Established Kidney Cell Lines of Normal Adult Bovine and Ovine Origin.* (24111)

S. H. MADIN AND N. B. DARBY, JR. (Introduced by C. Lamanna)
Naval Biological Laboratory, School of Public Health, University of California, Berkeley

The literature contains few references to established cell lines derived from normal adult domestic animal tissues. Drew(1) reported an established cell (epithelioid) obtained from normal kidney of adult female white rabbit, and Brion and Gruest(2) reported isolation of epithelial cells from calf kidney, which could be maintained *in vitro*. In addition to these, Haff and Swim(3) reported cell lines from normal adult rabbit muscle and testicle, and a normal adult rabbit kidney cell line, all of which were fibroblastic. Westwood *et al.*(4) reported cell lines from embryo rabbit kidney, Parker *et al.*(5) from rabbit kidney and embryo chick. Stice (unpublished) developed an established swine kidney cell which has been used extensively in this laboratory. No further reports have been found in the literature concerning cell lines obtained from normal adult domestic animal tissues.

It is the purpose of this paper to describe 2 newly established cell lines, one obtained from a normal adult bovine kidney (MDBK), and the other from a normal adult ovine kidney (MDOK).

Materials and methods. Nutrient medium (LA) consists of modified Earle's salt solution and lactalbumin hydrolysate, fortified with lamb serum, as follows: Sol. 1, modified Earle's NaCl 70 g, KCl 4 g, $CaCl_2$ 2 g, $MgSO_4 \cdot 7H_2O$ 2 g, NaH_2PO_4 1.4 g, dextrose 10 g, penicillin (potassium G) 2 g, streptomycin sulfate 1 g, polymixin B 1 g, phenol red (0.2% stock sol.) 10 ml, distilled water to 1000 ml. Sterilize by filtration and dispense in 100 ml amounts. Sol. 2: lactalbumin enzymatic hydrolysate 50 g, distilled water 1000 ml. Dispense this suspension in 100 ml amounts and sterilize by autoclaving at 10 lbs for 10 min. Sol. 3: sodium bicarbonate 75 g, distilled water 1000 ml. Sterilize by autoclaving at 15 lb for 15 min. The complete nutrient medium is obtained as follows: Sol. 1, 100 ml; Sol. 2, 100 ml; Sol. 3, 6-12 ml; lamb serum (sterile) 100 ml; distilled water (sterile) 800 ml.

Preparation of glassware and rubber stoppers. The method described by Madin *et al.* (6) was used throughout. *Preparation of primary tissue cultures.* Original cultures, from which all cell lines were derived, were prepared according to the methods of Madin *et al.*(6). *Cell resuspension fluid.* The saline (ATV) is a modification (A. J. Hackett, unpublished), of that described by Marcus *et al.* (7) and contains versene and trypsin as follows: (10X conc.) : NaCl 80 g, KCl 4 g, dextrose 10 g, $NaHCO_3$ 5.8 g, trypsin† 5 g, versene‡ 2 g, penicillin, potassium G 2 g, streptomycin sulfate 1 g, polymixin B 1 g, phenol red 0.2% stock sol. 10 g, distilled water to 1000 ml. This solution is sterilized by filtration and dispensed in 100 ml amounts. All solutions, unless otherwise stated, are stored at −17°F.

Preparation of cell lines. A vigorous culture of the primary cell growth is selected as cell source for passage. Usually 3 Blake bottles are used, one to serve as source of cells for continuous culture studies and 2 as a reserve cell source. The nutrient fluid is discarded, and the culture is quickly rinsed once with ATV, following which 25 ml of fresh ATV is added to aid in cell liberation. The bottle is placed in incubator at 37°C for approximately 15 min. The suspended cells are centrifuged 5 min. at 1000 rpm, resuspended in 10 ml of fresh nutrient media, and vigor-

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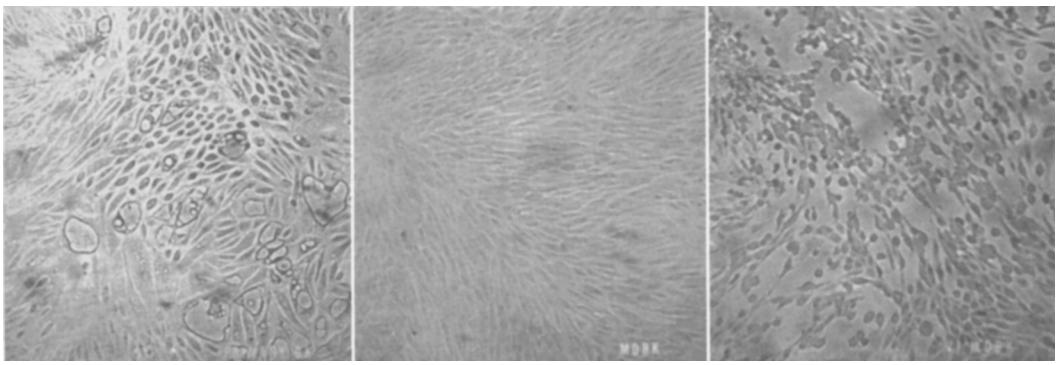
† Trypsin-1:250 Difco Laboratories, Detroit.

‡ Versene-disodium ethylenediamine tetraacetate, Fisher Scientific Co., Fairhaven, N. Y.

