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Dispersion and Cultivation of Renal Cells After Short-Term Storage of Kidneys

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A new method for the preparation of cell suspensions from human newborn kidneys is described. It involves the use of a mixture of trypsin-ethylenediaminetetraacetic acid and collagenase. The cell yields obtained after tissue dispersion by this method were significantly greater than those obtained after dispersion with either trypsin or ethylenediaminetetraacetic acid alone or in combination. When kidneys were removed 12 h or more postmortem from refrigerated cadavers, higher cell yields were obtained from renal tissue stored overnight at 4 to 6 C in CMRL ATM (Healy and Parker, 1966), as compared to cell yields obtained from kidneys processed immediately upon removal. This observation was confirmed by controlled experiments performed with rabbit kidneys.

Primary cell cultures of newborn human kidneys are widely used in virology. However, the methods of tissue dispersion developed for animal kidneys do not produce high enough cell yields when applied to human material. This results in suboptimal utilization of difficult-to-obtain tissue. To complicate the matter, postmortem excision of human kidneys can very rarely be performed immediately after death and is, by necessity, performed at various intervals postmortem. Therefore, the development of a reliable, high-cell-yield method for dispersion of human newborn kidney obtained after varying postmortem intervals appeared desirable. To this end, a series of dispersion and cultivation experiments on newborn kidneys was performed. Different tissue dispersion procedures were tested under commonly encountered laboratory conditions. This included testing the effect on cell yields of different postmortem intervals and short-term storage at 4 to 6 C, and determining cell yields from kidneys removed at predetermined intervals. Since it was impossible to perform studies on fresh human kidneys, a series of experiments was performed on rabbit kidneys. These served as controls.

MATERIALS AND METHODS

Specimen collection. Kidneys were removed aseptically from 24 newborn and stillborn cadavers between 3 and 36 h after death, according to previously described techniques (2). Immediately after excision, the kidneys were decapsulated, hemisectioned, step-sectioned, and placed in sterile double jars containing CMRL 1415 ATM (1) to which 200 μg of streptomycin and 200 units of penicillin/ml were added. The CMRL 1415 ATM is a non-sodium bicarbonate-buffered medium which maintains buffering capacity in an air phase. This characteristic makes it suitable for the collection of specimens in facilities which do not have a ready source of CO_2/air mixture necessary for the maintenance of pH in sodium bicarbonate-buffered media. The media were refrigerated.

Rabbit kidneys were obtained from six-week-old New Zealand White rabbits (1.5 to 1.9 kg of weight) killed by an air embolism. The carcasses of these animals were maintained for 12 h at 4 C. The kidneys were removed at the end of this period. One of the kidneys was dispersed immediately after removal, whereas the other one was placed in CMRL 1415 ATM and stored for 24 h in a refrigerator. It was then dispersed in an identical manner to the first kidney. Freshly removed rabbit kidneys were not studied in these experiments, since the dispersion and cultivation data on fresh rabbit kidneys were reported previously (3).

Dispersing solutions. The dispersion solution for human kidneys was Dulbecco phosphate-buffered saline without Ca or Mg, to which 0.25% (wt/vol) of trypsin, 0.02% (wt/vol) ethylenediaminetetraacetic acid (EDTA), and 0.1% (wt/vol) collagenase were added. However, for comparison, in the first three cases shown in Table 1, one-half of the tissue was dispersed with trypsin-EDTA solution. For dispersion of rabbit kidneys, only trypsin-EDTA solution was used. Unlike human kidneys, addition of collagenase did not appreciably increase the cell yield from rabbit kidneys. The dispersing solutions were prepared immediately before use from 5% tryspin stock solution and 10% EDTA.

