(solid line and right-hand ordinate), the maximum amount of luciferin activity was then obtained from cells harvested during the dark period. In this case, a sinusoidal-type function was observed, with the increase in extractable luciferin beginning before the end of the light period. Likewise, the decrease anticipated the onset of the light period and was, therefore, not triggered by it. With this technique of harvesting, the changes in luciferin activity in extracts continue to occur in the absence of an environmental light-dark cycle with cells kept in constant dim light (*6*).

An apparently analogous inhibition of flashing and concomitant conservation of luciferin can also be caused by exposing the cells to bright light (Table 2). If cells are exposed to bright light during the middle of the dark period (that is, at the time when the capacity is maximum for emitting light by flashing in response to agitation), the light-emitting capacity of the living cell drops sharply (Fig. 2). The present results demonstrate that this effect is not due to a destruction of luciferin by light—which had been considered to be one of the likely possibilities—but to an inhibition of the ability of the cell to respond to stimulation. The amount of luciferin extractable from cells harvested in the middle of the dark period thus increases dramatically upon exposure to a very bright light (Table 2), in parallel with the inhibition of the ability of the living cell to emit light following stimulation.

It is well known that extraction procedures often initiate chemical changes which can obscure certain aspects of cell biochemistry. The example encountered here seems analogous to one in muscle where glycolysis may be stimulated during extraction and cause production of lactic acid equivalent to that following exhaustive stimulation in vivo (*7*). The breakdown of certain compounds during extraction also is encountered and can be frequently attributed to the release or activation of specific enzymes, or to both. Similarly, a differential loss of enzyme activity during extraction may occur. For example, changes in the extractable activity of specific enzymes correlated with stages in the developmental pattern of the slime mold were attributable to differential inactivation of particular enzymes during the extraction process (*8*).

In conclusion, a daily variation in the bioluminescent flashing which occurs during extraction obscures the phase and amplitude of a daily rhythm in cellular luciferin. When this variation is inhibited, the time of maximum intracellular luciferin activity corresponds closely to the time of maximum bioluminescence in vivo (*9*).

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5. The temperature must be increased with minimal agitation. The growth flask was placed in a 46°C water bath and the culture was warmed (without shaking) for about 4 minutes to 34°C. The temperature required to obtain the effect is confined to a relatively narrow range. Below 32°C no marked differences from controls were observed, and extracts of cells which had been brought to temperatures of 37°C or higher had less luciferin activity.
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Isolation of a Viral Agent from Pseudocowpox Disease

Abstract. *A virus with characteristics similar to the pox group was isolated in tissue culture from bovine skin and oral lesions typical of pseudocowpox. The properties of this agent, certain of which differ from vaccinia virus, suggest that the isolate is the etiologic agent of the pseudocowpox syndrome.*

Pseudocowpox, or milker's nodules, or paravaccinia (*1*), is a disease distinguishable from cowpox in that it does not confer immunity to vaccinia (*2*). This report describes the first isolation of a cytopathogenic virus obtained from pseudocowpox in cattle.

Skin lesions were excised from a cow's teat and her calf's mouth. (Identical results were obtained with the excision of lesions from the teat alone.) A pool of three pseudocowpox lesions was ground and diluted with Earle's salt solution. The clarified suspension was inoculated into primary cultures of fetal bovine testes and a cytopathic effect was observed after 10 days. The destruction of the cell sheet was complete in 12 days. The agent has now been carried for eight consecutive passages in primary cultures of bovine testes. The initial appearance of the cytopathic effect is a rounding and increased granularity of the cells. This is followed by further degenerative changes with subsequent cellular detachment from the glass in a patchwork pattern.

Infected cells stained with hematoxylin and eosin show large cytoplasmic inclusion bodies similar to those seen with pox virus infection. Preparations stained with acridine orange and examined in a fluorescence microscope show that the cytoplasmic inclusion and cell nuclei fluoresce green (Fig. 1). Treatment of infected cells with 100 μg of desoxyribonuclease per milliliter for 60 minutes at 37°C eliminated the green cytoplasmic fluorescence. These results suggest that the virus contains DNA. The eighth passage in bovine testes cells had a titer of $10^{4.7}$ $TCID_{50}$/ml (tissue culture infectious dose). This titer was obtained 14 days after inoculation. The virus forms smooth, round plaques 4 to 5 mm in diameter 10 days after plating. No decrease in titer was noted upon exposure of the virus to 4°C for 1 or 24 hours; to 37°C for 20 or 60 minutes; and to 56°C for 30 minutes. Treatment at 60°C for 30 minutes resulted in a complete loss of activity. Treatment of a virus suspension with diethyl ether (final ether concentration, 20 percent) for 18 hours at 4°C caused no appreciable loss of virus activity.

Hemagglutination did not occur at 4°C or at room temperature with human O red cells, or bovine, rat, hamster, guinea pig, monkey, or vaccinia-susceptible chicken erthrocytes.

Infectious fluid from bovine testes

cells was passed in fetal bovine kidney (primary) or in *Cercopithecus* monkey kidney cells (BS-C-1 Hopps) with production of complete cytopathic effect and acridine orange positive staining of cytoplasmic inclusions. No attempt was made to maintain the virus serially in nonbovine cultures. Lesions were not produced on the chorioallantoic membrane of 10-day-old embryonated chicken eggs inoculated with virus obtained from infected bovine testes tissue culture. Similarly, no virus was recovered from embryonated eggs inoculated in the allantoic fluid. Neither antiserum to vaccinia virus nor antiserum to vesicular stomatitis virus (Indiana or New Jersey strains) neutralizes the isolate.

