EXPERIMENTAL ECTHYMA CONTAGIOSUM (ORF)*

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Ecthyma contagiosum (Orf, scabby mouth, contagious pustular dermatitis) is a virus infection of sheep and goats which is occasionally transmitted to man. Observation of an 18 year old girl (21) with the disorder aroused our interest in the virus and prompted the study of some of its properties. The purpose of this communication is to report the results of a series of experimental inoculations of the virus of ecthyma contagiosum into a number of different animal species. The order of the report will include (A) review of the literature pertaining to experimental ecthyma contagiosum (B) materials and methods (C) experimental disease in sheep (D) experimental disease in rabbits (E) negative inoculation experiments in mice, guinea pigs and the chorioallantoic membrane of the developing chick embryo (F) discussion and (G) conclusions.

A. REVIEW OF THE LITERATURE PERTAINING TO EXPERIMENTAL ECTHYMA CONTAGIOSUM

Aynaud (2) produced lesions in sheep after inoculation of the virus of ecthyma contagiosum into scarified skin, scarified buccal mucosa and scarified cornea. Sheep failed to develop evidence of disease after ingestion of infectious material and lesions did not appear after intradermal, intravenous and intracerebral inoculation of the virus. Lesions were produced in goats and calves by inoculation of the virus into scarified skin but lesions failed to appear after inoculation of rabbits, guinea pigs, white rats, white mice, pigs, dogs, cats, chickens, pigeons and frogs. In scarified sheep skin the site of inoculation became red after an incubation period of three days. By the fourth day the lesions were elevated and on the sixth day the central portion of the lesions became white. Crusts formed soon thereafter and in approximately twenty days the crusts fell off, leaving a barely perceptible scar. Microscopically, ballooning degeneration was observed in the epidermis and the lesions were thought to be similar to those of cowpox. The sheep were immune to further inoculation with the virus of ecthyma contagiosum but Aynaud was unable to demonstrate antibodies in the serum of the animals. There was no cross-immunity against cowpox.

Glover (9) confirmed most of the findings of Aynaud concerning the disease in sheep, but he could not demonstrate keratitis after inoculation into the cornea. He described the microscopic appearance of the disease in sheep in more detail, stressing the presence of ballooning degeneration in the vesiculopustular phase and mentioning the occurrence of papillomatous features when lesions occurred on the lips. Experimental infection in sheep was followed by complete immunity.

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which lasted at least eight months. He failed to produce lesions in the rabbit, guinea pig, mouse, fowl and pigeon.

Howarth (12) failed to produce the disease after inoculation of the guinea pig, cow, pig, rabbit and dog. Sheep were immune for several months after a single inoculation.

Blanc and Martin (4) described serial passage of a mild cutaneous disease in rabbits inoculated with ecthyma contagiosum virus. They stated the disease could be transmitted to dogs, monkeys and men. Later (5) they reported a disease in rabbits characterized by paralysis, fever, diarrhea and death after intracerebral inoculation of the virus.

Boughton and Hardy (7) described lesions in sheep but failed to produce disease after inoculation of the rabbit, guinea pig, calf and dog. They found that immunity lasted in sheep for at least 13 months but they failed to find evidence of immunity in newborn lambs born of immune mothers.

Newsome and Cross (17) failed to produce lesions in guinea pigs and rabbits.

Marsh and Tunnicliff (15) stated that a mild disease occurred in lambs after reinoculation, indicating that only partial immunity was produced by the first inoculation.

Selbie (20) reported that a papillomatous disease could be produced in rabbits and that a vesicular disease occurred in guinea pigs if the original virus from sheep was first passed through rabbits. After several rabbit passages, however, the virus failed to produce lesions in sheep which might mean the virus had been altered by rabbit passage or that some virus other than ecthyma contagiosum was involved.

Carne et al (8) found that sheep were completely immune after a single inoculation of the virus.

Blakemore et al (3) stated that rabbits develop a mild disease after inoculation and they described the disease in one human after experimental inoculation. They indicated that immune sera contained complement fixing and agglutinating antibodies and at times flocculating antibodies but they failed to demonstrate neutralizing antibodies. The virus was observed by means of the electron microscope but its appearance under the electron microscope was not described. Complete immunity followed the experimental disease in lambs.

Lyell and Miles (14) failed to produce lesions on the scarified rabbit cornea. They found minute lesions on the chorioallantoic membrane inoculated with first passage material and suspensions of this membrane produced lesions in sheep (the dilutions were such that it is possible the virus did not proliferate on the chorioallantoic membrane). Second passage on the chorioallantoic membrane produced fewer lesions and questionable lesions were seen on third passage chorioallantoic membranes. No lesions were seen on fourth passage membranes.

