persisted and even increased at higher temperatures of incubation, whereas at the latter pH levels, hemagglutinating ability fell rapidly as temperature rose. The altered hemagglutinating activity may indicate a change in the protein coat. This point is under study.

Absence of hemagglutinating ability does not necessarily mean that infectious virus is not present, since a titer of 10^3 newborn hamster infectious doses is required before hemagglutinating activity is demonstrable(5, 6,9,10). The agent of hemagglutination appears to be the virus particle itself as determined from density gradient ultracentrifugal and electron microscopic studies. No other particles have been seen even when agglutinated red cells are used as the source of materials for electron microscopy(5,6,13). Therefore the stability of the hemagglutinating property of H-1 virus probably indicates the great stability of the virus particle itself, a corroboration of laboratory observations (*e.g.*, H-virus stored for 3 years at refrigerator temperature has retained its original HA and infectivity titers).

Summary. The hemagglutinating property of H-1 virus is stable from pH 2 to pH 11 at 4°, 25°, and 37°C. With increasing temperature, loss of titer gradually occurs, particularly at the extremes of the pH ranges. Hemagglutinating activity is still detectable after treatment at 80°C at pH 5 and pH 6.

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Use of Cell-Banked Monkey Kidney Cells for the Isolation of Respiratory Viruses.* (30021)

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For primary isolation and growth of many of the myxoviruses in cell culture systems, kidney cells of Old World monkeys appear to have an advantage in sensitivity when compared with other practically available cells. There are, however, certain problems which attend the use of primary monkey kidney (PMK) cell cultures, such as lack of a convenient and flexible source of supply, and uncertain status of various lots in respect to growth characteristics and latent viruses. In an effort to circumvent these problems, our laboratory has made use of secondary monkey kidney cell cultures (SMK) prepared from freezer-banked PMK cells which have been grown, frozen, and stored after the method of Stulberg(1). This report will describe the procedure for preparing these cells, our experience using them in studies of respiratory viruses over a period of 4 years, and the results of tests which indicate that such SMK cells match sensitivity of PMK cells when used for the primary isolation of parainfluenza viruses types 1, 2, and 3.

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Materials and methods. Source of MK cells. Two lots of cells were prepared from

suspensions of trypsinized kidneys obtained from a commercial source. All others were derived from kidneys of 2-year-old Rhesus monkeys, weighing about 8 lb, obtained from a small colony where they had been kept for 6-12 months while being used for nutrition studies.

Solutions and media:

Growth:

0.5% lactalbumin hydrolysate in Hanks' balanced salt solution with 5% calf serum

Maintenance:

Equal parts of Eagle's Minimal Essential Medium and Medium 199 with no added serum

Freezing:

Eagle's Minimal Essential Medium + 15% calf serum and glycerol 5%, or dimethylsulfoxide 7.5%

Collecting:

Phosphate buffered saline + 0.5% bovine serum albumin

All serum was inactivated by heat at 56°C for 30 minutes. All media and solutions contained 250 U Penicillin, 0.25 mg of Streptomycin and 1 mg of Fungizone per milliliter. SV_5 antiserum was added to growth medium to a final concentration of 0.2%.

Hemadsorption tests. Hemadsorption (HA) tests were performed with guinea pig erythrocytes as described by Chanock *et al*(2).

Virus identification. Hemadsorbing viruses were identified by the hemadsorption inhibition method(2) using antisera prepared in this laboratory.

Preparation of SMK cells. Kidneys were trypsinized according to the method of Youngner(3), the packed cell volume determined, and a 1:300 dilution made of these cells in growth medium. Forty-five ml aliquots of cell suspension were seeded into 32 oz prescription bottles, and these were incubated at 36°C for 5 days with 2 changes of growth medium. Confluent cell growth was usually achieved by day 5.