A 2.5% (wt/vol) stock collagenase solution was

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TABLE 1. *Summary of results obtained with dispersion and cultivation of newborn infant kidneys obtained at various intervals postmortem and following short-term storage at 4 to 6 C*[a]

Exp no.	Age of donor[b]	Time from death to autopsy (h)	Time between removal of kidney and dispersion (h)	Total time from death to dispersion (h)	Tissue wt	Cell yield (×10⁶ cells/g of tissue)	Cell growth in culture
1	SB	12	1	13	14	30* 70	Yes
2	22 h	21	1	22	14	5* 18	Yes
3	33 h	3	2	5	13	20* 51	Yes
4	4 mos.	25	5	30	40	21	Yes
5	12 days	3	1	12	12	39	Yes
6	4 h	9	2	11	6	26	Yes
7	SB	9	4	13	12	0	No
8	1 day	4	3	7	10	70	Yes
9	6 h	22	3	25	8	60	Yes
10	28 h	8	3	11	30	11.6	Yes
11	2 h	4	3	7	7.6	49.6	Yes
12	4 days	5	2	7	11.3	36.1	Yes
13	43 days	6	14	20	15.7	7.4	Yes
14	1 day	27	23	40	29	31.4	Yes
15	4 days	14	23	37	29	10.3	Yes
16	1 day	20	27	47	7.5	72.8	Yes
17	SB	16	27	43	10	32	Yes
18	10 mos	14	23	37	47	6.5	No
19	NB	6	20	26	9.9	18	Yes
20	3 days	18	24	42	11	42.4	Yes
21	30 h	7	26	33	13	11.2	Yes
22	20 h	36	27	63	12	55	Yes
23	3 days	6	26	32	10	20	Yes
24	4 h	17	20	37	4.5	194	Yes

[a] Except in cases 1 through 3 the tissue was dispersed with trypsin-EDTA-collagenase solution. In the first three cases one-half of the tissue was dispersed with trypsin-EDTA. *, Trypsin-EDTA yields.

[b] SB, Stillborn; NB, newborn.

prepared in Dulbecco phosphate-buffered saline and sterilized by passage through a Selas Fluotronic MT-FEF-8 filter of 0.27 μm pore size. The collagenase solution was kept frozen (−15 C) in small aliquots until the time of use.

Dispersing technique. The dispersing technique was described previously (3, 4).

RESULTS

Table 1 summarizes the results obtained from each of the processed human kidneys. In the first three experiments one-half of the tissue was dispersed with trypsin-EDTA solution and the other half with trypsin-EDTA-collagenase solution. The results obtained indicated a 2.5-fold increase in the cell yield when collagenase was added to the dispersing solution, as contrasted to trypsin-EDTA solution alone. Therefore, subsequently only trypsin-EDTA-collagenase solution was used for the dispersion of human tissues. The cell suspensions obtained with trypsin-EDTA-collagenase were free of viscous clumps which were encountered in cell suspensions obtained by dispersion with trypsin-EDTA solution. The morphologic appearance of primary cell cultures obtained from suspensions of trypsin-EDTA-collagenase dispersed cells was similar to that observed for renal cells dispersed by other methods (Fig. 1).

In the cases in which kidneys were removed within 12 h after death and processed within a few hours, the average cell yield was 44.2×10^6 cells/g of tissue. When kidneys were removed 12 h or longer after death, and also processed within a few hours, the average cell yield was 33×10^6 cells/g of tissue. However, it was observed that when kidneys removed longer than 12 h after death were subsequently stored overnight in CMRL 1415 at 4 to 6 C and then processed, the average cell yield per gram of tissue increased to 60.6×10^6. In all except two cases (10-month-old baby and a stillborn) a full cell sheet was obtained seven days after seeding as little as 25,000 cells/ml (1,000 cells/cm^2) in F20

