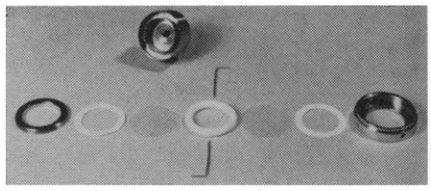

Fig. 1. Exploded view of modified Sykes-Moore chamber prior to assembly. The components are assembled in the order shown, from right to left: outer ring of Bellco Sykes-Moore chamber; ¾- by 1-inch paper washer; 25-mm circular cover slip; 17-210 Teflon O-ring with two bored holes; upper cover slip; upper paper washer; and inner ring of chamber. The stainless steel pins are shown opposite the holes in the O-ring. The die used for cutting the paper washers is shown at the left above. [L. Anderson]

to permit clonal growth was demonstrated by the following experiments carried out with the Chinese hamster cell line C14 FAF28 (*7*), growing in Eagle's medium supplemented with 20 percent fetal bovine serum (*8*). Replicate inocula containing approximately 50 cells were delivered into each of three chambers and three 6-cm petri dishes. The cultures were incubated at 37°C for 7 days, the petri dishes being held in an atmosphere of 5 percent CO_2; the dishes and the lower cover slips of the chambers were then stained, the colonies counted, and their diameters measured. Since the diameter of a colony is roughly proportional to cell number, this quantity serves as an index of cell multiplication rate. From the results given in Table 1 it may be seen that the plating efficiency is similar in the two systems. However, the colonies in the perfusion chambers were significantly larger than those found in the dishes. The slower growth in the dishes may be attributed to the unavoidable changes in *p*H which result from opening and closing the door of the CO_2 incubator, or to the larger volume of medium (5 ml) compared to that in the perfusion chambers (1 ml).

Table 2. Rate of growth of colonies of C14 FAF28 Chinese hamster cells in modified perfusion chambers. Fifty cells were inoculated per chamber, and after 24 hours, 18 locations with one or more cells were chosen for study. Colonies arose at ten of these, and the number of cells per colony was estimated microscopically at intervals of 24 hours. The number of cells per colony at 96 hours was estimated from stained preparations.

Colony No.	Cells per colony at times shown			
	24 hours	48 hours	72 hours	96 hours
1	1	2	5	10
2	1	2	3	11
3	8	13	26	72
4	2	4	5	12
5	7	8	12	36
6	1	4	12	32
7	2	2	8	17
8	5	9	18	54
9	5	15	38	130
10	6	16	45	136
Total	38	75	172	510
Mean generation time (hours)*	24.8	20.4	15.5	

* MGT = $0.301t/(\log N_t - \log N_0)$, where t = 24 hours.

The rate of growth, measured as the increase in cell number per colony, has been assessed in such chambers by direct microscopic observation. Chambers charged as those described above were incubated for 24 hours and then examined microscopically. The locations of single cells and small groups of cells were noted, and a count was made of the number of cells present at each location at intervals of 24 hours thereafter.

Of 18 locations studied, growth and colony formation occurred in ten; in the others either a single large cell was present at 96 hours, when the experiment was terminated, or the single cell died and detached from the cover slip. The yield of colonies from the observed locations of single cells corresponds to a plating efficiency of 0.55, consistent with that found in the experiment shown in Table 1. The observed numbers of cells per colony, for those locations at which growth occurred, are given in Table 2. Calculation of the mean generation time for the sample of colonies studied indicates a decrease from 24.8 hours for the first 24-hour period to 15.5 hours for the period from 72 to 96 hours. These results are consistent with generation times estimated from phase contrast time-lapse films of growing colonies. It should be noted that Hsu *et al.* (*9*) have reported a minimum generation time of about 12 hours for these cells using conventional culture vessels, and larger inocula.