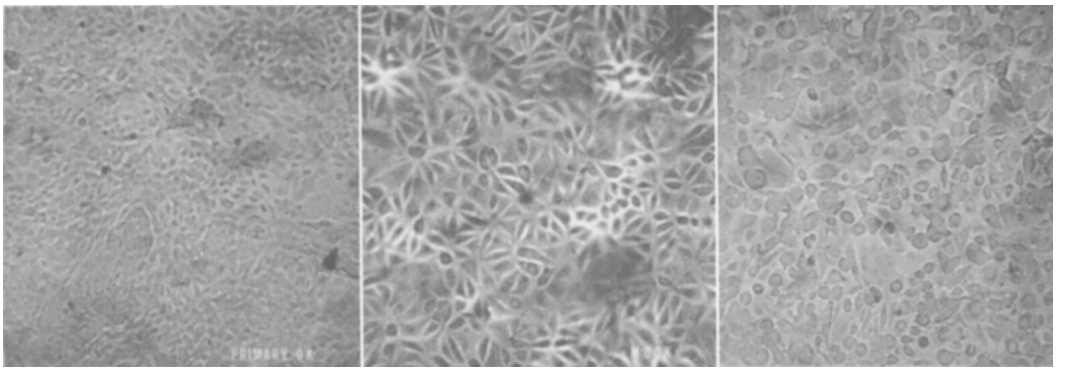


a b c

FIG. 1. a. Primary bovine kidney cells, 100×. b. Established bovine kidney cells in 32nd subculture (MDBK), 100×. c. Vesicular stomatitis virus-infected MDBK cells, 100×.

ously aspirated in a pipette until a homogeneous suspension is obtained. A cell count of this suspension is made with the aid of a hemocytometer and subsequent dilutions are made in nutrient media to yield a final concentration of approximately 5 x 10^4 cells/ml. Approximately 65 ml of this mixture is then added to each Blake bottle used to constitute the new passage. The cultures are incubated at 37°C for 7 days at which time they are satisfactory for repassaging. To obtain maximum growth, the nutrient fluid is changed on the 3rd or 4th day.

History of cell lines. The MDBK cell was carried through the first 16 serial passages on lactalbumin-Tris medium described by Madin *et al.*(6), with 30% lamb serum. The 17th through 22nd passage was made using the same medium with 10% lamb serum. All subsequent passages were made with LA medium described earlier. This particular culture exhibited no striking changes in morphology at any single passage. However, starting from a number of morphologically different cell types, a fibroblast-like cell became predominant at the 9th or 10th passage, and remained stable without any significant morphological changes throughout succeeding passages. Cultures inoculated with 5 x 10^4 cells/ml yield 10^7 cells/ml within a 7-day period at current passage level. The appearance of cells at 1st and 32nd passages is shown in Fig. 1a and 1b. The MDOK cell was serially passaged 8 times in the medium described by Madin *et al.*(6) with 30% lamb serum. 10% lamb serum was used for the 10th passage, and all subsequent passages were made with LA medium containing 10% lamb serum. The MDOK cell in contrast to MDBK began exhibiting increasing numbers of fibroblast-like cells from 4th passage on, and began to decrease in growth rate. Despite appearance of fibroblast-like cells the culture remained mixed fibroblast-epithelial until 12th passage. During the first 12 passages the cells had been transferred every 7 to 14 days. The 12th passage was distributed in several 3 oz. prescription bottles because of a decrease in growth rate and loss of transferable cell population. Attempts to passage the combined cells of 2 or 3 prescription bottles grown for 14, 28, and 42 days, respectively, failed. Sixty-day-old cultures, however, were capable of serial passage. It should be emphasized that while the nutrient fluid was changed approximately twice a week, only the 60-day-old cultures were capable of being subcultured continuously. At the end of the 60-day period epithelial-like cells predominated, eliminating the fibroblast-like cell which had previously been present. This epithelial-like cell formed the basis for the 13th passage, gradually increasing in growth rate over the next 3 passages, until by the 16th passage the current cell appeared firmly established. All succeeding passages have been marked by a uniform growth rate similar to the MDBK cell. The current MDOK cell is morphologically dissimilar both in shape and size to the culture isolated directly from lamb kidney (Fig. 2a and 2b). Apparently a



a b c

FIG. 2. a. Primary ovine kidney cells, 100×. b. Established ovine kidney cells in 30th subculture (MDOK), 100×. c. Vesicular stomatitis virus-infected MDOK cells, 100×.

marked change in cell morphology occurred primarily during the 12th passage.

Results. The current cell lines have been serially passaged 50 and 40 times for the MDBK and MDOK, respectively. Extensive investigations to determine the "type" of cell proliferating in either cell line have not been carried out. The MDBK cell appears somewhat spindle shaped and may be fibroblastic in nature, while the MDOK cell appears more epithelial-like.

Both cells support the *in vitro* growth of vesicular stomatitis virus types Indiana and New Jersey (Fig. 1c and 1b) and infectious bovine rhinotracheitis virus. McKercher (unpublished) states that the MDOK cell will support the growth of the virus of sheep bluetongue. Warren and Cutchins(8) examined 23 different viruses for their ability to multiply in embryonic bovine cells and found 9 which were able to propagate. It is suggested that both cells may be of particular aid to those interested in general host-parasite relationships.

Summary. Two newly established cell strains capable of continuous cultivation, one derived from adult bovine kidney (MDBK) and one from adult ovine kidney (MDOK), are discussed. It is suggested that the strains may be useful for those interested in veterinary virology in particular and virus-host relationships in general.

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