Pask et al (19) performed a series of inoculations in human volunteers. They described the production of lesions with macular, papular, vesicular and crusted phases. The disease began four to six days after inoculation, lasted 24 to 28 days and healed without scar formation. Regional lymphadenopathy occurred but systemic symptoms were absent. In most instances immunity followed
inoculation but in a few persons reinoculation produced a mild disease. Microscopically the lesions resembled those produced by the pock viruses but definite inclusions were not seen. They mentioned papillomatous features of the lesions in lambs, and complete immunity followed a single inoculation in these animals. They observed minute lesions in the first passage on the chorioallantoic membrane, questionable lesions on second passage and no lesions after three or more passages.

Lloyd et al (13) found elementary bodies in sheep lesions which resembled those seen in human smallpox. They failed, however, to see elementary bodies in tissue from a human lesion of ecthyma contagiosum. Questionable lesions were observed on first passage chorioallantoic membranes and no lesions were seen on subsequent passages. Sheep developed complete immunity after a single inoculation of the virus.

Asakawa et al (1) noticed fever and mild apathy in sheep inoculated with the virus of ecthyma contagiosum. Sheep in general were immune when reinoculated but they suspected the immunity might be transitory. They could not produce lesions after inoculation of chorioallantoic membranes (nine blind passages), rabbits, mice, guinea pigs and hens.

Mott (16), in a personal communication, stated that sheep are not completely immune after a single inoculation and that reinoculation of the virus of ecthyma contagiosum produces a mild disease.

In summary the literature on experimental ecthyma contagiosum indicates that inoculation of the virus regularly produces disease in sheep, goats and man. Monkeys, dogs and cattle may be susceptible to the virus. There is considerable difference in data concerning the susceptibility of the rabbit, and it seems very doubtful that the virus is able to proliferate on the chorioallantoic membrane of the chick embryo. A wide variety of other animals is probably not affected by the virus. The virus can apparently pass through Berkefeld V, Chamberland L2, Mandler No. 7 filters and membranes having pore diameters of 600 to 900 millimicrons (1, 2, 7, 8, 9, 12, 15, 17, 19). Tissue and crusts from lesions are usually infectious in dilutions up to 1:50,000. The nature of the disease in sheep and man has resulted in its classification with the pock-producing dermatotropic virus diseases. Immunity, with few exceptions, has been reported as being complete after one attack of the experimental disease. Little is known about the mechanisms of immunity but there is some fragmentary work indicating the presence of circulating antibodies.

B. MATERIALS AND METHODS

1. Source of virus

Ecthyma contagiosum virus was obtained from two sources, ML and K.

ML virus. Tissue was aseptically removed from a lesion of ecthyma contagiosum on the forearm of an eighteen year old girl (M. L.) on May 10, 1953 (21). Cultures of the tissue on blood agar, thioglycollate broth and Sabouraud's medium were negative and MacCallum and hematoxylin and eosin stains of fixed tissue failed to show evidence of bacteria or fungi. The tissue was stored at minus 70 degrees Centigrade (carbon dioxide ice box) until
November 11, 1953, when a portion of the tissue was removed and ground in a sterile porcelain mortar. Phosphate buffer (pH 7.38) containing 500 units of penicillin and 500 micrograms of streptomycin per cc. was added to the ground tissue to make a 1:10 dilution by volume. A portion of the 1:10 dilution was diluted further to make 1:100, 1:1,000 and 1:10,000 dilutions which were then used for inoculation of Sheep I and II. The remainder of the 1:10 dilution was mixed with equal parts of nutrient broth and divided into nine tubes, which were placed in the carbon dioxide ice box for future use. This material was used in all the first passage inoculations of rabbits, guinea pigs, mice and chorioallantoic membranes and it will be referred to in those sections as 1:20 ML virus.

K virus. A known virus of ecthyma contagiosum was very kindly supplied by Dr. I. O. Mott, Head of the Section on Viral and Rickettsial Diseases, Animal Disease Station, Agricultural Research Center, Beltsville, Maryland. Four serial passages of the virus in sheep had been performed at the Agriculture Research Center between 1947 and 1952. Tissue had been removed from the lip of the fourth passage sheep (No. 190) on February 7, 1952, and stored in buffered glycerin phosphate pH 7.6 at minus 70°C. The tissue containing the virus was received by us in October 1953. It was stored in the carbon dioxide ice box until April 26, 1954, when the vial was opened and a piece of tissue was ground in a sterile mortar. Phosphate buffer (pH 7.4) containing 500 units of penicillin and 500 micrograms of streptomycin per cc. was added to make a 1:10 dilution by volume. A portion of the 1:10 dilution was used at that time to inoculate Sheep III and the remainder was stored in the carbon dioxide ice box for future use. Cultures on blood agar, Sabouraud's agar and thio-glycollate broth from the 1:10 suspension showed growth of a gram negative rod and a non-pathogenic fungus (a member of the aspergillus group).