Thus, where the microscopic method to be employed demands rapid growth of isolated cells, the Sykes-Moore chamber with Teflon gasket can provide the necessary environmental control and proper optical conditions. We are presently using the chambers in studies of interphase synthetic processes with the flying spot microscope, and for other studies of clonal growth (*10*).

JEROME J. FREED

Institute for Cancer Research, Philadelphia 11, Pennsylvania

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7. This cell line derives from a pseudo-diploid clone isolated in the laboratory of Dr. George Yerganian, Children's Cancer Research Foundation, Boston, Mass. We should also like to thank Dr. T. C. Hsu, M. D. Anderson Hospital, Houston, Tex., for providing our original stock of these cells.
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Milker's Nodules: Isolation of a Poxvirus from a Human Case

Abstract. *A virus of the pox group was isolated from a lesion on a patient with milker's nodules by use of tissue cultures of bovine cells. The virus shows marked biologic and serologic differences from vaccinia virus, and it is presumably the etiologic agent of the milker's nodule syndrome.*

In 1799 Jenner proposed that there are two forms of human "cowpox" infection, only one of which, "classical cowpox," conferred immunity to variola (*1*). The other form, "spurious cowpox," is presumably the infection now generally known as milker's nodules, but also referred to as paravaccinia or pseudocowpox. This disease is clinically distinguishable from cowpox and does not yield a virus in the animal and egg systems used for the isolation of vaccinia virus. In contrast to patients with cowpox, patients with milker's nodules do not develop immunity to vaccinia (*2*), and vice-versa. Milker's nodule lesions, however, do contain inclusion bodies and elementary bodies characteristic of poxviruses (*3*). This report describes the first isolation of a poxvirus from a milker's nodule in tissue culture.

A 17-year-old white male (T.J.S.) had been milking cows, some of which had papular lesions on their udders. The patient, who had been vaccinated

with vaccinia several years previously, developed three painless nodules on his right hand and forearm, clinically and histologically diagnosed as "milker's nodules."

One of the lesions was removed by punch biopsy and ground up with 9 ml of physiological saline solution; 0.05-ml samples of the clarified suspension were inoculated into various monolayer tissue cultures. Rabbit kidney, cercopithicus monkey kidney, and human-embryo kidney cultures inoculated with this suspension remained normal for the 18 to 30 days of observation. Human-embryo lung fibroblasts (strain W1-26) similarly inoculated showed severe rounding by the 6th day; however, cytopathic effects were not produced on passage in this tissue. Primary bovine-kidney cultures inoculated with the suspension showed cytopathic effects at 12 days; 2 days later nearly all cells had been destroyed. Passage of this culture material to primary bovine-fetal kidney, diploid bovine conjunctiva, and human embryonic fibroblasts (strain MAF) produced cytopathic effects in all cultures within 1 to 3 days. The virus has now been carried for 15 consecutive passages in each of the bovine cultures, with rapid development of cytopathology in all passages. This agent has been designated the T.J.S. strain.

The cytopathic changes in bovine cultures begin with the development of scattered, refractile, round cells and enlarged, finely granular cells throughout the tissue sheet, particularly at the edge. Within the next few days, there are increasing numbers of foci of the large granular round cells, as well as many floating pyknotic cells. The overall picture resembles that caused by vaccinia, except that the focal round cells produced by vaccinia in the bovine conjunctiva cultures tend to be smaller and shriveled. Giemsa-stained cultures showed metachromatic cytoplasmic inclusion bodies roughly the size of the nucleus. Titers of infected bovine culture fluids were generally 10^3 to 10^4 tissue culture infectious doses per 0.1 ml; the endpoint dilutions required 12 to 14 days before cytopathic effects appeared. Virus from the serial passage lines produces partial or complete cytopathic changes in various nonbovine tissue cultures, including rabbit kidney, primary human-embryo kidney, and human-embryo fibroblasts, but the effects cannot be reproduced on second passage in these tissues. In rhesus-monkey kidney cultures the virus produced cytopathic effects in three consecutive passages, but with progressive increase in the latent period.