The primary outgrowth of cells was then trypsin-dispersed, sedimented, and resuspended in freezing medium at a final cell concentration of 2 million/cc. The cell suspension was packaged in screw-cap vials and frozen in a Canalco slow-freeze apparatus which reduced the temperature of the freezing-unit bath 1°C per minute from ambient to −20°C and then rapidly to −70°C. The vials of frozen cells were stored in a mechanical freezer at −90°C until needed.

For making SMK cell cultures, one or more vials were rapidly thawed at 37°C, diluted with growth medium, and planted 0.5 ml/tube in 15 × 200 mm tubes. The growth medium was the same as that used for the growth of primary cells except that the bicarbonate concentration was very low (15 mg%). Most lots could be used at a 1:15 dilution of the freezer-banked material but occasional lots gave better growth when diluted slightly less. These tubes showed islands of confluent growth after 3 to 4 days, at which time they were changed to maintenance medium and considered ready for use.

Results: Yield of cells. Over the past 5 years, a total of 28 lots of monkey kidney have been prepared by this method. Two of these were not considered usable, one because of a yeast contamination, and the other because of rapid deterioration of the secondary cell cultures, presumably due to Simian virus. The remainder were used for making SMK cell cultures. Most lots provided enough material to make between 5 and 7 thousand tubes and were used over periods as long as 4 months.

Simian viruses. All lots have appeared to carry Simian viruses. A varying fraction of inoculated tubes, seldom as many as 10%, developed progressive cytopathic changes, consisting of groups of cells with extensively vacuolated cytoplasm or gradually enlarging areas of syncytium formation. Often tubes which initially showed the vacuolated cells subsequently developed large syncytial areas. These changes always appeared more rapidly and extensively in cell cultures incubated at 36°C as contrasted to 33°C, and in rolled cultures as contrasted to stationary cultures. These cytopathic changes were seldom seen before 12 days of incubation and did not preclude the growth of various respiratory viruses in the affected tube. They were transmissible to human kidney and human diploid cells and were suppressed by serum of the animal from which the kidneys were obtained. It should be emphasized that cell cultures which showed these changes did not

hemadsorb guinea pig erythrocytes. Indeed, no hemadsorbing Simian viruses were encountered in any of the 26 lots of material used and, in noting this, it might be recalled that although SV_5 antiserum was present in the growth medium of the primary and secondary cells, it was not present in the maintenance medium of the secondary cell cultures.

Spectrum of viruses growing in SMK cells. This type of cell culture was used for attempting virus isolation from specimens collected from children with acute respiratory infections. Such cultures, incubated at 33°C or 36°C on roller drums, yielded various myxoviruses including influenza A_2 and B, parainfluenza 1, 2, and 3 and mumps virus and similar cultures incubated stationary at 33°C in the laboratory of Dr. Hamre have yielded parainfluenza type 4(4). Other viruses isolated in this cell culture system include adenoviruses, herpes virus, R. S. virus and rhinoviruses, although SMK cells are clearly of second choice as compared to HEp-2 or WI-38 for the isolation of these agents.

Comparison of PMK and freezer-banked SMK cells in the isolation of certain myxoviruses. Secondary MK cells prepared by these methods were compared to PMK cell cultures in respect to sensitivity for the primary isolation of certain myxoviruses. In an effort to minimize variables, the comparisons were made with PMK and SMK cells derived from kidneys of the same monkey. To do this, tubes as well as bottles were seeded with the trypsinized cells of a pair of kidneys. The tubes were grown at 36°C for 2 to 3 days, and after a change in medium, held at room temperature while the cells in the bottles were undergoing further outgrowth, trypsinization, and freezing. Tubes of SMK cells were then made from the frozen material after it had been in storage for 24 hours. When these SMK cells had grown for 2 days, the tubes of primary cells were returned to 36°C incubation. Both sets of cells were then permitted further growth, changed to maintenance medium and inoculated simultaneously in the comparisons to be described.

