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Characterization of an Established Line of Canine Kidney Cells (MDCK).*† (31293)

CHARLES R. GAUSH, WALTER L. HARD‡ AND THOMAS F. SMITH
(Introduced by W. O. Read)
Department of Microbiolgy, School of Medicine, University of South Dakota, Vermillion

During the last few years, an established line of canine kidney cells (MDCK) has been used in several laboratories to study virus-host cell relationships(1-4). The line was established in 1958 by Madin and Darby(5) but to our knowledge has never been characterized. This report describes the growth, immunologic and cytogenetic properties and preliminary data on its virus susceptibility.

Materials and methods. The MDCK cells were grown in lactalbumin medium(6) or Eagle's MEM containing 10% calf serum and nonessential amino acids. These cells were obtained from D. T. Imagawa of the University of California Medical Center and were designated MDCK-USD. Cultures incubated for 7 days at 35-36°C developed confluent monolayers of epithelial cells which were dispersed with trypsin-versene (TV) solution (6). The cells from one 7-day culture were resuspended in growth medium with a split ratio of 1:10 which was gradually increased to 1:40. The medium was changed after 3 and 6 days of incubation. The cells were grown in antibiotic-free medium after the 50th passage and were tested for the presence of *Mycoplasma* at periodic intervals. We also obtained an MDCK line from M. D. Hoggan at National Institutes of Health which we designated MDCK-NIH and a line from the American Type Culture Collection which we designated MDCK-ATCC. The latter 2 lines were grown in Eagle's MEM as described with a split ratio of 1:40.

The plating efficiency of MDCK-USD cells was determined by placing a known number of cells in growth medium and incubating them in 60 mm petri dishes for 2 weeks at 35.3°C in an atmosphere of 5% CO_2. The colonies formed were stained with crystal violet and counted.

Virus suspensions were prepared in MDCK-USD, KB and chick fibroblast (CF) monolayer cultures or in 9-day embryonated eggs. To determine virus susceptibility of MDCK-USD cells, Influenza (PR8), Vesicular Stomatitis Virus (VSV), Western Equine Encephalomyelitis (WEE), Sindbis, Semliki Forest Virus (SFV), Poliovirus Type 1 and Vaccinia Virus were inoculated into 7-day monolayer cultures at virus multiplicities of at least 1. After a suitable period for adsorption, the inoculum was removed and replaced with Eagle's MEM without serum and the cultures incubated for 5-7 days.

Anti MDCK-USD serum was prepared by disrupting 1.2×10^6 cells/ml in phosphate buffered saline (PBS) and injecting the debris into several guinea pigs *via* the intramuscular and intraperitoneal routes. Two additional injections of antigen were given at 1 and 2 months and the animals bled 1 week after the last injection. All sera were heated at 56°C for 30 minutes and stored at —62°C. We used the method of Brand and Syverton(7) to confirm the species of origin of this cell line. Preimmunization sera were used as controls.

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† Portions of this work are from the Master's Thesis submitted to the Department of Microbiology by Mr. Smith.

‡ Dept. of Anatomy.

Cells in the logarithmic phase of growth were treated with 0.10 μg colcemide (Ciba)/ml of culture fluid and incubated at 37°C for 2 hours. Harvested cells were washed in Hanks' BSS, treated with distilled water for 10 minutes and fixed with methyl alcohol-acetic acid. The fixed cells were air dried on glass slides and stained with Giemsa. Since selection of plates for counting is especially critical in species with large chromosome numbers, only those metaphase plates were considered in which chromosome spreading was confined to an area not larger than the field area for photography with Zeiss Photoscope at 400×. Photographs representing a 3000× enlargement were used for all counting and typing procedures.

Cells were preserved by suspending them in medium containing 30% calf serum and 5% glycerol. The suspension was stored at 4°C overnight and then frozen at −62°C. Frozen cells were thawed rapidly in a 45°C water bath, diluted 1:10 with growth medium and transferred to culture bottles.

Results. Observed as unstained monolayers, the MDCK-USD cells retained their epithelial morphology and property of contact inhibition after 103 passages. The medium was changed to Eagle's MEM during the 40th passage but this did not affect growth or morphology.