A 7- to 12-mm erythematous reaction was produced 24 hours after intradermal inoculation of 0.1 ml of undiluted virus in rabbits. No vesicles or ulcers were observed. Early and late virus passage did not produce any sign of disease in suckling mice inoculated intraperitoneally and intracerebrally.

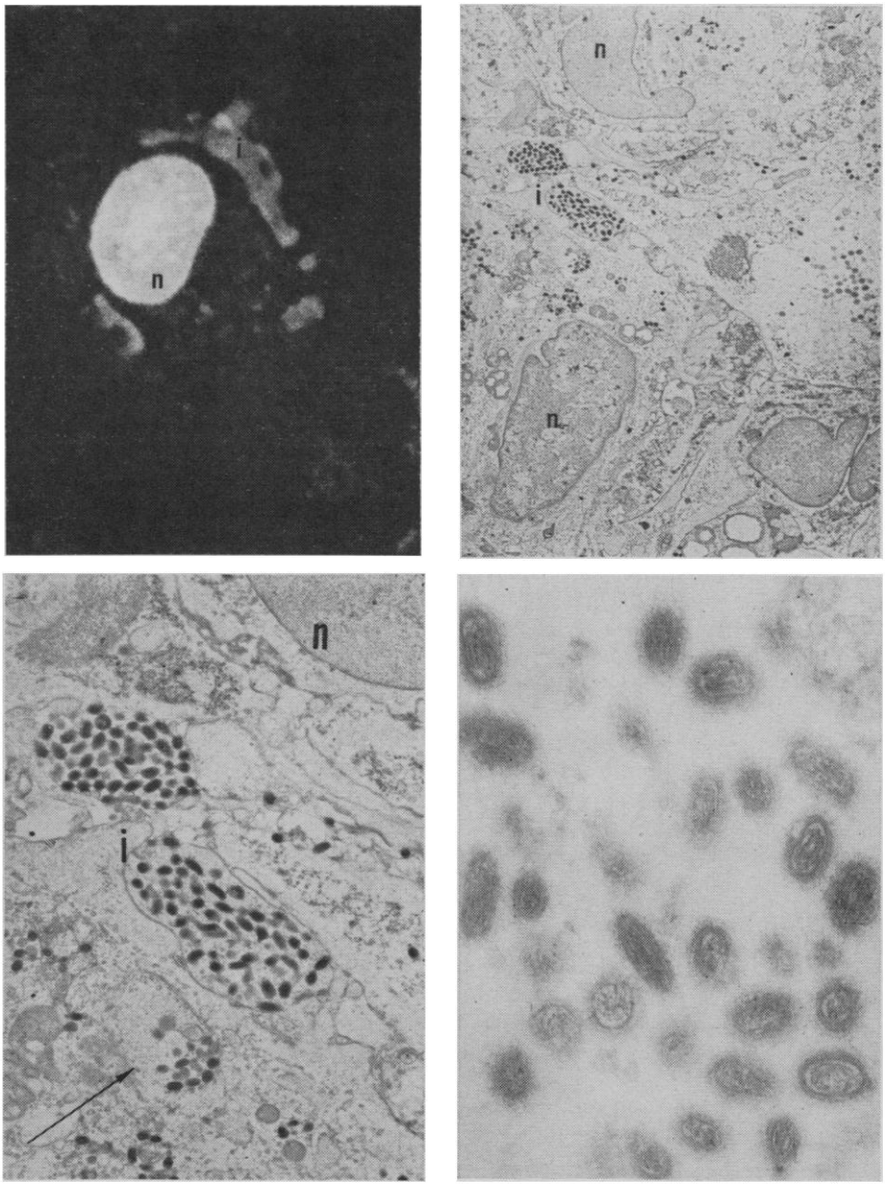

Fig. 1 (top left). Fluoresence micrograph of infected cell showing cytoplasmic inclusion bodies: *n*, nucleus; *i*, inclusion body (× 730). Fig. 2 (top right). Electron micrograph of infected cell showing inclusion bodies containing virus particles: *n*, nucleus; *i*, inclusion bodies (× 1530). Fig. 3 (bottom left). Higher magnification of inclusion bodies shown in Fig. 2. Note the dense central core in some of the particles. Arrow indicates ruptured membrane with free virus in the cytoplasm (× 8760). Fig. 4 (bottom right). High magnification (× 54,000) of infected cell showing virus detail. The central structured core is evident.

Preparations were made for electron microscopic study from cultures of bovine testes 2 days after virus inoculation. After fixation in osmium tetroxide and formalin, followed by dehydration and Epon embedding, thin sections were stained with 5-percent uranyl acetate and examined in a RCA EMU-3G electron microscope. Densely stained, oval-shaped particles were observed in clusters in the cytoplasm (Fig. 2). These clusters of particles were surrounded by a membrane. In some instances, the membrane appeared to have ruptured with a spilling out of the particles into the cytoplasm (Fig. 3). Micrographs taken at high magnification (Fig. 4) revealed the oval particles to have a structured central core surrounded by an outer coat. Control uninfected preparations did not reveal these oval-shaped bodies. Preliminary results of electron microscope examination of the virus after staining with 2-percent phosphotungstic acid revealed the oval-shaped particle to consist of subunits arranged in parallel rows. The particle measured 150 by 290 μ in osmium-fixed tissue sections.

The agent appears to be a virus belonging to the pox group. Its size and morphologic character are consistent with others of that group (*3*). It contains DNA and forms cytoplasmic inclusions. A study of the relationship of the virus with the agent of bovine papular stomatitis (*4*) and a detailed study of its morphologic development are in progress (*5*).

Note added in proof. Since the completion of this report, a paper by Friedman-Kien, Rowe, and Banfield has appeared (*6*). Characteristics of the agent described are identical to our isolate and it is apparent that the two agents are the same.

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