The specimens from patients used in these comparisons had been obtained from children with acute respiratory illnesses. Aliquots had been previously inoculated into various cell cultures, including SMK, and the remainder of the specimen frozen and stored. For the following studies, original specimens previously found to be positive for parainfluenza virus were chosen. For the simple reisolation attempts, specimens were rapidly thawed at 37°C and 0.1 ml inoculated into each of 2 cell culture tubes of the PMK and the SMK. For infectivity titrations, the thawed specimens were centrifuged at 2,000 *g* for 10 minutes, 10-fold dilutions made in collecting medium, and 0.1 ml of the dilution inoculated into 2 tubes of each kind of MK cells. The inoculated tubes were incubated on a roller drum at 36°C and observed for a total of 21 days with viewing for development of cytopathic changes and HA testing on days 5, 8, 11, 13, 18, and 21. Second passages were made of negative tubes at day 13.

Reisolation of virus from known positive original specimens. The first comparison was made by attempting reisolation of parainfluenza viruses from patient specimens which had previously demonstrated to be positive. The specimens tested had been in freezer storage for varying periods of time: those with numbers <5000 at —20°C for 7-19 months; higher numbered specimens at —90°C for 6 months or less. The results of this test, using 15 specimens are presented by virus types in Table I where the day is indicated when clearly positive CPE or HA was first noted in each of the 2 tubes of PMK and SMK inoculated. Four para-1, four para-2, and seven para-3 specimens were tested. Two of the 4 parainfluenza type 1 specimens did not yield virus. For the 2 which were positive results were similar in the PMK and SMK cells. It will be noted that all of these specimens had been in storage at —20°C. Three of the 4 parainfluenza type-2 specimens were positive and again the results in PMK and SMK cell cultures were similar except that in specimen #4782, one of the 2 tubes of SMK did not yield virus and the other showed questionable hemadsorption on first passage, but was not clearly positive until second passage. Only one of the parainfluenza-3 specimens which

TABLE I. Comparison of PMK and SMK in Reisolation of Parainfluenza Viruses from Original Specimens.

Virus	Spec #	Day 1st CPE or HA PMK	SMK
Parainfluenza Type-1	3632	–	–
		"	"
	3637	–	–
		"	"
	3689	8	5
		5	"
	3877	5	5
		"	8
Type-2	4176	–	–
		"	"
	4782	13	18*
		"	–
	5008	8	8
		"	11
	5092	11	11
		8	8
Type-3	4186	–	–
		"	"
	4652	11	11
		"	"
	4664	–	–
		"	"
	4671	–	–
		"	"
	5006	11	8
		"	11
	5028	11	11
		"	"
	5042	11	8
		"	11

* Day 5 of 2nd pass.

had been stored at —20°C yielded virus and in this case PMK and SMK were similar. All of the parainfluenza-3 specimens which had been stored at —90°C were positive and again results were identical in PMK and SMK cell cultures.

Infectivity titrations of original specimens. To make a more definitive comparison, the infectivity of original specimens was titrated in PMK and SMK cell cultures. The results of these comparisons for parainfluenza viruses types 1, 2, and 3 are presented in Table II.

Seven original specimens known to contain parainfluenza virus type-1 showed similar titers of virus in both the PMK and SMK cells. Parainfluenza virus type-2 titers were also similar in both PMK and SMK cells. Virus was not recovered in either cell type from specimen #5914 at a 10^{-1} dilution, the lowest dilution tested.

Infectivity titers of 8 parainfluenza type-3 specimens in PMK and SMK cells were again similar, although less uniform. One specimen, #6008, did not yield virus in either type of cell; and with another, #5061, the upper limit of infectivity was not reached in either type of cell. In one instance, #5158, the titer in SMK was 2.5 logs less than it was in PMK.

Isolation attempts from other specimens. Since all of these comparisons were conducted with specimens which had initially been found to contain myxovirus by testing on SMK, the strains used might be considered as selected for ability to grow well on SMK. In an effort to examine this possibility, 17 specimens which had been previously tested in SMK and found to be negative were retested with simultaneous inoculation into PMK and SMK. All were obtained from patients early in the course of acute respiratory illnesses: 5 had illnesses which were undifferentiated and diffuse; 6 had croup; 3 pneumonia; and 3 bronchitis. All of these specimens were negative in both PMK and SMK cultures on retesting.