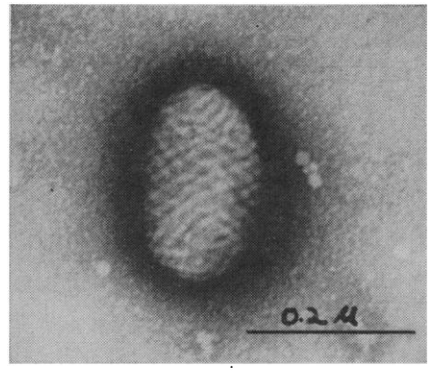

Fig. 1. Milker's node elementary body stained with phosphotungstic acid at *p*H 4.9 (× 114,000).

The virus was stored at −70°, −20°, and 4°C, for 3 weeks with no decrease in activity. Infectivity was abolished by treatment with chloroform for 10 minutes (*4*).

Virus of both the third and eighth tissue culture passage was nonpathogenic when inoculated intraperitoneally and intracerebrally into 1-day-old mice, and onto the scarified cornea of four young adult rabbits. Intradermal inoculation of rabbits produced an erythematous, firm papule at the inoculation site which developed within 24 hours and persisted for 8 to 10 days, with no vesiculation or ulceration; a suspension of such lesions removed on the 5th day yielded no virus in tissue culture and eggs, and was negative on intradermal passage into fresh rabbits. Rabbits which had received combined corneal and intradermal inoculation with the virus showed no resistance to vaccinia virus inoculated onto the same cornea and into the regions where the dermal papules had developed. Conversely, rabbits surviving vaccinia infection were not resistant to induction of the papule by the T.J.S. virus but were immune to vaccinia.

The virus grown in tissue culture, as well as the original specimen, produced no distinct lesions on the chorioallantoic membrane of 10-day-old embryonated hens' eggs examined on the 8th day after inoculation, and virus could not be recovered from suspensions of these membranes by inoculation of tissue culture or rabbit skin.

No hemagglutination could be demonstrated in tests with chicken erythrocytes susceptible to vaccinia hemagglutinin.

Fluid from infected bovine kidney cultures, maintained without serum, was used as a complement-fixing antigen in tests of sera obtained from the patient at various intervals. On the day of the biopsy the titer was 1:5; 3 weeks later, 1:80; and 10 weeks after the biopsy, 1:20. The second serum was negative at a dilution of 1:10 against a comparable control antigen; the first and second sera were negative at a dilution of 1:20 in a hemagglutination-inhibition test with vaccinia virus. The complement-fixing antigen did not react with guinea pig antisera against vaccinia and herpes simplex viruses.

Electron-microscope studies were made of thin sections of the original lesion and of negatively stained particles from infected culture fluids. Sections of the lesion showed changes similar to those described for milker's nodule lesions by Timmel (*3*); however, our measurements (120 by 280 mμ) of the virus particles were larger than the largest elementary bodies measured from his pictures (96 by 200 mμ). The negatively stained particles showed a spiral structure (Fig. 1) like that described for orf and bovine papular stomatitis viruses (*5*); the T.J.S. virus particles showed 13 bands running in one direction, corresponding to that reported for bovine papular stomatitis virus.

Our data indicate that a poxvirus differing in many respects from vaccinia and cowpox viruses has been isolated from a clinically typical case of milker's nodules. The possible relationship of the virus to the agent of bovine papular stomatitis, and of both agents to the milker's nodule syndrome, remains to be determined (*6*).

ALVIN E. FRIEDMAN-KIEN
Dermatology Branch, National Cancer Institute, Bethesda 14, Maryland

WALLACE P. ROWE
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases

WILLIAM G. BANFIELD
Laboratory of Pathology, National Cancer Institute

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6. We thank H. C. Turner for assistance in development of the complement fixation test.

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