2. Sheep inoculations

The sheep were obtained from a flock which had never been known to have ecthyma contagiosum. Sheep I and II were obtained on November 2, 1953, and initial inoculations were performed on November 11, 1953, while the animals were kept in separate pens at the medical school. These animals were moved in March 1954 to separate pens at the University Farm. Sheep III, IV and V were purchased in March 1954 and kept in separate pens at University Farm. Initial inoculations were performed on Sheep III, on April 28, 1954, Sheep IV on May 31, 1954, and Sheep V on April 19, 1954. Sheep VI was added to the group on October 6, 1954, and inoculated on October 13, 1954.

The lambs were four or five months old at the time the original inoculations were done, but since the experiment was carried on over a period of one year, some animals were approaching maturity near the end of the experiment. Inoculations were performed by dropping suspensions of infectious material on the glabrous skin of the axilla, groin, or tail after cleaning with ether and scarifying with sterile sandpaper. The inoculum was gently rubbed into the scarified skin with a sterile blunt glass rod or a sterile glass pipette. In most instances a control site on the same animal was cleansed, scarified and otherwise identically treated except that no virus was inoculated. Control sites were used to gauge the reaction to simple trauma and to test for any possible spread of the virus from one scarified site to another. In none of the sheep did the control site develop the experimental disease. The inoculation sites were observed at daily intervals, with few exceptions, until the end of the disease, and photographs and biopsies were taken at frequent intervals. The animals were kept in separate pens, fed and watered with separate containers and separate sterile instruments were used to perform biopsies, thus reducing contact between animals to a minimum.

ML virus (from the girl) was inoculated into the skin of three sheep (I, II and IV). A 1:10 dilution of this virus suspension was inoculated into the left axilla, left groin and under surface of the tail of Sheep I on November 11, 1953. The right groin and right axilla served as control sites. The same day (November 11th) Sheep II was inoculated as follows: Left axilla 1:100 dilution, left groin 1:1,000 dilution, under surface of the tail 1:10,000 dilution, right groin and right axilla control sites. Sheep IV was inoculated with ML virus on May 31,
1954, as follows: Right axilla 1:20 dilution, left axilla 1:2,000 dilution, right groin 1:200 di-
lution, left groin 1:200 dilution.

ML virus, after three serial passages in rabbit skin, was inoculated into the skin of the
right groin of Sheep V on April 19, 1954 (procedure described in detail later under 3. Rabbit
Inoculations). On the same day this sheep was inoculated with other materials as follows:
Right axilla third passage chorioallantoic membrane, left axilla third passage ascites tumor,
under surface of tail third passage mouse brain and left groin control.

K virus (from the Agriculture Experiment Station) was inoculated into the skin of
Sheep III on April 26, 1954, as follows: Right axilla 1:10 dilution, left axilla 1:100 dilution,
right groin lower area 1:1,000 dilution, right groin upper area 1:10,000 dilution, left groin
control. On October 13, 1954, K virus was inoculated into the skin of Sheep VI as follows:
Right axilla 1:100 dilution, right outer groin 1:1,000 dilution, right inner groin 1:10,000
dilution. ML virus, which proved inactive, was inoculated at the same time into the left
axilla and left groin of Sheep VI.

Reinoculations of the sheep in the study of immunity and cross-immunity were per-
formed in the same manner as the initial inoculations. In all instances the disease from a
previous inoculation had healed before the animal was reinoculated. The pattern of re-
inoculation varied from time to time which made it possible to tell if an uninoculated site
on a given animal was capable of developing immunity from inoculation elsewhere and if
a previously inoculated site could respond with a mild form of the disease.

3. Rabbit inoculations

Domestic albino rabbits were obtained from a local rabbit breeder. Rabbits I and II
were full-grown, III, IV, V and VI were half-grown and VII and VIII were one-quarter
grown. Rabbits I and II were obtained in December 1953, III, IV, V and VI in January
1954 and VII and VIII in March 1954. Rabbits I, II, VII and VIII were kept singly in sepa-
rate cages but III and IV were kept in one cage and V and VI in another.

ML virus (ground tissue diluted 1:20 with equal parts of nutrient broth and phosphate
buffer pH 7.3 containing 500 units of penicillin and 500 micrograms of streptomycin per
cc.) was used to inoculate first passage rabbits. Subsequent passages (second and third)
were performed using rabbit skin which had been removed by biopsy of the inoculation
sites. The tissue was stored in the carbon dioxide ice box until the day of inoculation when
it was removed, thawed and ground in a sterile mortar. P & S* buffer was added to make a
1:10 dilution by volume.

