The papules are about the size of a hemp seed and at first are filled with a water-like content. Within a few days this changes into pus. The pocks which are now mature rupture and there remains a brownish base.

"An efficient treatment for this disease is difficult to attain. The best remedy which we know for pox is and remains the following: 'Make the sick rabbit shorter by a head.'"

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EXPLANATION OF PLATE 37

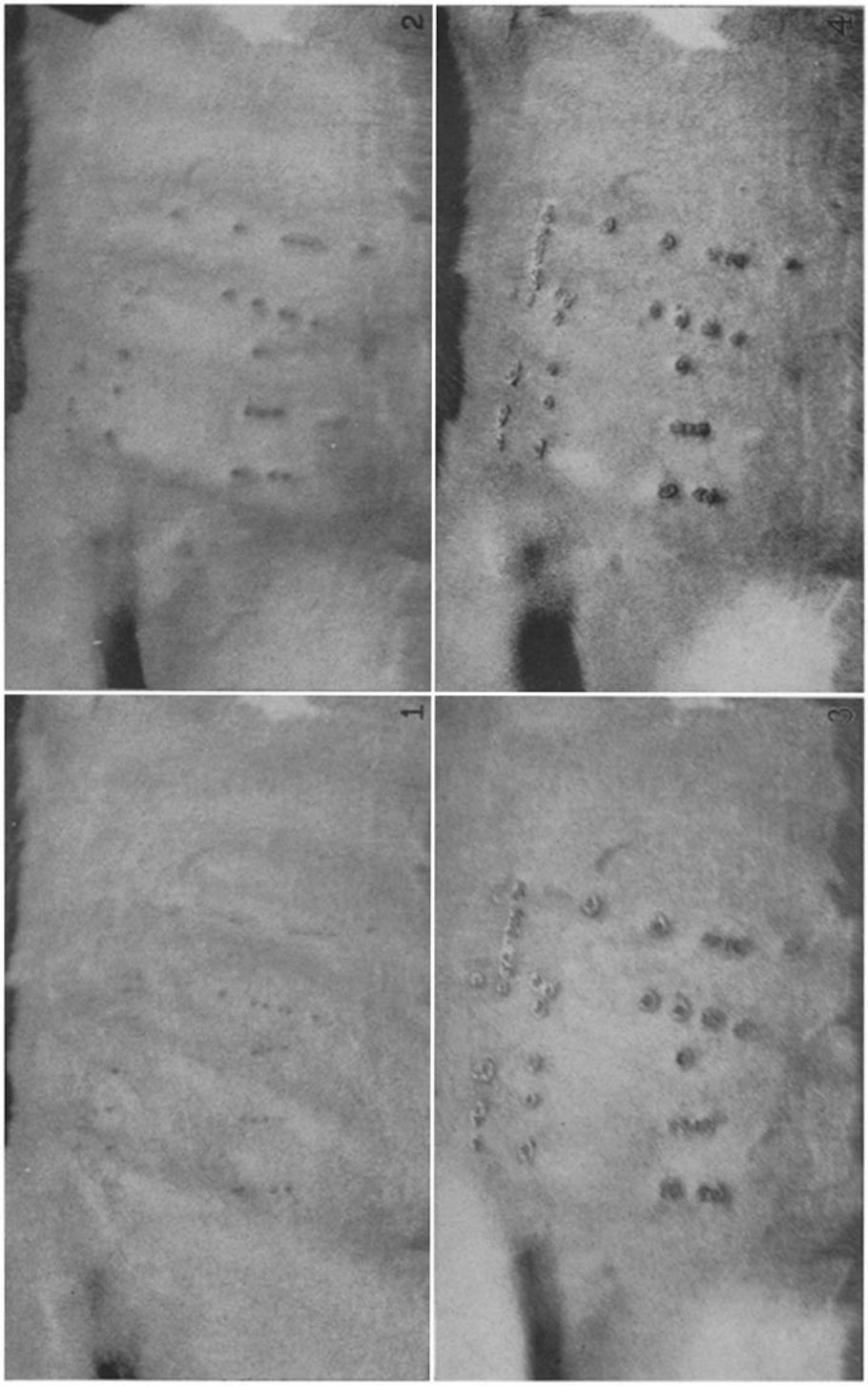
The cutaneous reaction of the calf to inoculation with rabbit pox virus. 1.0 cc. unfiltered tissue virus emulsion was rubbed into a scarified skin area on the side of the body.

FIG. 1. 3 days. Several small pinkish papules in the lines of scarification. Some of the lesions show slight vesicle formation.

FIG. 2. 5 days. The papules have increased in size and many new lesions have developed. A few are not definitely in the scarified lines. Hemorrhage and necrosis are prominent features of many lesions and edema is also present in some of them. There are a number of small umbilicated vesicles containing a semi-translucent whitish material; the overlying skin is beginning to scale.

FIG. 3. 8 days. Large umbilicated papules with pronounced necrosis and hemorrhage and crust formation. While most of the lesions are discrete, some have coalesced.

FIG. 4. 10 days. Beginning regression. The lesions are firmer and dryer with thick tenacious black or reddish black crusts. Some of them show fine scaling of the overlying skin.



Photographed by Joseph B. Haulenbeek

(Pearce *et al.*: Etiology of rabbit pox. V)