taneous disease. Of 10 mice inoculated with material from the second egg generation (after intervening lyophilization), 4 died on the fifth day and 6 on the sixth day, with swollen foot pad and autopsy findings similar to those of the spontaneous disease. This culture has been carried through 6 egg generations, with intervening storage in glycerol. Penicillin and streptomycin have been added since the third egg generation. It has continued to reproduce the same proliferative pocklike lesion of the chick membrane, and the same disease in mice not previously exposed.

Intracytoplasmic, eosinophilic inclusion bodies have been observed in tissues from both infected mice and eggs. They were, however, not as frequently found in the tissues of infected mice as might have been expected from the literature on infectious ectromelia. Moreover, because of their lack of specificity, definitive diagnosis should depend not on the presence or absence of inclusion bodies, but on serological or immunological methods.

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An Outbreak of Mouse-Pox (Infectious Ectromelia) in the United States: II. Definitive Diagnosis[1]

J. J. Trentin and B. A. Briody[2]

Department of Anatomy and *Department of Microbiology, Yale University School of Medicine, New Haven, Connecticut*

The immunological similarity of the viruses of infectious ectromelia and vaccinia was first reported by Burnet (*1–3*) on the basis of the following observations:

a) The virus of infectious ectromelia, growing on the chick chorioallantois, produces a soluble hemagglutinin which agglutinates the same spectrum of fowl erythrocytes agglutinated by vaccinia hemagglutinin.

b) Both ectromelia and vaccinia hemagglutinins are inhibited by either ectromelia or vaccinia immune sera, but not by normal sera.

c) Vaccinia can be used to immunize mice effectively against ectromelia.

On this basis Burnet suggested that infectious ectromelia is the murine representative of the mammalian pox diseases, and proposed (*4*) that the term "mouse-pox" should be used as a synonym for infectious ectromelia. This work has been confirmed and considerably extended by Fenner (*5–11*), who adopted "mouse-pox" to describe the disease, but retained "ectromelia" to describe the causative virus.

Definitive diagnosis of the disease described in the preceding article (*12*) was made by demonstration of vaccinia antihemagglutinin in the sera of convalescent mice, and by successful cross-immunization with vaccinia against the isolated etiological agent.

Approximately 2 months after the mortality rate had returned to normal, 18 mice that had lived through the epizootic were bled and the sera tested for inhibition of the hemagglutinin produced by the Nelson and Levaditi strains of vaccinia virus. Six of the sera inhibited 4 agglutinating doses of vaccinia hemagglutinin at dilutions of 1–20 to 1–80. Nine of the sera showed no inhibition of vaccinia hemagglutinin at a dilution of 1–10. At the same time 14 sera from a group of mice separated from the main colony during the course of the epizootic, but before showing manifestations of the disease, were tested. One showed inhibition of vaccinia hemagglutinin at a dilution of 1–48. The remaining 13 were negative at a dilution of 1–6.

TABLE 1

CROSS-IMMUNIZATION BY VACCINIA (NELSON) AGAINST THE ISOLATED ETIOLOGICAL AGENT

Intranasal vaccinia	No. of mice	Specific mortality following foot pad inoculation 19 days later
None	30	20/30
1–100 Dilution	29	6/29
1–5 Dilution	29	3/29

For cross-immunization, mice were used from a source without previous known exposure to either vaccinia or ectromelia. Two groups received intranasal inoculation, under ether anesthesia, of vaccinia (Nelson) at dilutions of 1–5 and 1–100. A third group received no vaccinia. Nineteen days later all three groups were challenged by foot pad inoculation of the etiological agent, isolated as previously described (*12*). Those groups exposed to vaccinia showed definite protection (Table 1). The experiment was repeated, using a different strain of vaccinia (Levaditi) and a 40-day interval between intranasal inoculation and foot pad injection. The mice receiving vaccinia were again protected (Table 2).

Mouse-pox is enzootic in laboratory mice in Europe. The U. S. has been remarkably free of the disease. Importation of the virus is prohibited by the U. S. Bureau of Animal Industry (*11*). Serological surveys for the presence of vaccinia antihemagglutinin in mice from different breeding stocks in the neighborhood of New York City and Boston failed to reveal a single positive result (*11*).