Corneal inoculations were performed by inoculating 0.05 cc. of the virus suspension into
the right cornea, which had been cross-hatched with a sterile 27 gauge needle. Inoculation
into the skin was performed by rubbing 0.1 cc. of the virus suspension into a site on the
right side of the abdomen where the skin had been shaved and scarified with sterile sand-
paper. In each instance the left cornea and left side of the abdomen were treated identically
with the exception that virus was not inoculated.

The skin and cornea of Rabbits I and II were inoculated on December 8, 1953, and the
site of inoculation on Rabbit II was biopsied on December 16. Second passage rabbits
(III, IV) were inoculated (skin and cornea) January 9, 1954, and the lesions of Rabbit III
were biopsied on January 20. Third passage rabbits (V, VI, VII and VIII) were inoculated
March 31, 1954. The skin of all third passage animals was inoculated but the only third
passage rabbit receiving corneal inoculation was V. The skin lesions of Rabbit V (third
passage) was biopsied on April 9. The material was stored in the carbon dioxide ice box
until April 19, when it was removed, thawed, ground in a sterile mortar, diluted to 1:10
with P & S buffer and inoculated into the scarified skin of Sheep V.

* Phosphate buffer pH 7.38 containing 500 units of penicillin and 500 micrograms of
streptomycin per cc.
4. Guinea pig inoculations

Six immature colored guinea pigs were used in this portion of the experiment. Four animals (two males and two females) were inoculated with virus suspension and two used as controls. In each guinea pig both sides of the abdomen were shaved and scarified with sandpaper and both corneas were cross-hatched with a 27 gauge needle. One-tenth of one cc. of 1:20 ML virus suspension was inoculated into the right side of the abdomen and 0.025 cc. onto the right cornea, the left side of the abdomen and the left cornea serving as control sites in addition to the two control animals. The animals were observed 28 days without local or systemic evidence of disease.

5. Mouse inoculations

Immature white mice (CF, from Carworth Farms) were used in this portion of the experiment. The mice were divided into three groups: (A) Intracutaneous and intraperitoneal inoculations (B) Intracerebral inoculations and (C) Inoculation of mice bearing Ehrlich ascites carcinoma.

A. Intracutaneous and intraperitoneal inoculations. Five mice were used in this group. Abdominal skin was shaved and scarified with sterile sandpaper and 0.025 cc. of 1:20 ML virus suspension inoculated on the skin of the right side leaving the left side as a control. At the same time 0.1 cc. of virus suspension was inoculated intraperitoneally. Lesions did not appear on the skin, which was observed almost daily for 28 days, and the animals remained in perfect health for four months at which time they were sacrificed.

B. Intracerebral inoculations. Five mice were inoculated intracerebrally with 0.025 cc. of 1:20 ML virus suspension on January 20, 1954. Five days later the animals were sacrificed and the brains removed and stored in the carbon dioxide ice box. Two days later the brains were ground in P & S buffer to make a 1:10 dilution and this material was used to inoculate five more mice intracerebrally. The procedure was repeated another time to make three passages. None of the mice showed evidence of disease. Third passage brain emulsion inoculated into the skin of a non-immune sheep (V) failed to produce lesions of ecthyma contagiosum in the sheep.

C. Mice bearing Ehrlich ascites carcinoma. Five mice were inoculated intraperitoneally with Ehrlich ascites carcinoma. After ascites was observed the mice were inoculated intraperitoneally with 0.1 cc. of 1:20 ML virus suspension. Ascites fluid was harvested after five days and reinoculated into five more mice. After three of these animals developed ascites the fluid was removed and inoculated into a third group of five mice. Ascites fluid from the third group of five mice was harvested and inoculated into the scarified skin of a non-immune sheep (V) but lesions of ecthyma contagiosum failed to appear.

Five mice were inoculated intraperitoneally with Ehrlich ascites carcinoma, five with ecthyma contagiosum virus and five with ecthyma contagiosum virus plus Ehrlich ascites carcinoma. The five mice receiving only ecthyma contagiosum virus remained well and the other ten mice died of the ascites carcinoma at the expected time. This pilot study showed no effect of ecthyma contagiosum virus on the course of Ehrlich ascites carcinoma and the study was discontinued.

6. Chorioallantoic membrane inoculations

Chorioallantoic membranes of ten or eleven-day old developing chick embryos were exposed using standard techniques. One-tenth of one cc. of a tissue suspension was dropped onto the membrane and the egg reincubated for three days before harvesting. The membranes were observed carefully for possible specific lesions, and the embryos were checked for viability and gross abnormalities. After harvesting, the membranes were placed in the carbon dioxide ice box until further inoculations were performed, at which time the membranes were thawed, ground in a sterile mortar and diluted with P & S buffer to 1:10 dilution by volume. At each passage control membranes were inoculated in identical fashion with the test membranes except virus-containing tissue was not used.
First passage chorioallantoic membranes were inoculated with ML virus diluted to 1:20 using equal parts of nutrient broth and P & S buffer. The inoculations are presented in table form as follows:

<table>
<thead>
<tr>
<th>Passage</th>
<th>Date</th>
<th>No. of Eggs</th>
<th>No. of Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1/22/54</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>II</td>
<td>2/5/54</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>III</td>
<td>2/10/54</td>
<td>10</td>
<td>5</td>
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</table>

Third passage membranes were stored in the carbon dioxide ice box from February 13, 1954 to April 19, 1954, at which time they were thawed, ground and made up to a 1:10 dilution by volume with P & S buffer and inoculated into the scarified skin of Sheep V with negative results.