Growth curves for representative passages were prepared by counting cells from 3 replicate cultures in a hemocytometer each day. Plotting cell counts *vs* time revealed that cell division was logarithmic between 2 and 5 days (Fig. 1). In this experiment, the increase in cell number was almost 100-fold and the population doubling time estimated from the curve was 19.2 hours. We found that by further diluting the cells at each passage we could gradually increase the split ratio to 1:80 and still obtain a fully grown monolayer in 7 days. We also found that the concentration of calf serum could be reduced from 10% to 5% without seriously affecting the growth rate. In the plating efficiency experiments, 95% of the inoculated cells grew into macroscopic colonies. We were not able to detect *Mycoplasma* in this cell line.

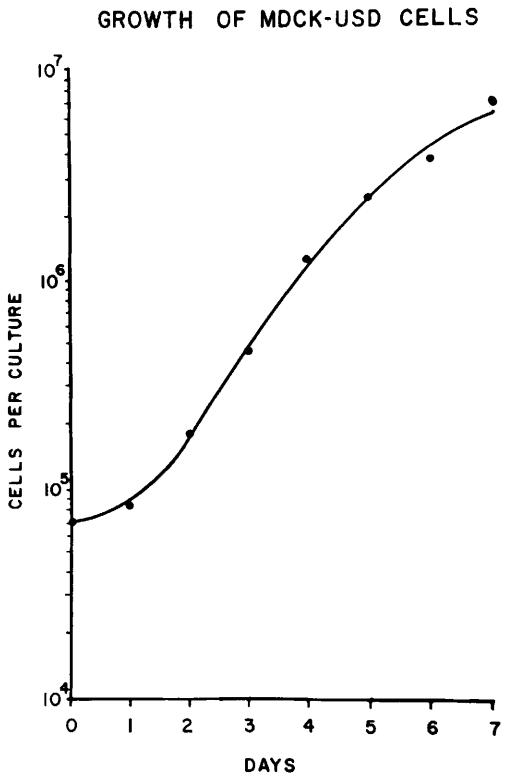

FIG. 1. Growth of MDCK-USD cells in Eagle's MEM, 10% calf serum at 35-36°C. Medium was changed on 3rd and 6th day.

TABLE I. Confirmation of Species of Origin of an Established Line of Canine Kidney Cells (MDCK-USD).

Erythrocyte species	Preimmunization serum 1*	2	3	Anti-MDCK-USD serum 1	2	3
Human	<5†	<5	<5	<5	<5	<5
Monkey	<5	<5	<5	<5	<5	<5
Mouse	<5	<5	<5	<5	<5	<5
Canine	<5	<5	<5	160	320	160

* Guinea pig number.
† Hemagglutinating units/ml serum.

Immunological identification of MDCK-USD cells was performed using human, mouse, and monkey red cells in addition to canine red cells since they represent the species of origin of the most commonly used cell lines. Table I shows that only the canine red cells were agglutinated by the anti MDCK-USD serums thus confirming the stated species of origin.

