Development of a Camel Kidney Cell Strain and Its Use in Virology

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A strain of camel kidney cells was developed and carried in serial passages. The subcultures were slow-growing in the early passages and were composed of heterogeneous cell population. By the 35th passage, the growth rate increased, and more homogeneous cells, mostly of the epithelioid type, were seen. The cell strain was highly susceptible to West Nile, Sindbis, vesicular stomatitis, adeno, and vaccinia viruses, and also was susceptible to herpes simplex, rinderpest, measles, and canine distemper viruses.

Primary camel kidney cell culture has not found a widespread application in modern virology as a tool for isolation or identification of animal viruses because camel kidneys are not always available. To our knowledge, no cell line derived from primary camel kidney cells or from any other tissue of this animal has been reported and is available in routine virology.

The present report deals with the growth and susceptibility of a camel kidney cell strain to some animal viruses.

For study of susceptibility of viruses to this cell system, cell culture tubes were seeded with $10^6$ cells suspended in 1 ml of growth medium. When a confluent sheet of cells was formed, the growth medium was removed, and cells were washed twice with warm phosphate-buffered saline (PBS) before infection with selected viruses. The Vero line of stable vervet monkey cells (8) used in this study was the same strain used in a previous report (3) and was kindly supplied by J. L. Melnick. The particular characteristic of this strain of Vero was its defectiveness in interferon production (1). Vero cell was grown in Melnick medium supplemented with 5% calf serum. After inoculation, the Vero cell cultures were maintained in Melnick medium with 2% calf serum. To perform the infectivity assays, the subcultures of cells at 5th to 50th passage were infected in tubes with vaccinia, adenovirus, and herpes simplex as deoxyribonucleic acid (DNA) viruses and with vesicular stomatitis, sindbis, West Nile, rinderpest (Kabete O vaccine strain; 5), measles virus (Sugyama strain) adapted to calf kidney cells (2), and canine distemper as ribonucleic acid (RNA) viruses. When cytopathic effects (CPE) were observed in three successive passages, the cultures were harvested by two cycles of freezing in dry ice and thawing and were stored at $-65$ C for titration. All viruses had been previously adapted to Vero cells, and titrations were done in this cell line. In the case of rinderpest, measles, and canine distemper viruses, cover-glass monolayers of camel kidney cells, cultured inside Leighton tubes, were infected with the viruses already adapted to camel kidney cells. At intervals, cover slips were removed from the incubator, washed three times with PBS, and stained with hematoxylin-eosin stain as described by Reissig et al. (6).

The primary cultures, up to the 10th passage, presented mixed cell types but appeared to contain predominantly fibroblastic cells which grew slowly. A confluent sheet of cells was formed only after 20 days. Under trypsin treatment, the cells were not easily detached from the glass surface. The subcultures were made every 3 weeks with a weekly change of 50% of growth medium in each bottle. There was a gradual increase in growth rate during the succeeding passages. From the 15th passage, the cells were subcultured once every 2 weeks. Additional subcultures were made with the same growth medium until passage 35 was obtained. The split ratio was usually 1 to 3. At this point, changes in cell structure and growth rate were evident. In general, from the 35th passage henceforth a confluent sheet of cells was formed by a weekly subculture. The growth medium and the split ratio were not changed. Chromosome patterns of the new cell strain were analyzed at the 2nd and the 52nd subculture passage level by the technique of Rothfels and Siminovitch (7) modified by Moorhead et al. (4).

Chromosomes of more than 200 cells were microphotographed and counted. As far as the 52nd passage, it was found that the chromosome change was slight, not exceeding 3 to 5% of total cells counted. The chromosomes of a normal
camel cell seem to be 37 pairs; in the transformed cells, this number was doubled. According to this result, the camel cell strain was not yet established; however, the growth pattern of cells had changed. At the 38th passage, a large number of morphologically different cell types appeared, and epithelioid cells became predominant throughout succeeding passages.

The susceptibility of camel kidney cells to several DNA and RNA viruses was examined. The type of test virus, passage level in camel cell, intensity of CPE of each virus type, and the titer of harvested viruses are listed in Table 1. Vaccinia virus infected the whole cell sheet in less than 24 hr and destroyed all of the cells in 36 hr. Similar changes which also followed the complete de-

### Table 1. Susceptibility of camel kidney cells to some animal viruses

| Virus                      | Strain                                      | Camel kidney passage | Cytopathic effects | Infectivity titer (log 10 TCID₅₀/ml) in Vero cells |
|----------------------------|---------------------------------------------|----------------------|--------------------|----------------------------------------------------|
| Vaccinia                   | Calf lymph, 5th passage in Vero             | 5                    | ++++               | 6.0                                                |
| Adeno                      | Type 5, 4th passage in Vero                 | 6                    | ++++               | 5.5                                                |
| Herpes simplex             | 4th passage in Vero                         | 6                    | +++                | 6.0                                                |
| Vesicular stomatitis       | New Jersey serotype, 3rd passage in Vero    | 3                    | ++++               | 7.5                                                |
| Sindbis                    | 3rd passage in Vero                         | 3                    | ++++               | 8.25                                               |
| West Nile                  | 3rd passage in Vero                         | 3                    | ++++               | 8.75                                               |
| Rinderpest                 | Kabete 0, 7th passage in Vero               | 6                    | ++++               | 5.5                                                |
| Measles                    | Sugyama, 76th passage in bovine kidney cell and 6th passage in Vero | 6 | ++            | 4.0                                                |
| Canine distemper           | 3rd passage in Vero                         | 6                    | ++                 | 4.0                                                |

*a Symbols: ++++, the whole sheet was destroyed; +++, most parts of cell sheet were affected; +++, scattered foci of CPE.

![Syncytium in camel kidney cells, subculture 36, 9 days postinfection with measles virus, Sugyama strain.](image)
Construction of the cell sheet in 3 to 7 days were produced by adenovirus, vesicular stomatitis, sindbis, West Nile, and herpesviruses. The CPE caused by rinderpest or canine distemper virus consisted of scattered small syncytia which developed in 7 days to cover the parts of the cell sheet. The development of morphological cell changes from infection with measles virus, Suyama strain, was slow. After four blind passages, small syncytia in limited numbers were observed beginning 5 days after infection. At this time, the maintenance medium was changed, and cultures were incubated for another 5 days. The CPE progressed, but the whole sheet still was not affected. When cultures of camel kidney cell on cover glass were infected with rinderpest, measles, or canine distemper viruses and were stained with hematoxylin-eosin, large multinucleated syncytia together with eosinophilic cytoplasmic inclusion bodies were observed (Fig. 1), but intranuclear inclusions were rare and were seen only in later stages of infection when most of the cells were detached from the glass surface. A point of interest was that no change occurred in the sensitivity of camel kidney cells to selected numbers of viruses through serial subcultures. As a matter of fact, the infectivity titer of rinderpest or vaccinia viruses was the same when subcultures of camel kidney cells were infected at the 5th or at the 48th passage.

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