7. Bacteriologic and mycologic controls

Each time inoculations were performed the tissue suspensions (virus-containing and control) were cultured on blood agar, thioglycollate broth and Sabouraud's agar. All inoculations using ML virus in sheep as well as all inoculations of rabbits, guinea pigs, mice, and chorioallantoic membranes were performed with tissue suspensions that showed no growth on the above media. As mentioned before, K virus inocula grew a gram negative bacillus and a non-pathogenic fungus (a member of the aspergillus group) in spite of the addition of penicillin and streptomycin.

Secondary infection of the inoculated sites in sheep and rabbits appeared to play no rule in the experimental disease in these animals. The scarified inoculation sites had practically healed in all animals before the lesions of ecthyma contagiosum appeared and the scarified control sites never showed evidence of significant infection. The only exception was the appearance of secondary infection in the scarified undersurface of the tail of one sheep and inoculations of this site were discontinued early in the experiment.

C. EXPERIMENTAL DISEASE IN SHEEP

The inoculation of ML virus produced lesions in sheep that were grossly and microscopically identical with lesions produced by K virus.* The ML suspension contained a lower concentration of virus than the K suspension. The inoculation of 1:10 and 1:100 dilution of ML virus suspension produced plaques composed of confluent lesions. The 1:1,000 dilution produced lesions in lines at the center of the inoculation site and discrete lesions at the edge, and the 1:10,000 dilution produced only four discrete lesions. The inoculation of 1:10, 1:100 and 1:1,000 dilutions of K virus produced lesions in confluent plaques and although a plaque was formed with the 1:10,000 dilution, it was evident that the disease was not as severe in this dilution. Later inoculation with K virus (presumably when the virus was losing some of its potency) resulted in the production of discrete lesions with the 1:10,000 dilution.

The description of the gross and microscopic features of the disease which follows includes observations derived from sheep inoculated with either virus. The non-specific traumatic reaction to scarification practically disappeared by the end of two days and by the third day bright red macules were present in the lines of scarification. The macules became elevated by the fourth day and

* The course of the disease produced by K virus was apparently uninfluenced by the presence of the gram negative bacterium and the non-pathogenic fungus mentioned on page 277.
Fig. 1. Ecthyma contagiosum in Sheep IV eight days after inoculation of a 1:200 dilution of the virus. Note the iris-like character of some of the lesions and the arrangement of the lesions in the lines of scarification.

Fig. 2. Same lesion as shown in Fig. 1 eleven days after inoculation. The lesion is beginning to form a crust but the multilocular, honey-combed character is still apparent in the central portion.
FIG. 3. Ecthyma contagiosum in Sheep IV twenty days after inoculation of a 1:20 dilution of the virus. In the lower portion of the lesion the heavy crust has been removed revealing the warty, papillomatous base.

FIG. 4. Photomicrograph of six day old lesion in Sheep IV. Note the ballooning degeneration and basket-weave appearance of the uppermost portion of the rete.
Fig. 5. Photomicrograph of eleven day old lesion in Sheep IV. The superficial, stuck-on pustule has largely fallen away in fixing but it is still evident on the upper right. The pseudo-epitheliomatous and granulomatous base of the pustule can be seen.

Fig. 6. Photomicrograph of nine day old lesion in Rabbit V. Round cell infiltrate and vascular changes are present in the dermis.
by the sixth day the lesions were seen as dull, brick-red, relatively flat-topped papules. By the seventh day the central portion of the lesions took on a gray-white appearance and by the end of the ninth day the lesions were gray-white except for a narrow red rim. The lesions at this stage appeared to contain pus but incision failed to show the expected pustular content. The epidermis was white and sodden, however, and a small amount of turbid, milky fluid lay beneath it. Between the tenth and fourteenth days the gray-white appearance was gradually replaced by a brown crust and the underlying base of the lesion assumed a granulomatous character. By the sixteenth day the crust was dark brown, thick and firmly adherent and when forcibly removed was seen to cover a warty, papillomatous base. The lesions underwent gradual involution from this point until the twenty-eighth day when most of the crusts and papillomatous character had disappeared. As late as the forty-second day tiny areas of crusting could still be seen at some of the inoculation sites. The skin remained pink and slightly thickened a few days after loss of the crust and finally healed without scar formation. At no time were the sheep systemically ill as far as could be determined from their behavior.