Karyotypes were determined for the 33rd,

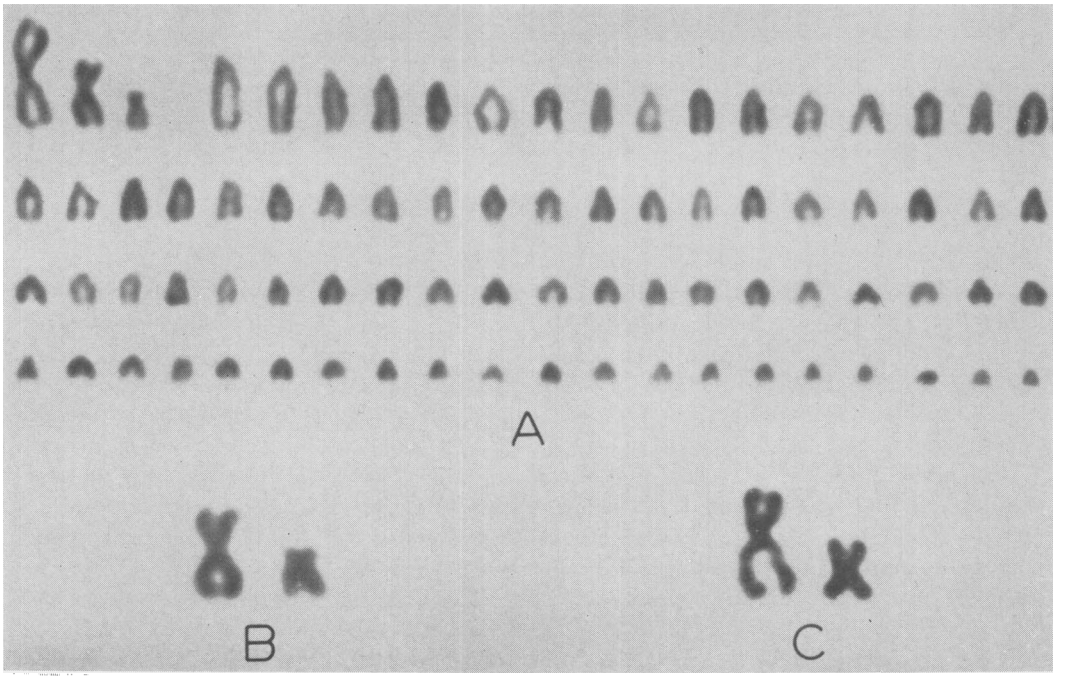

FIG. 2. Karyotypes of MDCK lines: A, MDCK-USD-97; B, MDCK-ATCC-50; C, MDCK-NIH-7; only the full karyotype of the USD line is shown since the autosomes of ATCC and NIH cannot be distinguished from USD. Number of chromosomes in A is 79.

61st, 69th and 97th passage of MDCK-USD, the 7th passage of MDCK-NIH and the 50th‡ passage of MDCK-ATCC. We observed no identifying characteristics that would permit pairing of homologous autosomes and therefore arranged them in order of decreasing size (Fig. 2A). Similarly, it was impossible to distinguish between cell lines on the basis of the autosomes and only the karyotype of MDCK-USD is shown. However, we observed consistent differences in marker chromosomes of the metacentric type (Fig. 2: A, B, C). The MDCK-USD line (Fig. 2A) contained a large metacentric chromosome which could be considered the X but it is mediancentric while the X of primary canine cultures is submediancentric(8-10). There were 2 additional submediancentric chromosomes in the USD line which we considered too small to be X chromosomes since they are both smaller than the largest autosomes. The ATCC line was characterized by a large metacentric and a much smaller subtelocentric chromosome (Fig. 2B). The NIH line also contained only 2 chromosomes of the metacentric type; the larger was a submediancentric which resembled an X and a smaller metacentric (Fig. 2C). There are several reports which indicate that a Y chromosome is present in male somatic cells in primary culture and that it is the smallest member present(8-10). However, we were unable to identify a Y chromosome in any of the cell lines examined even though they were established from a male animal(5).

The modal number of chromosomes was 79 for the ATCC line and 87 for the NIH line (Fig. 3). We found that the modal number of the USD line fluctuated and in earlier passages a stem line with 81 chromosomes appeared to be selected. However, in the 97th passage a predominant stem cell was not observed (Table II). The earlier passages of the USD line suggested a bimodal population centering around stem cells of 78-79 and 81 chromosomes.

In virus infected MDCK-USD cells, cytopathic effects were observed in 24-48 hours

‡ The 49th passage from establishment was received from ATCC; this represents the 1st passage in our laboratory.

TABLE II. Distribution of Chromosome Numbers in Various Passages of MDCK-USD Cells.

Cell passage	Total cells	% of cells with the following chromosome numbers: 76	77	78	79	80	81	82
33	24	12.5	0	16.6	25.0	0	45.8	0
61	14	0	0	7.1	0	21.4	64.3	7.1
69	29	3.4	0	10.3	6.9	0	75.8	3.4
97	27	0	11.1	11.1	22.2	18.5	25.9	11.1

with the PR8, VSV and Vaccinia viruses but not with Poliovirus, Sindbis, SFV or WEE. The replicating viruses were subcultured until the total cumulative dilution greatly exceeded the titer of the original inoculum. With PR8 virus, this dilution is presently 10^{-31} and the virus yields have infectivity titers of 10^5 $TCID_{50}$/ml in MDCK-USD cells and hemagglutination titers as high as 1280 hemagglutinating units/ml. A more detailed study of the replication of these viruses in MDCK-USD cells is in progress.