Fenner (*11*) quotes two references (*13, 14*) and a personal communication (*15*) suggesting that the disease may previously have existed in the U. S. The

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[2] Present address: Department of Bacteriology, Hahnemann Medical College, Philadelphia, Pa.

first is extremely vague. The second is strongly suggestive. The third refers to endemic "ectromelia" in one of the mouse colonies of the Department of Anatomy of this institution. This suggests the possibility that the present outbreak may represent a sudden flare-up of a pre-existing disease. Fortunately, evidence is available regarding this plausible possibility. The term "ectromelia" has been used loosely in this department to refer to gangrenous lesions of the feet or tail, which sometimes resulted in loss of the extremities. Such lesions have appeared in small numbers at widely separated times over a period of many years, and have been associated with a low mortality rate. Such lesions may be caused by agents other than ectromelia virus (*16, 17*). No conclusive evidence exists that the lesions seen in this department prior to 1951 were caused by the virus immunologically related to vaccinia. On the contrary, evidence exists that they were not. Approximately 60 sera from a number of inbred strains of mice in the department were tested for vaccinia antihemagglutinin in 1948 and 1949, with negative results. On a number of occasions since 1949, but prior to the outbreak in 1951, mice bearing gangrenous lesions of the feet or tail have been examined.

TABLE 2

CROSS-IMMUNIZATION BY VACCINIA (LEVADITI) AGAINST THE ISOLATED ETIOLOGICAL AGENT

Intranasal vaccinia	No. of mice	Specific mortality following foot pad inoculation 40 days later
None	15	11/15
1–5 Dilution	14	0/14

The sera from these mice did not contain vaccinia antihemagglutinin. Serial passage in mice of an extract from these lesions failed to induce any signs of an infection when injected intraperitoneally or into the foot pad, and no pocks were produced when the extract was inoculated on the chorioallantois of the chick embryo.

Because of confusion resulting from the use of the term "ectromelia," or even "infectious ectromelia," it is strongly recommended that the suggestion of Burnet and Fenner be universally adopted, and the term "mouse-pox" be used to refer to the disease of mice caused by the virus related to vaccinia.

If the virus of mouse-pox was not present in the Department of Anatomy prior to 1951, whence then did it originate? After the disease had made its apperance and had been diagnosed in this department, it was learned that an outbreak of what was apparently the same disease had occurred in another department of the university a year earlier, with loss of approximately 60% of that colony. It is known that mice were transferred from that colony to the Department of Anatomy approximately 2 months before the 1951 outbreak was noted.

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A Simplified Preparation of High-Polymer Desoxyribonucleic Acid

Maurice H. Bernstein[1]

Department of Zoology,
University of California, Berkeley

High-polymer desoxyribonucleic acid has customarily been prepared by the removal of contaminating protein from nucleoproteins extracted in strong salt solutions. Until recently the deproteinization has been accomplished by shaking with chloroform and centrifuging, according to the method described by Sevag *et al.* (*1*). More recently deproteinization procedures based on the removal of proteins by precipitation with detergents have been described (*2, 3*). The chief drawback in either case is that these are laborious and time-consuming, requiring several days for their completion. By combining the chloroform and detergent methods, a simple, rapid, and highly satisfactory procedure has been developed.

The source material (isolated nuclei or sperm) is extracted in 2.0 *M* NaCl, in the cold, and all subsequent operations are done in the cold. When the thick, viscous nucleic acid and protein extract is obtained, an equal volume of reagent grade chloroform is added, the material is shaken violently by hand or mixed in a Waring blendor at a slow speed. However formed, the resulting emulsion is centrifuged and the aqueous supernatant layer is decanted from the protein pad and the chloroform. To the aqueous supernatant solution, 1.25 volumes 1% detergent (Duponol C; Na dodecyl sulfate) are added, and then additional 2.0 *M* NaCl to give a final concentration of 1.0 *M* NaCl, and 0.5% detergent. The preparation now contains a white, murky precipitate that can be removed by centrifugation. Ultracentrifugation (25,000 × *g* for 30

[1] Fellow of the American Cancer Society, recommended by the Committee on Growth of the National Research Council. Present address: Carnegie Institution of Washington, Cold Spring Harbor, N. Y.