A description of the microscopic findings of the experimental lesions in sheep from biopsies taken on the fifth, eleventh and seventeenth days follows.

On the fifth day localized thickening of the epidermis and cellular infiltration in the dermis could be seen with the reversed ocular. At higher power there was mild acanthosis and a layer of three or four cells in the upper-most portion of the rete was under-going ballooning degeneration. These cells were swollen and pyknotic and there was a collection of fluid about their nuclei. In the areas where the process was most advanced, the contents of the cells were completely replaced by fluid and only the cell walls remained, producing a "basket-weave" appearance. The basal layer of the epidermis showed patchy liquefaction degeneration. There was a dense cellular infiltrate in the upper dermis and the infiltrate could be seen around some of the deeper vessels and appendages in the dermis. The infiltrate was composed largely of round cells but there were some polymorphonuclear leukocytes and reticuloendothelial cells. There were many newly formed blood vessels in the dermis and many of these vessels showed proliferative changes in their walls.

Lesions on the eleventh day of the disease observed with the reversed ocular showed a superficial "stuck-on" pustule covering a proliferating epidermis which in turn overlay a dense dermal infiltrate. Examination at higher power confirmed the presence of a very superficially placed pustule. It occupied a position which ordinarily would represent stratum corneum and resembled the pustule of impetigo contagiosum. At the base of the pustule were spotty areas of ballooning degeneration and "basket-weaving". The underlying epidermis presented well-developed pseudoepitheliomatous hyperplasia. The upper dermis was very cellular. Round cells and reticuloendothelial cells were present in profusion. There was considerable evidence of new blood vessel formation and many of the vessel walls were considerably thickened.

On the seventeenth day observation with the reversed ocular showed heavy
crusting and a striking degree of papillomatosis. Higher magnification revealed hyperkeratosis, parakeratosis and scattered abscess formation in the exaggerated horny layer. Pseudoepitheliomatous hyperplasia had largely given way to a high degree of papillomatosis. The elongated dermal papillae were densely cellular (round cells, reticulendothelial cells and young fibroblasts) and the dermal blood vessels showed thickened hyperplastic walls.

A series of reinoculation and cross inoculation experiments with the two viruses was designed to test the development of immunity to one strain of virus and cross immunity between the two strains. The results, dates of inoculation, animals and virus suspensions used are shown in tabular form.

### Results of repeated inoculations of ecthyma contagiosum in sheep

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Date Sheep Acquired</th>
<th>Dates Inoculations Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>Sept. '53</td>
<td>ML-4</td>
</tr>
<tr>
<td>III</td>
<td>March '54</td>
<td>ML-4</td>
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<td>IV</td>
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<td>MN</td>
</tr>
<tr>
<td>V</td>
<td>March '54</td>
<td>ML-4</td>
</tr>
<tr>
<td>VI</td>
<td>Oct. '54</td>
<td></td>
</tr>
</tbody>
</table>

ML = Virus from the girl.  
K = Known virus from sheep tissue obtained from Maryland.  
0 = No lesions produced by inoculation.  
? = Very questionable lesions of two or three days duration.  
1 = Few lesions lasting one week or less.  
2 = More severe disease lasting one to two weeks.  
3 = Disease lasting two to three weeks.  
4 = Full-blown disease lasting four weeks or more.  
MN = Inoculation with material from Milkers' nodule with no disease produced.  
* = The ML virus had lost its potency by October 1954 because no reaction occurred in the susceptible animal, VI, when it was inoculated with ML virus. There had also been some decrease in potency of the K virus suspension since the 1:10,000 dilution gave a strong reaction in June 1954, but very little reaction in October 1954.  
† = It is likely that the ML virus was inactive in September 1954.

The immunity to a given virus suspension was not absolute after a single inoculation but it became absolute for practical purposes in two animals (one after two and one after three inoculations). The two virus suspensions provided cross immunity to each other of about the same order as one virus suspension provided immunity against itself, an indication that the two virus strains, if not identical, were closely related. Inoculation in a partially immune animal resulted in a milder disease, fewer lesions, a more rapid development of pustular and crusted phases and little or no papillomatosis. Higher dilutions of the virus failed to produce lesions in the partially immune animals.

Sheep I and III were inoculated with tissue suspensions of milkers' nodules from three patients on March 29, 1954. No evidence of disease appeared at the
inoculation site and the animals remained systemically well. We interpreted these negative results to mean that the milkers' nodules did not contain an agent transmissible to sheep and that Sheep I and III could be used for further studies of ecthyma contagiosum (refer to F. Discussion for further remarks about the differences between milkers' nodule and ecthyma contagiosum).