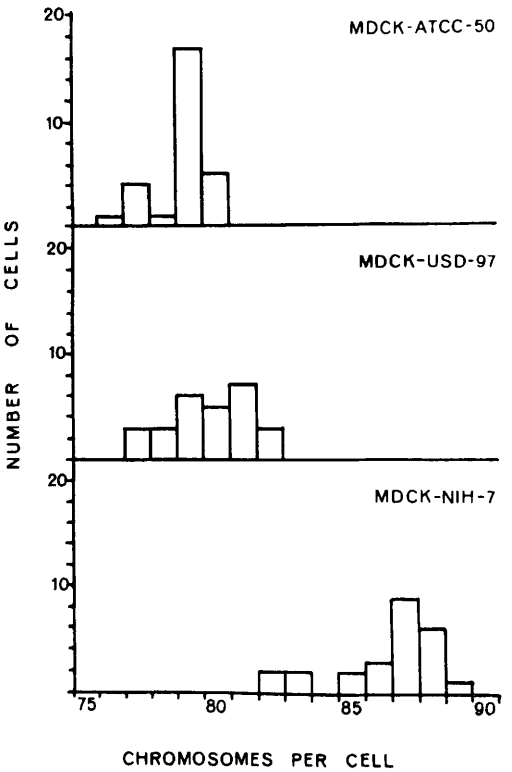

FIG. 3. Chromosome distributions in lines of MDCK cells. MDCK-ATCC-50 represents the 1st passage in our laboratory since it is known to have had 49 previous passages. Designations of the USD and NIH lines indicate number of passages in our laboratory since the previous history is not known.

Discussion. The MDCK-ATCC line of cells represents the parent line from which the USD and NIH lines were derived. We have encountered no difficulties in growing the USD line and have observed plating efficiencies of 95% as well as 100-fold increases in cell numbers. Our brief experience with the ATCC and NIH lines indicates that these cells also grow well. We have found the USD line suitable for studying the replication of some viruses.

The USD line was obtained from Imagawa after an undetermined number of passages and has been subcultured 103 times since acquisition 3 years ago. The NIH line has been in continuous passage for 5 years by Hoggan (personal communication) but the total number of passages is not known. The modal number of chromosomes in each line appears to be correlated with the length of time in culture. The stem cell of the NIH line contains 87 chromosomes after 5 years in continuous culture, the USD line 81 chromosomes after 3 years, and the ATCC line, 79 chromosomes in 50 passages from establishment. Thus the ATCC stem cell resembles the normal canine karyotype (2n = 78) with one additional metacentric autosome.

The numerical chromosome aberrations described above were accompanied by structural alterations which took the form of metacentric autosomes. These karyotypes can be compared with those of 2 canine kidney cell lines established from malignant tissue(11). One, from a thyroid adenocarcinoma, contained 5 metacentric chromosomes while the other, from a melanoma, contained as many as 34 metacentrics. This is analogous to permanent cell lines of murine origin in which the normal complement of autosomes is also acrocentric, the commonest morphological change being the appearance of metacentric

autosomes(12,13). The mechanism controlling the appearance of the metacentrics is not clear. Ruddle suggests that it may be due to terminal union of 2 acrocentrics(14) but other mechanisms cannot be excluded. However, the differences observed in number and morphology of metacentric chromosomes provides convenient markers for cell lines with otherwise indistinguishable acrocentric autosomes.

Summary. An established line of canine kidney cells (MDCK-USD) of male origin was shown to contain 3 metacentric chromosomes and possess a modal number of 81. It grew rapidly in Eagle's MEM, displayed contact inhibition and was susceptible to vaccinia, VSV and influenza (PR8) viruses. Two additional MDCK lines, the parent line and another derivative from other laboratories, were compared to the USD line and could be distinguished on the basis of modal number and morphology of the metacentric chromosomes observed in each line.

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Normal Acid-Base Composition of Cerebrospinal Fluid in Infants And Children.* (31294)

W. JOSEPH RAHILL† AND ROBERT W. WINTERS‡
(Introduced by Edward C. Curnen, Jr.)
Department of Pediatrics, Columbia University College of Physicians and Surgeons and the Babies Hospital, Columbia-Presbyterian Medical Center, New York City

Much recent work has focused attention upon the acid-base composition of the cerebrospinal fluid (CSF) and its relation to the acid-base composition of the blood. This relationship is of general interest in an understanding of the functional nature of the blood-CSF barrier and of specific interest in elucidation of the mechanism for the chemical control of pulmonary ventilation(1). To date all studies of blood acid-base relationships have been carried out on either experimental animals or on adult human subjects. In connection with studies in progress in this laboratory on the CSF acid-base status of infants and children(2), the need for precise definition of normal CSF acid-base composition of patients in these age groups became apparent. The present paper presents such data.

Methods. Patients. Two groups of patients were studied: (a) a group of 11 infants of low birth weight and (b) a group of 13 children. Clinical data on the infants are shown in Table I. Birth weight varied from 1050 to 2100 g. All were clinically normal, having been continuously observed since birth in the Premature Nursery of the Babies Hospital. Because of the lack of a precise range

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