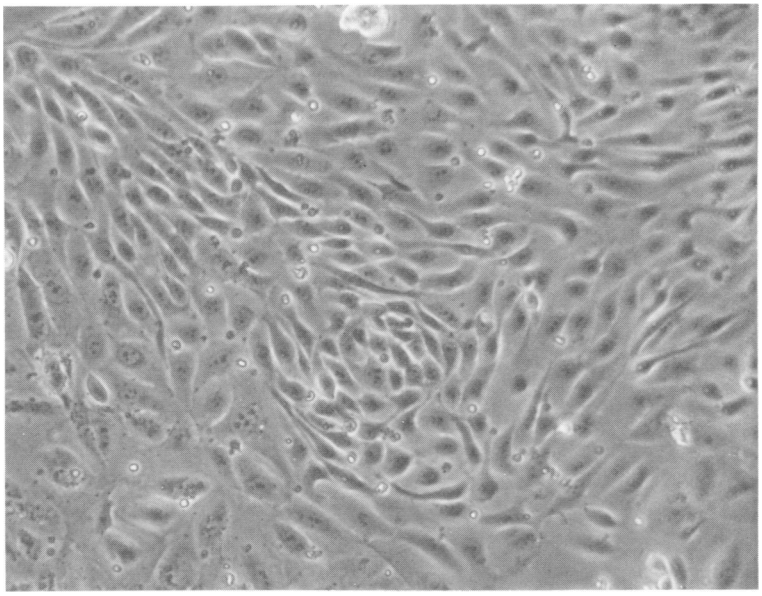

FIG. 1. *Primary human kidney culture 96 h after seeding. Phase contrast ×100.*

Falcon flasks. No differences in cell growth could be detected when tissues were cultured in storage medium instead of fresh medium.

Table 2 summarizes the results obtained with rabbit kidneys. In all of the experiments higher cell yields were obtained after storage of the kidney for 24 h at 4 to 6 C in CMRL 1415 ATM (paired Student's t-test $P<0.005$).

TABLE 2. *Cell yields per gram of rabbit kidney (10^6 cells/g of tissue) obtained 12 h postmortem*[a]

Expt no.	Kidney processed immediately after removal	Kidney processed after overnight storage
1	29.3	44
2	20.5	27.2
3	30.8	38.4
4	33	46.5
5	16.7	33
	$\bar{X}$ 26.06 ± 7.06 standard deviation	$\bar{X}$ 39.02 ± 8.57 standard deviation

[a] One kidney was processed immediately after removal. The second kidney was stored 24 h in CMRL 1415 at 4 to 6 C.

DISCUSSION

An increase in cell yields from newborn human kidneys was obtained by the addition of collagenase to the conventionally used trypsin-EDTA-dispersing solution. The mechanism of the increase of cell yields seems to be due to the digestion of a viscous material formed when newborn human kidneys are dispersed with trypsin-EDTA alone. This viscous material apparently entraps cell clumps, thus reducing the cell count. The formation of the viscous material seems to be peculiar to human kidneys, and was not observed in monkey, rabbit, rat, hamster, or dog kidneys (4).

Viable cell suspensions could be obtained from newborn human kidneys (removed from refrigerated cadavers) even when the kidneys were removed as late as 36 h after death. However, not unexpectedly, higher cell yields were obtained from newborn kidneys removed within 12 h after death as compared to those removed later than 12 h postmortem. Curiously, it was noted that the cell yields from the kidneys removed between 12 and 36 h postmortem could be increased by overnight storage of kidneys in synthetic medium at 4 to 6 C. This observation, made on randomly available human material, was confirmed by experiments with paired rabbit kidneys. When the rabbit kidneys were processed immediately after death, a cell yield of 76×10^6 cells/g of tissue was obtained (3). In this study, when the kidneys were removed 12 h after death, the cell yield was only 23.3×10^6 cells/g of tissue. However, if the 12-h postmortem kidneys were incubated overnight at 4 to 6 C in CMRL 1415 ATM, the cell yield rose to an average of 37.7×10^6 cells per g of tissue. Although no definite explanation can be offered for the increase in cell

yield after storage, it is possible that storage rendered the cells less susceptible to injury by dispersing enzymes. Since cells grow well in the storage medium and do not show signs of toxicity, this effect is apparently not due to the elution into the medium of toxic substances during storage.

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