D. EXPERIMENTAL DISEASE IN RABBITS

As the non-specific reaction to scarification subsided (5–7 days) the inoculated skin was seen to be slightly more erythematous than the control sites in all animals. Erythematous maculopapular lesions about 1 mm. in diameter were seen at the scarified sites in 7–9 days after the inoculation. The lesions were discrete and inconspicuous and varied in number from three to a dozen. The lesions disappeared without scar in three to five days. Discrete maculopapular lesions were noticed on all animals except IV (one of two second passage animals) and VII (one of four third passage rabbits). Corneal lesions were not seen in any of the rabbits.

Microscopic examination of lesions of ecthyma contagiosum in rabbits failed to reveal significant changes in the epidermis (biopsies were performed on one rabbit of each of the three passages and microscopic sections were available for the eighth, ninth and eleventh days after inoculation). The dermis contained a round cell infiltrate ranging in number from a few cells to dense collections. In some of the sections dermal edema was evident and reticuloendothelial cells were increased in number. New blood vessel formation and proliferation of the vessel walls was seen in most of the sections.

Inoculation of tissue suspensions prepared from third passage rabbit lesions into a non-immune sheep resulted in typical lesions of ecthyma contagiosum (see F. Discussion for further comments).

E. NEGATIVE INOCULATION EXPERIMENTS IN GUINEA PIGS, MICE AND THE CHORIOALLANTOIC MEMBRANE OF THE DEVELOPING CHICK EMBRYO

Under the conditions of the experiment there was no evidence of proliferation of the virus of ecthyma contagiosum in guinea pigs (skin and cornea), mice (skin, peritoneum, brain), chorioallantoic membrane, and mice bearing Ehrlich ascites carcinoma. The inoculation of third passage mouse brain, third passage ascites carcinoma and third passage chorioallantoic membrane suspensions into the skin of a susceptible sheep failed to produce lesions. Emphasis must be placed on the ability of the 1:20 ML virus suspension used in the inoculations of these animals to produce lesions in the skin of sheep and rabbits, indicating an active virus was used in the initial inoculations.

F. DISCUSSION

The material removed from the 18 year old girl appeared to contain the virus of ecthyma contagiosum because it produced a disease in sheep which was identical grossly and microscopically with the disease produced by a known ecthyma contagiosum virus and because there was very suggestive evidence of
cross-immunity between the two viruses. In addition, the sheep from which the girl apparently contracted her disease presented the typical gross and microscopic picture of ecthyma contagiosum. These facts would seem to justify a description of the disease based on composite results from inoculation of ecthyma contagiosum virus from two separate sources.

Ecthyma contagiosum in sheep presents a series of unique and characteristic microscopic changes. Ballooning degeneration much like that seen in the pock diseases (herpes simplex, herpes zoster, varicella, variola, vaccinia) occurs early in the course of the disease. Pseudoepitheliomatous changes are prominent during the mid portion of the disorder and finally, a pronounced papillomatous picture appears near the end of the disease. In addition, the heavy dermal infiltrate of round cells and the striking vascular proliferation deserve emphasis. In its several stages the disease could be classed with the pock diseases, the infectious granulomas or the papillomas, depending upon the age of the lesion at the time of examination. The changing picture of the lesions with the passage of time probably represent changing host-virus relationships which may be amenable to study at a future time.

Another very interesting feature of the disease is the gradual development of immunity to reinoculation of the virus. The first inoculation produces the full-blown disease in all its phases. The second inoculation results in a disease of shorter duration in which the pustular and granulomatous stages occur much sooner, the papillomatous phase is absent or very mild and higher dilutions of virus fail to produce disease. The third inoculation may cause a very mild disease with still more telescoping of the several phases of the disease or no disease at all. This description of the changing nature of the disease upon reinoculation of the virus is based upon a study of only six sheep, but a total of 46 inoculations of active virus were performed in these animals and 80 biopsies were studied ranging from the fourth to the forty-second days after inoculation. It seems safe to say that the above trend is a real one. Little is known about the mechanisms of immunity in ecthyma contagiosum and studies are planned to throw some light on this problem.

In the process of reinoculation of the sheep, some of the inoculations were performed at skin sites which had been inoculated once or twice before. These sites were capable of developing an accelerated, mild form of the disease. Conversely, in the process of reinoculation fresh sites were inoculated in animals which had been inoculated in other remote areas once or twice. The new sites developed lesions but the disease was accelerated and mild and corresponded to what one would expect considering the strength of the inoculum and the immune state of the animal. These findings indicated immunity was not confined to the exact site of inoculation in the skin but could be demonstrated by inoculation of previously uninoculated, remote skin sites.