To summarize these comparisons, PMK and SMK cells appeared to be equally sensitive when used to reisolate parainfluenza types 1, 2, and 3 from original specimens.

TABLE II. Infectivity Titers* of Original Specimens as Determined in PMK and SMK Cells.

Virus	Spec #	Infectivity titer per 0.1 ml* PMK	SMK
Parainfluenza Type-1	5058	2.5	3.5
	5240	3.5	3.5
	5514	3.5	3.5
	5531	4.0	3.5
	5545	2.5	2.5
	5814	4.0	4.0
	6047	2.5	2.5
Type-2	5057	3.5	3.0
	5109	1.5	1.5
	5914	<1	<1
	5922	2.5	2.5
	6043	3.5	3.5
	6067	3.0	2.0
Type-3	5061	≥ 4.5	≥ 4.5
	5168	3.0	.5
	5417	4.5	4.5
	5548	4.0	3.0
	5900	3.5	4.5
	6008	<1	<1
	6011	4.5	5.0
	6023	3.0	2.5

* Expressed as reciprocal $\log_{10}$ of initial dilution of specimen.

Although virus could not be reisolated from a number of specimens, this was true in both types of cells. Since most of the specimens from which virus could not be reisolated had been in storage at —20°C for at least 6 months, it seems probable that this failure was related to the conditions of freezing and storage rather than the cell system used.

Summary. A procedure for preparation of freezer-banked PMK cells has been described. Such cells provide a readily available source of SMK cell cultures of known characteristics in respect to growth and Simian viruses. The yield from one pair of monkey kidneys can provide seed for 5 to 7 thousand tubes of SMK cell cultures which may be made up and used as needed over a period of several months. The spectrum of viruses that will multiply and produce cytopathic changes or hemadsorption in such cell cultures appears to be similar to that in PMK cell cultures. In specific comparisons these cells appear to equal the sensitivity of PMK cells when used for the primary isolation of parainfluenza viruses types 1, 2, and 3.

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Venous-Arteriolar Response in the Canine Liver.* (30022)

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Increases in total vascular resistance as functions of elevated large vein pressures have been reported in the dog foreleg(1), kidney(2), and intestine(3,4), although decreases in resistance in certain vascular beds have been found(5-7).

Increases in resistance with elevated venous pressure have been explained in part on the basis of activated neural pathways(1), a myogenic response(4) and unknown factors (2). It was considered of interest to explore the influence of hepatic vein pressure on pressures and resistances in both hepatic artery and portal vein systems in the liver. These relationships were studied in the isolated, denervated, perfused dog liver with controlled perfusion rates of both inflow circuits. Results show a profound influence of hepatic vein pressure on hepatic artery resistance, suggesting the activation of a myogenic response.

Methods. Experiments were carried out on totally isolated, denervated dog-perfused livers. Adult mongrel dogs, intravenously anesthetized with sodium pentobarbital, 30 mg/kg, were used in all experiments. The liver was removed from the dog after being freed from all surrounding tissue with the exception of hepatic artery, portal vein and vena caval attachments. Inflow vessels were separately cannulated with plastic tubing following heparinization, 3 mg/kg. The liver was then freed and transferred to a strain gauge weighing device(8) and perfused at constant flow with Sigmamotor pumps. Flows were slowly increased until hepatic artery pressure and portal vein pressures were in the physiological range (4-11 mm Hg and 60-140 mm Hg, respectively), and the liver was maintained in the isogravimetric state. A large bore polyethylene cannula was then introduced into the orifice of the hepatic veins (a portion of distal and proximal inferior vena cava remained intact) and tied firmly in place. A small catheter (PE 150) was advanced through this orifice to monitor hepatic vein pressure within the liver sub-

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