A relationship between ecthyma contagiosum and milkers' nodule has been suggested (10). We feel the two diseases are unrelated. Milkers' nodules are acquired from cows and ecthyma contagiosum from sheep and in these natural
hosts the diseases do not resemble each other in any significant degree (6, 11, 21). The gross and microscopic features of the two diseases in uncomplicated form (i.e. no secondary infection) in man are dissimilar (6, 18, 21). Several authors have been unable to transmit milkers' nodules to rabbits (6, 18) and we were unable to produce lesions in sheep and rabbits in a pilot set of inoculations which will be described briefly. Two sheep (one which had received one inoculation with ecthyma contagiosum, and one which had not) failed to develop lesions after inoculation into the skin of tissue suspensions from milkers' nodules from three humans. Four rabbits (two which had been inoculated once with ecthyma contagiosum and two which had not) failed to develop lesions after inoculation with tissue suspensions from one patient with milkers' nodule. Experimental ecthyma contagiosum in rabbits was quite mild and unexciting. If the animals had not been observed carefully each day the disease most certainly would have been missed. It may be that the inconspicuous character of the lesions accounts for the failure of some authors to describe lesions in the rabbit after inoculation with the virus. There seems little doubt that the virus was able to grow in rabbit skin. If one considers that three passages were performed in rabbits, that each passage was diluted 1:10 in buffer and at each biopsy less than one-tenth of the lesion was removed, the third passage material used to inoculate the test sheep was diluted at least 1:200,000. The original material used to inoculate the first rabbit produced confluent lesions in sheep at a dilution of 1:100 and at a dilution of 1:10,000 four discrete lesions were seen. The confluent disease produced by the third passage rabbit material could hardly have been produced if the virus had not grown in rabbit skin.

The negative results after inoculation of ecthyma contagiosum virus into rabbit cornea, guinea pigs (skin and cornea), mice (skin, peritoneum and brain) and the chorioallantoic membrane of the chick embryo are in agreement with most of the reports on experimental ecthyma contagiosum.

CONCLUSIONS

1. The inoculation of ecthyma contagiosum virus into the scarified skin of sheep produced lesions which progressed through macular, papular, vesicular (microscopic only), pustular, crusted and papillomatous stages and healed in approximately 28 days without scar formation.

2. The inoculation of ecthyma contagiosum virus into sheep did not result in complete immunity unless two or more inoculations were performed.

3. The inoculation of ecthyma contagiosum virus into the scarified skin of rabbits produced small maculopapular lesions which disappeared in three to five days without scar formation. The inoculation of tissue suspensions from third passage rabbit lesions produced typical lesions of ecthyma contagiosum in a susceptible sheep. The inoculation of infectious material into scarified rabbit cornea failed to produce evidence of disease.

4. The inoculation of ecthyma contagiosum virus into mice (scarified skin, intracerebrally and intraperitoneally), guinea pigs (scarified skin and cornea)
and the chorioallantoic membrane of the chick embryo failed to produce evidence of disease.

5. The microscopic appearance of lesions of ecthyma contagiosum in sheep and rabbits has been described.

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REFERENCES

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DISCUSSION

DR. HARVEY BLANK (New Brunswick, N. J.): This is an excellent painstaking study which answers many questions about an interesting disease that occurs from Iceland to Australia. The caliber of the work is indicated by the authors' success in passing the disease in rabbits, whereas most other workers, including ourselves, have failed probably because of a failure to do blind passages as Dr. Wheeler and his group have done. Selbie in England however succeeded in this some years ago (J. Path. Bact. 58: 199, 1946). Our own experience (unpublished) and that of others is in agreement with the failure of growth in the embryonated egg, rabbit's eye or guinea pig.

I understand that following deliberate reinfection you were able to induce a second accelerated episode of the disease. I would like to ask if you feel that the partial immunity which exists would have been sufficient to protect animals against natural infection? Would you discuss this and also the fact that the common practice in sheep countries of a single vaccination for the lambs appears to provide practical immunity?

DR. CLAYTON E. WHEELER (in closing): It would have been easy to overlook the disease in rabbits. The lesions did not appear for approximately a week after inoculation of the virus and they lasted three to five days. The lesions were tiny and inconspicuous and at first we thought they were non-specific in nature. We had planned to do blind passages if there was no evidence of disease in rabbit skin so we continued the work and eventually showed, as the body of this paper brings out, that the virus was able to proliferate in rabbit skin and that a mild disease resulted.

Large numbers of sheep have been vaccinated in Texas using a single inoculation of live virus and the results indicate that immunity develops in sufficient degree to provide effective protection on the range. Reinoculations in our sheep were done using serial dilutions of virus. The 1:10 dilution usually resulted in a relatively mild disease lasting approximately two weeks. The 1:100 dilution resulted in a still milder disease. The 1:1000 dilution often failed to induce disease and lesions usually did not appear after reinoculation with the 1:10,000 dilution. Definite evidence of immunity was found after a single inoculation of the virus but the immune mechanisms could probably be overwhelmed if a large enough dose of virus was introduced.